TECHNICAL MYCOLOGY:

THE UTILIZATION OF MICRO-ORGANISMS IN THE ARTS AND MANUFACTURES.

A PRACTICAL HANDBOOK ON

FERMENTATION AND FERMENTATIVE PROCESSES FOR THE USE OF BREWERS AND DISTILLERS, ANALYSTS, TECHNICAL AND AGRICULTURAL CHEMISTS, PHARMACISTS, AND ALL INTERESTED IN THE INDUSTRIES DEPENDENT ON FERMENTATION.

BY

DR. FRANZ LAFAR,

Professor of Fermentation-Physiology and Bacteriology in the Imperial Technical High School, Vienna.

TRANSLATED BY CHARLES T. C. SALTER.

VOL. II.—**EUMYCETIC FERMENTATION.**

PART II.

With 50 Figures in the Text.

LONDON:
CHARLES GRIFFIN AND COMPANY, LIMITED;
EXETER STREET, STRAND.
1910.

PRINTED BY
BALLANTYNE & COMPANY LTD.
TAVISTOCK STREET COVENT GARDEN
LONDON

TABLE OF CONTENTS.

DIVISION II.

EUMYCETIC FERMENTATION.

Section XIII.—Yeast Nutrition and Yeast Culture.

Chapter XLIX.—Mineral Foodstuffs.

Chapter L.—Organic Foodstuffs. The Requirements in Respect of Oxygen.

Chapter LI.—Cultivation and Reproduction of Yeast.

Chapter LII.—The Effect of Certain Technically Important Chemical Influences of Yeast.

Section XIV.—Life History and Variability of the Saccharomycetes. Classification of the Saccharomycetes and Schizosaccharomycetes.

By ALBERT KLÖCKER.

Chapter LIII.—The Life History of Saccharomycetes in Nature.

Chapter LIV.—Variability and Heredity in Saccharomycetes.

Chapter LV.—Classification of the Families Saccharomycetaceæ and Schizosaccharomycetaceæ.

Section XV.—Morphology and Classification of Certain Technically Important Higher Ascomycetes and Allied Forms.

Chapter LVI.—Morphology and Subdivision of the Family Aspergillaceæ.

By PROF. DR. CARL WEHMER.

Chapter LXI.—Saccharomycetes Apiculatus.

By PROF. DR. H. MÜLLER-THURGAU.

Chapter LXII.—The Moniliæ and Oidia.

By DR. H. WICHMANN.

Section XVII.—The Enzymes and Enzyme Actions of Yeast.

Chapter LXIII.—Alcoholase.

By DR. RUDOLF RAPP.

Chapter LXIV.—The Chemistry of Alcoholic Fermentation.

By DR. ARMINIUS BAU.

Chapter LXV.—Enzymes Decomposing Disaccharides and Polysaccharides.

By DR. A. BAU.

Chapter LXVI.—Endotryptase and Philothion.

By DR. M. HAHN AND DR. LAFAR.

ERRATUM

Page 379, line 1, "*Malrosporium*" should be "*Macrosporium.*"

TECHNICAL MYCOLOGY.

SECTION XIII.

YEAST NUTRITION AND YEAST CULTURE.

CHAPTER XLIX.

MINERAL FOODSTUFFS.

§ 257.—Ash Content and Ash Analysis.

Since the requirements of the Eumycetes in general in respect of ash constituents have already been fully dealt with in chapter xli., it may seem superfluous to the reader to revert to the question of the mineral needs of the yeasts, all of which belong to that group. It should, however, be remembered that the matter was then treated from a purely physiological standpoint, the problem being to ascertain what mineral substances are essential to the structure of the Eumycetes, and therefore also yeasts. The question, however, comes into the region of practical economics, so soon as we have to deal with ferments (*e.g.* yeasts) that are required in large quantities for industrial purposes. In this case it is no longer sufficient to know that certain ash constituents are indispensable, but it also becomes necessary to ascertain how these requirements can be satisfied in practice, and to investigate the conditions under which the activity of these ferments can be raised to the maximum by a suitable selection of the sources of supply of the mineral foodstuffs under consideration.

From this practical standpoint we shall deal more fully, in the present chapter, with three elements : calcium, phosphorus, and sulphur, which have been, up to the present, more fully investigated than any others with regard to their influence on the development and activity of yeast. On the other hand, our knowledge on the importance of potassium must be characterised as scanty, and therefore this foodstuff (also known to be essential) must necessarily be dismissed in a few words. From experiments conducted by H. Becker (I.) it appears that the quantity of potash in the nutrient solution influences the degree of attenuation. A beer wort containing naturally 0.071 per cent. of potassium (K), attenuated to 56.4 per cent., whereas in the case of two parallel samples, the potassium content of which was raised to 0.078 and 0.085 per cent. by the addition of potassium carbonate, attenuated, under

similar conditions, to only 52.2 and 48.9 per cent. respectively. Hence the increased potassium content of the wort resulted in a reduced attenuation. In tests carried out by R. KUSSEROW (I.), the addition of dipotassium phosphate (K_2HPO_4) had no apparent influence on the fermentation of the wort; but here the effect of the potassium alone could not be judged, owing to the simultaneous presence of the phosphoric acid of the salt. According to the observations of C. G. MATTHEWS (II.), potassium is more readily assimilated when a portion is present in the condition of sulphate than when the phosphate alone is used. Compare also the statement of A. MAYER (I.) on this point.

Before proceeding to discuss the importance of the aforesaid three elements in connection with the life and action of yeast, it will be necessary to deal with two points of special interest to the chemist and fermentation technologist, namely, the percentage and the quantitative and qualitative composition of the ash constituents of yeast.

With regard to the ash content, numerous determinations are available, a few of which, referred to the dry matter of the yeast, are given below:

PERCENTAGE OF ASH IN YEAST.

Top fermentation Yeast . . .	7.65	2.5	8.9	5.5	11.5
Analyst—	Mitscherlich.	Schlossberger.	Bull.	Bèlohoubek.	HESSENLAND (I.).
Bottom Yeast . .	7.51	3.5	5.3	8.1	8.07
Analyst—	Mitscherlich.	Schlossberger.	Wagner.	Schützenberger and Destrem.	C. Lintner, Munich.
Bottom Yeast (*cont.*) .	7.61	8.76	7.7	10.1	
Analyst—	C. Lintner, Weihenstephan.	Seyffert, St. Petersburg.	BÉCHAMP (VI.)	Hessenland.	

These figures are not all equally reliable. For instance, the remarkably low figures given by Liebig's pupil, J. SCHLOSSBERGER (I.) are due to the fact that the yeast samples were not only washed very clean with plenty of water, before drying and incinerating, but were also "purified" with cold and hot water, and with ether, as though dealing with a precipitate of the character of ferric hydroxide, instead of delicate cells with permeable walls. Other workers, though proceeding with greater care, have mostly overlooked the fact that the sedimental yeast they obtained from the brewery for the purposes of their experiments does not consist exclusively of yeast cells, but contains all sorts of other matters (§§ 245, 254, 255), only a few of which are removed by washing and sifting, so that a considerable difference exists between the ash of yeast, *per se*, and that of ordinary sedimental yeast.

PERCENTAGE COMPOSITION OF YEAST ASH.

	Pressed Yeast.			Top Yeast from White Beer.	Bottom Yeast, Munich.			Weihenstephan Bottom Yeast.			
	Mitscherlich.	Bèlohoubek.	Champion and Pellet.	Bull.	Liebig.		Mitscherlich.	Béchamp (VI.)		Lintner.	
K_2O . .	39.5	38.68	23.33	35.2	29.1	30.6	28.3	28.79	31.52	38.45	26.07
Na_2O .	—	1.82	16.63	0.5	2.5	—	—	1.93	0.77	—	2.26
CaO . .	1.0	1.99	1.36	4.5	2.4	2.1	4.3	2.49	2.39	2.85	7.58
MgO . .	6.1	4.16	5.23	4.1	4.1	4.2	8.1	6.54	3.77	5.80	6.34
Fe_2O_3 .	—	0.06*	*	0.6	—	—	—	7.34?	2.73**	0.51	0.70
P_2O_5 . .	53.8	51.09	49.06	54.7	44.8	48.5	59.4	53.87	53.44	48.19	54 31
SO_3 . .	—	0.57	trace	—	2.1†	—	—	6.38	5.05	0.62	0.31
SiO_2 . .	trace	1.60	1.88	—	14.4	—	—	trace	trace	1.26	0.92
Cl . .	—	0.03	trace	0.1	—	—	—	?	?	—	—
Not determined	—	—	††2.15	—	—	—	—	—	—	—	—
	100.4	100.0	—	99.7	99.4	85.4	100.1	107.34	99.67	97.68	98.49

? An error. * Trace of MnO. † Incl. Chlorine and CO_2. ** Trace of Al_2O_3. †† Incl. Fe_2O_3.

The foregoing objections apply equally to the percentage composition of the two kinds of ash; and for this reason the percentage results of the series of ash analyses performed on wine yeast by Braconnot must be omitted. Furthermore, the results of analyses of commercial pressed yeast, by Champion and Pellet, and A. Bèlohoubek (II.), in the table (vol. ii. p. 193) must not be taken as unconditionally accurate, since this class of yeast contains a considerable amount of (ash-bearing) starch, the proportions being in these two instances 10 and 14 per cent. respectively.

For the purpose of criticism we will take the first column of figures. The 53.8 parts of phosphoric acid require 107 parts of lime for complete saturation, whereas only about 40 parts of that substance are present, *i.e.* only sufficient to neutralise 21.6 parts of P_2O_5. The remaining 28.4 parts cannot be completely combined by the other bases, viz., 1.0 CaO and 6.0 MgO, so that $21.6 - (0.8 + 7.1) = 13.7$ parts of P_2O_5 are left in a free state. This excess of free phosphoric acid accounts for the acid reaction of yeast ash, or the solution thereof, which reaction was first observed by Quevenne (I.). Being able to resist the action of heat, this free phosphoric acid effects the expulsion of sulphuric acid during incineration, and consequently the latter acid will not be found in the ash unless measures have been taken to ensure its combination and protection. Such measures were first adopted by Béchamp (VI.) in 1871, but were neglected by Mitscherlich (III.) in 1845, and even by J. Liebig (II.) in 1870, although it was already known that yeast contains an appreciable quantity of sulphur. In fact Liebig himself found 0.69 per cent. of sulphur in the dry residue, whilst Mitscherlich, Reichenbach, and Dempwolff gave the figures 0.6, 0.57, and 0.39 per cent. respectively. The same objection must be made with regard to the results of analyses by H. Seyffert, given in subsequent paragraphs.

In connection with the phosphoric acid, careful procedure is also necessary to prevent its partial reduction to (volatile) phosphorus during the initial carbonising stage of the incineration process. The possibility of this loss is apparently not precluded even in the Mitscherlich method. With regard to the 14.4 per cent. of silica mentioned in the fourth column, it is uncertain whether this is entirely due to accidental contamination of the sample with sand, &c. (vol. ii. p. 48). It is therefore evident that it is by no means an easy task to secure a perfect analysis of yeast ash; and it is desirable that the hiatus, resulting from this cause, in our knowledge of yeast and its vital activity, should be filled up as early as possible.

§ 258.—The Importance of Lime.

We cannot expect to find any reliable particulars regarding the ash requirements of yeast at a period when the authorities were

of opinion that this "ferment" was nothing more than a proteid body in a state of incipient decomposition. Even T. A. QUEVENNE (I.), who did not dispute that yeast possesses the characteristics of a living organism, regarded the ash of same as something quite immaterial—an accidental impurity.

Since, as already mentioned, the proteids also contain ash, it was not possible to affirm the necessity of mineral foodstuffs for the development of yeast, until a nutrient medium had been discovered in which the nitrogenous food constituents were present in a state of combination free from ash. This was first successfully accomplished by PASTEUR (VII.), who proposed to employ the readily procurable ash from beer yeast for the purposes of artificial culture in nutrient solutions in the laboratory.

The further problem of the number and character of the indispensable inorganic elements was not attacked by Pasteur, the first to do this being ADOLF MEYER (I. and V.), who ascertained, by numerous culture experiments, that the mineral foodstuffs indispensable and sufficient for the development of yeast are potassium, magnesium, iron, phosphorus and sulphur.

The resulting indirect assumption that lime is unessential conflicts with the practical experience, of brewers especially, that worts and mashes poor in lime give a very defective fermentation. In view of this circumstance, the preceding sentence requires modification in so far that, though lime may not be essential to the actual growth of yeast, it forms an indispensable adjunct or stimulant when the fermentative power of the yeast is concerned. The part played by lime in this connection is still unknown, and its investigation would be a very thankworthy task. Possibly it combines with and nullifies the action of the poisonous oxalic acid, which was shown by C. WEHMER (V.) to be a common and important final product of the metabolism of numerous fungi, including *Aspergillus glaucus*, *A. niger*, *Penicillium glaucum*, *Mucor mucedo*, *Rhizopus nigricans*, *Phycomyces nitens*, *Peziza Fuckaliana*, &c. In the case of these fungi, the acid can probably be rendered innocuous by the magnesium present, though the larger quantities produced by the far greater fermentative activity and substantive transformations resulting in the case of yeast, cannot be properly dealt with by that base alone. A more careful examination (which does not seem to have been undertaken as yet) of the gradual increase in formation of oxalic acid during the fermentation of beer wort, and the determination of the nature of the base, or bases, with which this acid is combined, will, it is hoped, bring us a step nearer to solving the problem of the importance of lime in connection with yeast.

As the result of wide experience, brewers are agreed that yeast which has been grown in worts poor in lime quickly degenerates, and in particular is incapable of producing "break" in the wort. This defect is frequently experienced in breweries com-

Material.	Ash contents in dry residue.	Percentage composition of Ash.									
		K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	CuO.	P_2O_5.	SO_3.	SiO_2.	Cl.
Bavarian wort, according to Lintner	—	41·20	0.03	4.50	2.20	—	—	31.50	trace	20.40	trace
St. Petersburg pale wort, according to Seyffert	0.24	32.99	1.60	1.63	9.21	0.44	—	45.78	0.32	6.88	0.10
YEAST A :											
1. Obtained direct from Germany .	9.71	32.66	0.59	4.48	4.94	0.05	—	55.75	trace	0.51	0.11
2. After being used seven times .	8.94	35.22	—	0.54	5.23	0.45	—	56.75	trace	1.22	trace
3. Pure culture, slightly watered .	7.56	34.92	—	0.83	5.25	0.29	0.64	56.86	trace	0.80	trace
4. „ „ strongly wat red .	7.65	31.87	0.07	1.48	5.47	0.42	0.37	58.88	trace	0.98	trace
YEAST B :											
1. Pure culture, used once in wort treated with gypsum, slightly watered	6.82	31.76	trace	3.68	5.38	0.15	0.27	57.67	trace	0·62	trace
2. After being used twice in untreated wort ; strongly watered	5.01	29.06	0.14	2.42	5.70	1.21	0.38	60.30	trace	1.21	trace

pelled to use very soft brewing liquor (*i.e.* deficient in lime), and it is a common practice to counteract this evil by adding a little powdered (unburned!) gypsum to the mashing liquor (about 1½ oz. per 1000 galls.). Very satisfactory results are obtained: good head, thorough fermentation, good "break" and a firm sedimental yeast (provided the yeast employed is capable of furnishing such, *see* vol. i. p. 256). We are indebted to H. Seyffert (I.) for an interesting example, relating to a St. Petersburg brewery, working with a liquor containing only 1.3 parts of CaO per 100,000. After having failed to obtain satisfactory fermentation with a series of pure yeasts, of German origin, it was finally decided to examine the worts, and these were found to be deficient in lime, so that the yeasts grown in them became more and more impoverished in that constituent, as can be seen from the table (vol. ii. p. 196). (The column headed CuO will be dealt with later on.) The yeasts were famished in respect of lime, and even absorbed the small quantities of that base present in the water with which they were washed (vol. ii. p. 118).

H. Seyffert pushed his experiments to the furthest limit, by reducing the already small lime content of the wort still more by dialysis, before pitching it with yeast. In these circumstances a highly frothy fermentation (vol. ii. p. 184) ensued. His observations have also a certain value in connection with the pure culture of yeast, since they demonstrate that a successful result depends not only on the happy selection of a suitable race of yeast, but also on the favourable composition of the nutrient medium—a point already insisted on by Hansen (III.). Hence, in cases where the application of pure yeast does not fulfil expectations, one should not immediately condemn the innovation. The yeast can only furnish good results when a suitable medium is provided—and this is the task of the practical man.

§ 259.—The Phosphoric Acid Requirements of Yeast.

Yeast requires a good deal of phosphoric acid; and, as can be seen from particulars already given, a considerable amount of this acid is present in the ash. The figures given in the table on p. 193 agree with those reported by K. Lintner (IV.) as obtained at the Munich Experimental Station, and ranging between the limits of 3.21 and 3.84 per cent. of P_2O_5 (referred to dry matter), the mean being 3.61 per cent. All these values, however, are surpassed by 59.5 per cent. of P_2O_5 found in the ash of English top-fermentation yeast by A. C. Salmon and W. de Vere Mathew (I.).

Under ordinary conditions in the brewery the needs of the yeasts for phosphoric acid are satisfied by the phosphates and organic phosphorus compounds present in the malt, though in some cases the amount contained in the barley and malt is inadequate. As a rule, 0.9 per cent., calculated on the dry residue,

may be taken as the average phosphoric acid content in barley, though C. Lintner (I.) reports an instance of a Hungarian barley from the year 1877, which exhibited the remarkable low content of 0.58 per cent. and furnished worts with such a low attenuation, and sedimental yeast of such enfeebled fermentative energy, as to cause great trouble; whereas barleys of the same origin, but from the preceding year, and containing 0.67 to 1.06 per cent. of phosphoric acid, yielded readily fermentable worts under equal conditions. Given parity in the requirements of phosphoric acid content by the yeast, and equal treatment in brewing, a wort low in phosphoric acid will give a relatively far poorer beer. As a matter of fact the corresponding percentages, so far as they have been published up to the present, fluctuate within wide limits, 0.026 and 0.115 per cent. of P_2O_5 in the case of German beers. For this reason, as has already been shown by G. Holzner (I.), the proposal of J. Skalweit (I.) and Fritz Elsner (I.) to employ the phosphoric acid content of beer as a measure of its quality or purity, falls to the ground. Lessened attenuation, in consequence of a scarcity of phosphoric acid in the wort, is also frequently experienced in British (top-fermentation) breweries. The remedy applied in such cases is to fortify the wort with phosphates, potassium phosphate in particular. Care is, however, necessary not to employ an overdose, the observations of A. G. Salomon and W. de Vere Mathew (I.) apparently indicating that an excess of phosphates retards fermentation.—Among artificial adjuncts for such purposes, mention may be made of G. Funk and N. von Balogh's (I.) patented method of employing glycerophosphoric acid, $C_3H_5(OH)_2H_2O$, the calcium and magnesium salts of which are soluble in water.

Considerable advantages can be derived from these observations in the preparation of mead or honey wine. Honey is very poor in ash constituents and nitrogenous nutrition, the quantity being usually too small for even moderate development and fermentative activity of the contained yeast cells. The resulting difficulties, well known to all mead manufacturers, can be obviated by treating the honey with nutrient salts. The following recipe for the preparation of mead is based on researches carried out by G. Gastine (I.): About 230 grms. of honey are dissolved in 1 litre of water and treated with 5–7 grms. of a mixture of nutrient salts, composed of diammonium phosphate 100 parts, neutral ammonium tartrate 350, potassium bitartrate 600, magnesia 20, calcium sulphate 50, common salt 3, and tartaric acid 250 parts. The one part of sulphur, also recommended by this author, is, however, preferably omitted. The solution, prepared as above, is boiled up, and after recooling is pitched with wine yeast, which quickly incites a powerful fermentation that runs its due course. It should not be forgotten that an improvement in the flavour of the mead may be expected from the employment of a selected

race of yeast. As pointed out by Beyerinck (XVIII.) and others, yeast cells are rarely, if at all, present in the nectar of flowers or natural honey ; consequently an artificial addition of high-class yeast is really necessary for obtaining accelerated fermentation, and will be the more successful inasmuch as its action is barely interfered with by the relatively small proportion of other fermentative organisms present. A few observations in this connection have been made by E. Chuard (I.), and more exhaustive experiments by E. Kayser and E. Boullanger (I.). The latter workers also replaced the Gastine nutrient mixture by simpler and equally efficient adjuncts, namely, by treating 1 litre of diluted (24–27 per cent.) honey with either 1.5 c.c. of maltopeptone and 1.5 grms. of potassium tartrate, or with 1.5 c.c. of maltopeptone and 1 grm. of ammonium tartrate; or with 0.12 grm. of spongy peptone, 1.5 grms. of potassium tartrate and 1 grm. of ammonium phosphate. These workers also conducted some experiments in connection with "oenomel," a fermented mixture of honey and wine must.

The opinion expressed by H. Elion (I.) as to the variable requirements of different yeasts in respect of phosphoric acid, and the fluctuations in the resulting increase in fermentative activity, still needs confirmation.

L. Liebermann (I.–III.) asserted that a portion of the phosphoric acid present in the yeast cell is in the form of the meta compound, and, in fact, the same as that contained in nuclein (§ 252), because he believed he had succeeded in isolating barium metaphosphate both directly from yeast, and also from the nucleic acid separated from yeast by himself and B. von Bitto (II.). The analyses advanced in support of this view proved quite as untenable, under the criticism of Kossel (IV.–VI.), as was the case with the cognate characterisation of nuclein as a mixture of the metaphosphates of xanthin and allied bases with a proteid metaphosphate, and the resulting inference that the artificial nucleins prepared in this way are identical with certain natural nucleins. Nevertheless, the first of these hypotheses has been found accurate, Kossel (V.) himself having detected metaphosphoric acid among the decomposition products of the nucleic acid of yeast (vol. ii. p. 161). Its occurrence was also demonstrated by Alb. Ascoli (I. and II.) in the molecule of plasmic acid, one of the derivatives of that acid. On the other hand, no metaphosphoric acid has been detected in other nuclein bodies, such, for instance, as the paranuclein obtained by the action of pepsin on casein, and in the so-called leuconuclein.

Part of the phosphates or phosphoric acid consumed by yeast is excreted from the latter in the form of phosphocarnic acid. This interesting discovery, which, we are informed by B. Haas (II.), was made by J. Stoklasa, will, if found to be accurate,

facilitate the differentiation of natural and sophisticated wines by the chemist, the latter wines usually containing phosphoric acid solely in the form of (added) orthophosphates.

§ 260.—The Importance of Sulphur.

The value of sulphur in connection with the metabolism of yeast is still in complete obscurity. The fact that this substance is never absent in yeast samples justifies the inference that it is indispensable for the growth of the plant. It is almost impossible to prove this directly, *i.e.*, by cultivation experiments, because up to the present no one has succeeded in eliminating the sulphurous impurities (vol. ii. p. 48) from the comparatively large amount of sugar needed to furnish a sufficient crop of yeast for analytical purposes, which impurities—according to a calculation made by Adolf Mayer—suffice to supply the sulphur present in the proteid substances of the crop. The next problem on the list, namely, the nature of the assimilable sulphur compounds taken up by the yeast, also remains unsolved. All that can be said at present is that the sulphates (of calcium and magnesium), so greatly appreciated by the higher plants, appear ill adapted for the construction of the yeast cell. The sulphur in these salts is eliminated and expelled either as sulphur dioxide or even sulphuretted hydrogen. Further information on this point will no doubt be welcomed by fermentation technologists.

The first reliable information on the production of sulphur dioxide during alcoholic fermentation by yeast was supplied by Fr. Pfeifer (I.), who traced the gradual accumulation of this reduction product in fermenting beer wort, and obtained the following figures:

SO_2 Content in Mgrms. per Litre.

	Lager Beer.	Draught Beer.	
Wort from the filter-bag . . .	traces	traces	traces
At the beginning of fermentation .	11.1	7.6	2.9
In cask, after close of primary fermentation	11.7	8.8	3.7
After storage for a month . . .	12.5	7.7	?
On leaving the brewery . . .	?	9.6	4.7

In saccharose solutions, treated with the necessary nutrient salts (including ammonium sulphate), sterilised and inoculated with a large quantity of yeast, 11.4 mgrms. of SO_2 were detected at the end of five days, when fermentation was almost completed. The same results were obtained by B. Haas (I.) in his experi-

ments with fermenting wine must, though he raised the point that the collaboration of reducing bacteria was not impossible. In a fermenting must, seven weeks old, he found 49.4 mgrms. of SO_2 per litre; and two months later 576 mgrms. In view, however, of the determinations of Pfeifer, confirmed by the observations of S. Klaudi and A. Svoboda (I.), his statement that the reduction of the sulphates occurs only with thin sowings of yeast and sluggish fermentation cannot be accepted. E. Hotter (I.) also found 4.5–4.8 mgrms. of SO_2 per litre in cider and currant wines prepared in the laboratory, and certainly not sulphured.

These discoveries have no small importance for the fermentation technologist and foodstuff chemist, since, until recently, it was usual to consider that beer found to contain sulphur dioxide must have been made from strongly sulphured hops, or else treated with calcium sulphite as a preservative. Similar conclusions (cask sulphuring or washing with calcium bisulphite) were also formed with regard to wines found to contain sulphur, more especially since L. Roesler (I.) in 1885 stated that he had never succeeded in detecting sulphur dioxide in wine prepared in the laboratory so as to preclude these sources of sulphur. The present state of our knowledge shows that the matter is different and that great care must be exercised, in this direction also, in judging the results of analysis, a schooling in fermentation physiology being moreover indispensable. Both the sulphur dioxide produced during fermentation and that originating in the sulphuring of the casks is almost entirely converted into a state of combination during the storage of the wine (§ 79), so that, as was confirmed by M. Ripper (I.) and R. Kayser (I.), only extremely minute quantities of free SO_2, mostly inferior to 2 mgrms. per 100 c.c., are present in wine that is ready for bottling. This free dioxide alone comes under consideration in judging wine from the medico-physiological standpoint, and not that present as aldehydic sulphur dioxide, which is not merely innocuous to health—according to the researches of J. Marischler (I.)—but really essential to the bouquet of the wine. In consequence of the reducing power of the dioxide, the presence of larger quantities in beer or wine affects the results of sugar determinations with Fehling's solution, causing, as was first pointed out by Jos. Herz (I.), the sugar values to come out in excess of the truth.

In certain circumstances the reduction of the sulphates in the nutrient solution by the activity of yeast may proceed a stage further than the formation of the dioxide, namely, to the production of sulphuretted hydrogen. However, the first report on this point, as made by Crouzel (I.), was found inaccurate when tested by F. Gay (I.), though confirmed by the researches of Nastukoff (I.) with pure cultures. A solution of 10 per cent. of

saccharose and 0.5 per cent. of Gastine's nutrient salt mixture (§ 259), in which the calcium sulphate had been replaced by magnesium sulphate, was inoculated with pure wine yeasts from Portugal and Champagne, Brussels beer yeast, *Saccharomyces apiculatus* and *Sacch. Pastorianus*, all of which yeasts proved capable of producing the gas in question. The question, arising from this result, as to the dependence of the reduction phenomenon on the environment of the culture, was more closely examined by A. L. Stern (I.), who failed to discover any substance of *known* constitution capable of supplying the yeast with sulphur without the concurrent liberation of sulphuretted hydrogen. This agrees well with the fact, known to every chemist and fermentation technologist and first pointed out by Reischauer, that the distillates from beer very frequently contain sulphuretted hydrogen, or a compound exhibiting all the characteristics of the same.

Moreover, the formation of sulphuretted hydrogen by the reducing action of yeast is not confined to the sulphates; even sulphur itself may serve by combining with the hydrogen liberated from other substances by the activity of a yeast enzyme (philothion), which will be dealt with, in conjunction with other allied matters, in a subsequent chapter.

CHAPTER L.

ORGANIC FOODSTUFFS. THE REQUIREMENTS IN RESPECT OF OXYGEN.

§ 261.—Sources of Carbon.

The true water content, and therefore also the amount of dry residue (dry matter) in the yeast cell itself, is not yet accurately known. The figures cited in the literature are based on experiments performed, not on the cells only, but on samples of sedimental yeast from the brewery, or pressed yeast, neither of which, as we have already seen (vol ii. pp. 119, 175, 176), is of uniform nature, but includes a variety of organic and inorganic admixtures. The amount of dry residue in pressed yeast is determined, of course, by the amount of pressure employed. R. Kusserow (III.), in testing eight different samples, free from starch, found 22.1 per cent. as the minimum, 29.9 per cent. as the maximum, and 25.6 per cent. as the average water content. In practice, 26 per cent. is usually estimated.

The sp. gr. of the cells of pressed yeast was determined as 1.1 by P. Guichard (I.) in 1894, though the method, suspension of the cells in a mixture of alcohol and chloroform, was not perfectly reliable, and probably gave results in excess of the truth. On the other hand, the pycnometric method adopted by Kusserow for determining the sp. gr. of his eight samples of pressed yeast gives values that are probably too low. He weighs out exactly 10 grms. of the yeast, triturates them with a little distilled water in a porcelain basin, and swills the mixture into a pycnometer, which is then filled up to the mark with distilled water and weighed. Taking O as the weight of the whole and P the weight of the pycnometer filled with water alone, the difference O-P being equal to a, the sp. gr. of the sample of pressed yeast works out to $S = 10 : 10 - a$. Kusserow determined the maximum value as 1.1093 and the minimum as 1.0821.

The sp. gr. of the dry residue of these samples ranged from 1.580 and 1.491, with 1.509 as the mean value. By assuming (which is not strictly accurate) that the volume of the yeast sample is equal to that of the percentage, by weight (T), of the dry residue, plus that of the water content (W), and taking the above mean into consideration, we obtain the equation

$$\frac{W}{1} + \frac{T}{1.509} = \frac{100}{S}.$$

and, the sp. gr. of the sample being known, this gives the equation

$$T = 296.5\left(1 - \frac{1}{S}\right),$$

whilst, the dry residue being known, the sp. gr. of the yeast sample is found by the equation $S = 296.5 : (296.5 - T)$.

Contrary to the proposition advanced by Hayduck, however, it is unfortunately impracticable to determine the quantitative addition of starch in a sample of pressed yeast by this method, the difference between the sp. gr. of anhydrous starch (mean 1.65) and that of the dry residue of the yeast (1.509) being too small. This method is discarded with greater regret because the existing chemical methods, based essentially on the hydrolysis of the starch and the determination of the resulting sugar, are very unreliable owing to the fact that this treatment saccharifies the glycogen of the yeast as well as the added starch, and that the amount of the former (vol. ii. pp. 170, 171) is sometimes very large, occasionally exceeding that of the starch itself.

The ultimate composition of the organic matter in the dry residue of yeast cells is influenced by the mode of nutrition as well as by the kind and age of the cells, for which reason generalised values are unreliable. Moreover, the available analytical data on this matter have not been obtained by working with actual cells, but from the examination of pitching yeast or pressed yeast. Now the invariable presence of admixtures in these samples, already alluded to in § 257 as preventing the acquisition of reliable data on the ash constituents of the yeast cell, has a still greater adverse influence when the determination of the amount of carbon, hydrogen and oxygen in the cells is in question, many of these admixtures being low in or free from ash, and consisting solely of three or four of the elements just mentioned. Consequently the results of the ultimate analysis may differ between wide limits, according to the proportion of such impurities present. As instances of this, and not merely to comply with an injudicious demand for quantitative reports on the ultimate composition of yeast, a few results obtained in this connection are reproduced on p. 205. Although on the publication of the first analysis by Marcet the useless character of such figures was pointed out by Quevenne (I.) in 1838, similar results have been brought forward from time to time since. In fact, some workers have gone so far as to assume that an expression of the difference between top and bottom yeasts can be found in the results furnished by ultimate analysis—a view that is, of course, untenable.

Carbon compounds may be taken up by yeast for three purposes: (1) for alcoholic fermentation and other enzyme actions;

(2) to replace the energy dissipated by respiration; (3) for the formation of new cell substance in growing cells, or to replace constituents decomposed and excreted, in consequence of other metabolic changes in full-grown cells. The first of these three causes of the consumption of carbon compounds will be discussed thoroughly in a subsequent section, and will therefore be omitted from the present paragraph. The second has also been referred to on pp. 126, 127 of vol. ii., and will be supplemented later; so that we have at present only the third to deal with.

We are indebted more particularly to Emil Laurent (VI.) for a comprehensive investigation of a large number of carbon com-

Ultimate Percentage Composition of the Organic Matter of Yeast Referred to Dry Residue free from Ash.

Author.	Class of Yeast.	C	H	N	O	S	P
Marcet . .	Beer yeast	30.5	4.5	7.6		45.4	
Dumas (I.) .	,, ,,	50.6	7.3	15.0	—	27.1	—
Mitscherlich (III.) . .	,, ,,	47.0	6.6	10.0	?	0.6	—
Schlossberger (I.)	,, ,, ,, (top yeast)	49 8	6.7	12.4	31.1	—	—
Hessenland (I.)	,, ,,	48 6	7.1	7.8	36.6	—	—
,,	bottom yeast	49.3	8.2	10.5	32.0	—	—

pounds in respect of their suitability for supplying yeast with carbon. These researches were performed on a series of pure culture beer and wine yeasts, with both mineral nutrient solutions and gelatin nutrient media, and showed that the following substances can be absorbed and assimilated as sources of carbon: the acetates of potassium, sodium and ammonium; lactic acid and the lactates of these three bases and of calcium; malonic acid and its potassium salt; succinic acid and its ammonium salt; the potassium and calcium salts of glyceric acid; the calcium salt of glycerophosphoric acid; malic acid and its potassium and ammonium salts; dextro-tartaric acid and its potassium and ammonium salts; levo-tartaric acid; citric acid and its potassium and ammonium salts; mucinic acid; fumaric acid; aspartic acid; asparagin; glutaminic acid (all in the proportion of 1 per cent.); glycerin, mannitol, quercitol, glucose, fructose, saccharose, maltose, lactose, dextrin, salicin, amygdalin, and many others. On the other hand, the following were not assimilated by the yeasts (in sedimental and not film cultures): methyl-, ethyl-, propyl-, and butyl-alcohol (2–4 per cent.), formic acid and its potassium, sodium, ammonium and calcium salts, acetic acid, propionic acid

and its potassium salt, butyric acid, valerianic acid, stearic acid, oleic acid and its potassium salt, sodium butyrate, oxalic acid and its potassium and ammonium salts; the ammonium salts of benzoic acid, salicylic acid, and gallic acid; urea (all as 1 per cent. additions).

When yeast is cultivated as film cultures on the surface of the nutrient solution, and not under the conditions employed by Laurent, it is also capable of utilising alcohol, though chiefly, or even exclusively, by respiration, a method we are not considering at present.

Both in nature and in the practice of the fermentation industries, the carbohydrates form the usual and preferential material from which yeast obtains its requirements in respect of carbon, the chief part in this respect being played by certain **sugars**. The behaviour of yeast toward these latter, with regard to their assimilation as distinct from fermentation, has not, however, been sufficiently investigated, the results of Laurent's researches on this point being unsuitable for generalisation, since they apply solely to the species of yeast tested by him, and not to all the others. We are indebted to Beyerinck (XVIII.) for the discovery that the *Schizosaccharomyces octosporus*, found by him on currants, forms an exception to Laurent's rule, inasmuch as it is capable of assimilating maltose, glucose and fructose, but not saccharose, lactose, raffinose, arabinose, dulcitol, quercitol, erythritol and inositol. The antithesis of this species is *Saccharomyces Zopfii*, which, according to Artari (I.), can cover its needs in respect of carbon from saccharose, glucose and mannitol, but not from maltose, lactose, galactose, inulose or melampyrit. Similar behaviour to the last-named organism is afforded by a yeast discovered by Beyerinck (XXI.), and named by him *Saccharomyces fragrans* on account of the fragrant ester it produces. *Sacch. kefir* and Beyerinck's *Sacch. acetethylicus* assimilate glucose, fructose, maltose and saccharose, the last-named one utilising lactose as well. With regard to the suitability of this last disaccharide as a source of carbon, P. Mazé (I.) experimented with eleven stocks of yeast from soft cheese. These few examples will show that, also in respect of assimilation, the only way to obtain really applicable results is by working with pure cultures; and it is owing to the omission of this essential condition that both the experiments of C. von Naegeli (IV.) in the "seventies," and the publications of T. Bokorny and other workers must be left out of consideration here. Dextrin seems to form a good source of carbon for most yeasts; in Beyerinck's (XXI.) experiments it was only refused by a single species.

The suitability of the pentoses ($C_5H_{10}O_5$) as sources of carbon for yeast has not yet been examined by fermentation physiologists as thoroughly as might be desired in the interest of the fermentation industries. The parent substances of these sugars, namely,

the pentosans ($C_5H_8O_4$) occur in abundance as an important constituent of the vegetable cell-wall in cereals as well, B. Tollens and H. Glaubitz (I.) having found up to 8.9 per cent. (based on dry matter) in barley, 11.2 per cent. in malt, 8.7 per cent. in wheat, 11.1 per cent. in rye, and 5.8 per cent. in maize. The question whether and in what proportions the pentosans of barley undergo hydrolysis during germination has not been exactly determined, even in the exhaustive researches of Cross, Bevan, and Cl. Smith (I.), though it is certain that this occurs during the kilning of malt, varying amounts of furfural being produced, according to the working conditions. Tollens and Glaubitz state that about three-fourths of the pentosans of the malt are left in the grains by the mashing process in the brewery, partly, however, no longer in the form of pentosans, but as the resulting pentoses. These, though unfermentable (*see* chapter lxix.), may serve as sources of carbon for the yeast, provided the external conditions be favourable, as in the case of certain experiments conducted by H. van Laer (I.) and by Cross and Bevan (I). In other cases, however, as shown by Beyerinck (XXI.) with regard to arabinose in the case of *Schizosacch. octosporus*, they are utilised to only a small extent or not at all. Pentoses are formed, in still larger quantity than in brewing, in raw-grain distillery mashes, where the raw grain is dissociated by steaming for several hours under a pressure of 3–4 atmospheres.

Beyerinck's (XVIII.) proposal to divide the genus *Saccharomyces* into six sub-genera: *Glucomyces*, *Maltomyces*, *Lactomyces*, *Raffinomyces*, *Polysaccharomyces* and *Dextrinomyces*, in accordance with their characteristic behaviour toward the various carbohydrates, is scarcely feasible (*see* chapter ix.).

When more than one assimilable source of carbon is present in the nutrient medium, selective power (*see* p. 45, vol. i.) is exercised. In yeasts the study of this property—so far as sugars are concerned—is very difficult, and little progress has been made, because of the intervention of fermentative action in most cases, so that the separate determination of the amount of sugar consumed for the structural purposes of the cell cannot be performed with sufficient accuracy, if at all. A great influence on the ratio of the quantities of two or more nutrient substances in unit time is exercised by their relative diffusibility, this being also determinative when two or more fermentable sugars are at the disposal of, and being fermented by, the yeast. The selective power of yeast in fermentation has already been accurately tested, but the results must be postponed to chapter lxix., where also the extensive literature on selective fermentation will be cited. At present we can only take into consideration the discovery that, when two or more diffusible carbohydrates are present, the one exercising the greater osmotic pressure will diffuse more abundantly in the cell per unit of time, glucose, for

instance, more than fructose. According to E. Prior and H. Schulze (I.), the permeability of the cell membrane varies in the several species of yeast.

The amount of carbohydrates consumed in the formation of cells depends on the rate of reproduction, and therefore on the nature and extent of the influences controlling same. Pasteur (VII.), for a series of experiments, calculated the consumption for this purpose to be about 1 per cent. of the total saccharose consumed; and Balling (I.) stated that 5.323 parts of dry matter of yeast are formed for every 100 parts of wort extract (not only carbohydrates) disappearing in primary fermentation during the reproduction of bottom-fermentation yeast. In an experiment by Giltay and Aberson (II.), one part of yeast was obtained for every 3.8 parts of the total sugar consumed.

§ 262.—Inorganic Sources of Nitrogen.

When, in the course of his controversy with Liebig on the character of alcoholic fermentation, and on the nature of yeast as a living organism (*see* p. 121, vol. i.), Pasteur (XXIII.), in 1858, made his victorious discovery that this ferment is also active in a solution containing nitrogen solely in the form of ammonium tartrate, the term "yeast" was still very vague, the question whether wine yeast or beer yeast consisted of several species of organisms probably differing considerably in their foodstuff requirements had not come up for discussion, and there was no reliable means available for separating such a mixture of species into its components, and then examining the latter separately. Hence no clear light could be thrown on the matter by argument on the point of these observations. Liebig's (II.) statement in 1869 that he failed to obtain either fermentation or reproduction of the sowing, in an accurate repetition of Pasteur's experiment, was, in the opinion of the latter (XXIV.), sufficiently disposed of by the offer to perform the experiment again in the presence of any trustworthy person appointed by his opponent, and produce as much yeast as the latter "could reasonably desire." An objection urged by Millon (II.) was controverted in 1864 by Duclaux (XVI.), and by degrees Pasteur's assumption that yeast is able to satisfy its nitrogen requirements from inorganic sources exclusively, assumed the position of an unassailable law, observations to the contrary being reported with diffidence. A. Mayer (I.), whose researches on the nitrogen requirement of yeast in 1869 led him to adopt substantially the same opinion as Pasteur, observed—as did also the latter, and subsequently Naegeli (IV.) as well—that "the nutrition of yeast at the expense of ammonium salts always proceeds with somewhat greater difficulty than with nitrogenous yeast extract," and added, "in the former case *a larger number of well-organised*

yeast elements are required to induce fermentation." The limitation implied by the words italicised was not determined until twenty-two years later.

Wildiers (I.), in 1901, was the first to show, by the use of pure cultures of top-fermentation beer yeast of the *Sacch. cerevisiæ I.*, Hansen type, that neither fermentation nor yeast reproduction took place in 125 c.c. of a saccharified nutrient solution of mineral salts, when only a very small number of yeast cells were used for inoculation, *e.g.*, about as many as are contained in two drops of a culture grown in beer wort, or in 0.25–1.0 c.c. of a mixture of pressed yeast with ten parts of water. On the other hand, both fermentation and reproduction took place when the inoculation was accompanied by the addition of a few c.c. of a decoction of yeast, or of Liebig's meat extract, peptone or wort. From these observations Wildiers concluded that nitrogen in inorganic combination is insufficient for the needs of the yeast cell, the growth and fermentation also requiring a certain quantity of a special unknown substance, absent from inorganic foodstuffs, and which he proposed to term "Bios" (Gr. = Life). This substance is not an ash constituent; it is destroyed (rendered inactive) by boiling in 20 per cent. sulphuric acid, can be dialysed, is soluble in water, and can be extracted with this solvent from yeast (especially on boiling). Yeast, though containing bios, is incapable of elaborating it; so that when a small amount of yeast is taken for inoculation, the quantity of bios introduced into the mineral nutrient solution is insufficient for reproduction, whereas with a more plentiful inoculation enough is introduced to allow new cells to be formed at the expense of such as are moribund.

Owing to their highly important bearing on the study of the nutrition of yeast, these observations deserve a thorough experimental investigation; but at the outset they were yearly subjected to deprecatory criticism, as being opposed to the ruling dogma. Some asserted that the dependence of the result of the experiments on the amount of the inoculation was due to the presence, in Wildiers' nutrient solutions, of poisons, such as copper, derived (in traces) from the distilled water or present in the air of the laboratory, or ultramarine contained in the commercial saccharose used in the experiments, although Wildiers expressly stated that no difference in the results was obtained by working with invert sugar. To bios was ascribed the task of rendering these poisons innocuous, becoming thereby itself inactive and unsuitable as a yeast food, the further quantities, present only in larger sowings, being required for the needs of the cells. This opinion was tested, in a series of exhaustive experiments, by A. Amand (I.), who showed that bios does not play the part of an antidote.

One of the next measures was to obtain quantitative data on the new problem. Wildiers mainly judged the results of his

experiments by the quantity of carbon dioxide liberated from the cultures, and did not make any exact determinations on the number of cells sown and gathered. This, it should be distinctly observed, is a factor that does not affect the principle of the question; but in later researches it was impossible to forget, in the investigation of the conditions of cell reproduction, the quantity of the matter influenced, as well as the dimensions of the influence. This requirement was first satisfied by Al. Kossowicz (I.) in 1903. This worker, operating with pure cultures of *Sacch. ellipsoideus I.* (Hansen) and the distillery yeast, Race II. of the Berlin Experimental Station, found, for instance, that 200 cells of the former yeast sown in 100 c.c. of saccharified mineral nutrient solution increased to 140 million cells in fifty days. Subsequently (II.) he extended the work by sowing single cells; but in twenty-one out of twenty-two tests no development at all could be detected under the microscope, and the only positive result (which was very scanty) was probably due to the accidental introduction of a larger quantity of wort-gelatin along with the cell used for inoculation.

According to the observations of A. Amand (II.), the amount of bios in the nutrient solutions sown with yeast decreases very rapidly, and can then no longer be demonstrated in the cells, at least by the lixiviation method. J. Henry (I.), on the contrary, believed he had found yeast capable of forming new quantities of bios, a capacity certainly possessed by other fungi (*Penicillium glaucum* and a *Mycoderma*), as was first demonstrated by Kossowicz (I.). Thus, saccharified mineral nutrient solutions in which no development took place, owing to insufficient sowings (in parallel tests), gave both reproduction and fermentation on the *Eumycetes* in question being sown along with the yeast or had been previously grown in the solution and then killed off by heat before the introduction of the yeast. This observation, subsequently confirmed by A. Amand (II.), is valuable in connection with the interpretation of the results of previous workers. Commercial pressed yeast and wine yeast are almost always contaminated with *Mycoderma*, and the same is often the case with brewers' pitching yeast. These *Mycoderma*, however, as shown by Winogradsky (XI.) and Kossowicz (with different species), even in the case of rapid reproduction from a small sowing, are able to satisfy their nitrogen requirements from ammonium salts exclusively. Hence, when introduced with a sowing of yeast into mineral nutrient solutions, they develop first, in spite of their originally minute number, and then prepare the nutrient medium in the above sense for the purposes of the hitherto quiescent yeast. Ad. Mayer (I.) also reports that in his experiments (already mentioned on p. 542), "*Mycoderma vini*" almost invariably appeared. Hence, as is now admitted, his results do not apply solely to yeast, and show that the only way

to obtain reliable data regarding the nitrogenous nutriment of yeast is by the aid of pure cultures, no others being worth the trouble of undertaking.

The problem set out above is still too fresh for a final solution to have been found; and each day may reveal some new observation opening up a quite unexpected perspective; for which reason the matter has been very briefly treated here. However, one point is now well established, namely, that, in a saccharified nutrient mineral solution containing ammonia as the sole form of nitrogen, cell reproduction and fermentation can only commence when the number of cells introduced does not fall below a certain minimum, the absolute dimensions of which have not yet been definitely ascertained, and which is probably dependent on the other conditions of the experiment.

This, however, does not imply that yeasts unconditionally reject ammonium salts when the other conditions essential to development are present. On the contrary, they then exhibit a certain preference for ammoniacal nitrogen. This was already observed by Duclaux (XVII.) during his experiments on the fermentation of wine must in 1866, the nitrogen content falling from 120 mgrms. to a very small proportion per litre in consequence of its consumption by the yeast present. The question has also been studied by Müntz and Rousseaux, then by Roos and Chabert, and finally by J. Laborde, the last-named stating that, at 28° C., ammoniacal nitrogen is taken up more extensively than organic nitrogen, though the reverse was found to occur at 36° C. According to Artari (I.), *Sacch. Zopfii* is even satisfied with ammonium sulphate as the sole source of nitrogen. Further particulars respecting the behaviour of *Mycoderma* species toward ammonia salts will be given in chapter lx.

The nitrates, which are the best, and in some cases the only, sources of nitrogen for higher plants, are of no value for yeasts, except in a few cases, typified by Beyerinck's (XXI.) *Sacch. acetethylicus*. This was first proved by Ad. Mayer (I.); and the converse opinion expressed by Dubrunfant (III.), was disproved by E. Laurent (VI.). The injurious effect produced on yeast by the presence of such salts in otherwise favourable nutrient solutions seems due to the reducing action of the cells causing the formation of highly poisonous nitrites. One of the reasons of fermentation disturbances in molasses distilleries is certainly to be found in the presence of nitrates, which sometimes occur in large quantities in molasses. Similar results have also been found by L. Briant (II.) in breweries employing water rich in nitrates; and this worker mentions about 75 grains per gallon as the highest permissible limit of these salts. The influence of nitrates on attenuation has also formed the subject of experiments by Evans (I.). In a patented process for cultivating races of yeast capable of thoroughly fermenting dextrins (*see* chapter lxv.),

J. Effront (VIII.) is said to employ a nutrient medium containing nitrates as the exclusive source of nitrogen.

§ 263.—Organic Sources of Nitrogen.

In the worts, mashes, and musts used in practical fermentation, the yeast has not to depend on inorganic sources of nitrogen, but generally has at its disposal an abundance of readily assimilable organic nitrogen compounds. The suitability of a few representatives of this large class will be more closely considered in the following paragraphs.

Among the amides suitable as nitrogenous foodstuffs, special attention is merited by the acid amide of aspartic acid

$$(COOH—CH_2—CH.NH_2—COOH)$$

namely, asparagin

$$(COOH—CH_2—CH.NH_2—CO.NH_2),$$

this substance playing an important part in the mashes used in practice. It is always formed during the germination of seeds, and is therefore present in malt, and still more abundantly in malt culms. Even potatoes contain appreciable quantities. Finally, in addition to other amides, asparagin is one of the chief forms in which nitrogen occurs in the molasses of beet sugar works; proteins, on the other hand, being almost entirely excluded or eliminated by the method employed for obtaining the juice from the beet in the diffuser and by the purification process in the saturator. M. Hayduck (IV.) recognised asparagin as an excellent source of nitrogen in the nutrition of yeast; and this was confirmed by E. Laurent (VI.), G. Heinzelmann (IV.), H. P. Wijsmann (II.), and others. Yeast is capable of transforming asparagin into proteins, a property unshared by the animal organism so far as is known at present. The nitrogen of asparagin (which is worthless as a food for animals) is present in the potatoes made into distillery mash, is recovered in the distillery waste in the form of protein, and imparts to this waste product the character of a concentrated fodder for stall-fed cattle. In an experiment carried out by P. Petit (II.) with a nutrient medium containing asparagin and ammonium phosphate, it was found that top-fermentation yeast consumed twice as much of the former as was utilised by bottom yeast—a difference considered by the author to afford a means of differentiating these two yeasts. According to a comparison instituted by R. Kusserow (I.), on the relative influence of asparagin and peptone as the source of nitrogen in saccharified mineral nutrient solutions, the former substance accelerates fermentation and increases the yeast crop. The observation that when grown with the aid of asparagin, the cells of the sedimental yeast are not cohesive,

whereas in the case of peptone they unite into flocculent masses, which settle down less firmly than the others, was also confirmed by H. Lange (I.) with beer wort, his explanation being that the cohesion is due to precipitated peptone. A high selective power is exerted by *Schizosacch. octosporus*, which, according to Beyerinck (XVIII.) is suited only by the nitrogen compounds naturally present in raisins or malt, and not by ammonium salts, asparagin or peptone.

Among cereal grains, rye is characterised by a high percentage of proteins specially suitable for the formation of yeast protoplasm. This is one of the reasons why yeast manufacturers, particularly those working with the old (Vienna) method, prepare their mashes with an addition (usually one-third) of this grain. Its percentage content of nitrogenous substances fluctuates, however, betwen wide limits, 7.5 per cent. on the one hand and 15.3 per cent. on the other, being by no means exceptional figures—as was shown by Delbrück (IV.) in a highly interesting experiment. Manufacturers endeavour to counteract the resulting great variation in the amount of the yeast crop by employing mixtures of rye of different origin; but they would, no doubt, prefer to be able to determine the amount of these proteins in buying the raw material, and to value the latter accordingly. On this point, however, there are no data at present available. Among the proteins and allied substances mention should also be made of diastase, which is not merely capable of serving as a source of nitrogen when present as the sole nitrogenous constituent of the nutrient medium, but is also taken up and utilised by the yeast, and thereby caused to disappear, even when peptone and asparagin are also present in sufficient quantity. This observation, recorded by Heinzelmann (IV.), is of interest in connection with the fermentation of raw grain (maize, &c.) mashes, in which the unsaccharified dextrin is intended to be hydrolysed by diastase during the prolonged primary fermentation. In one experiment performed by Heinzelmann (V.), out of the 37 parts of diastase left from each 100 originally present at the end of the mashing process, 33.4 disappeared during fermentation, so that only 3.6 remained in the fermented mash.

When amides are present in nutrient media together with proteins and their nearest degradation products, the selective power of the cells (*see* p. 46, vol. i.) becomes manifest. In this connection a number of very instructive data are already available. The degradation products of protein are always present in brewery and distillery mashes, owing to the activity of the proteolytic enzymes in the malt used. In an experiment performed by C. J. Lintner (V.), out of the total nitrogen (0.092 per cent.) in the saccharified malt extract used as the nutrient medium, and composed of 0·062 per cent. of amides and 0.030 per cent. of proteins, &c., 0.036 per cent. (viz., 0.030 per cent. of amide nitrogen and only

0.008 per cent. of proteid nitrogen) was absorbed by the yeast. WAHL and HANTKE (I.) found the relative proportions of nitrogen absorbed (from wort) by the yeast, in the form of proteins, peptone and amides, were: 0.4 : 1.7 : 19.9 mgrms. respectively per 1000 c.c., the initial content in the wort being 9.0 : 27.4 : 52.0. In this case also amides were preferentially absorbed. Though P. PETIT and G. LABOURASSE (I.) consider that their observations justify an opposite conclusion, it follows from R. KUSSEROW's (IV.) experiments that the protein degradation products are better foodstuffs than the unaltered proteins. This partly explains the favourable influence of an addition of malt culms to the mash, especially in cases of sluggish fermentation of rich mashes. This throws new light on the influence of the kind of mashing process on the protein content of beer wort, and thereby on the progress of the development of yeast, as also the course of fermentation and the character of the resulting beer, especially in contrasting the two opposite processes of the infusion method on the one hand and the Bavarian thick-mash method on the other. ADALBERT FLÜHLER (I.) was the first to draw attention to this, and his work was continued by V. GRIESSMAYER (II.). C. J. LINTNER (V.) reported that these discoveries were confirmed by Ad. Ott.

HAYDUCK (IV.) carried out the first experiments worthy of mention on the ratio between the size of the yeast crop and the amount of the nitrogen content in the nutrient medium; and this worker found that asparagin, when present as the sole source of nitrogen, and to an extent not exceeding 0.25 per cent., in a saccharified solution of mineral salts, was completely absorbed by the added pressed yeast. With a larger quantity of asparagin present, more was consumed, but in all cases a portion remained undecomposed. On the other hand, A. L. STERN (I. and III.) found 0.025 per cent. of asparagin to be the maximum, no appreciable increase in the reproduction of the cells occurring with any larger quantity. A similar observation was made by P. THOMAS (I.) with regard to urea as a source of nitrogen. IWANOWSKI (II.) states that the degree of alcoholic fermentation in a saccharified mineral-salt solution varies inversely with the amount of peptone added as the source of nitrogen. On the other hand, in experiments with wine must containing 1 per cent. of added peptone, J. BEHRENS (XIII.) found that this addition assisted fermentation (by pure yeast).

The observations of D. DELBRÜCK (IV.), CES. FORTI (I.) and E. BOULLANGER (I.) show that the quantity of nitrogen absorbed from the nutrient medium is primarily dependent on the species of yeast employed. It also varies, however, with one and the same yeast, according to the other conditions of environment, being greater, for instance, in aerated and strongly agitated cultures. This was noted both by the last-named worker and also by C. F. HYDE (I.) who found that 23.8 per cent. of the nitrogen

originally present in a given wort was eliminated by means of a large sowing of yeast, coupled with rousing and agitation, whereas with a medium sowing without aeration only 17.2 per cent. was removed, and merely 15.8 per cent. with a small sowing and slight aeration.

The influence of the nature of the source of nitrogen is revealed by the observation made by A. L. Stern (V.) that in parallel cultures with equal initial nitrogen content, either in the form of asparagin, peptone or yeast extract, the relative quantities of this element eliminated by the yeast were 1 : 1.8 : 2.2. That the amount of sugar present in the nutrient medium also influences the absorption of nitrogen has been shown by P. Thomas (I.) and also by Stern (III.) The former observer found that a larger quantity of the urea offered as the source of nitrogen was assimilated in presence of 20 per cent. of dextrose than when only 10 per cent. of that sugar was available. In Stern's experiments the largest quantity of asparagin was assimilated from a mineral-salt solution containing 0.3 per cent. of that substance when the added dextrose amounted to 15 per cent. (the limits ranging from 0 to 30 per cent.); but when only 0.15 per cent. of asparagin was present the optimum quantity of sugar fell to 12.5 per cent.

Apart from the exceptions to be mentioned hereafter, all the worts, musts, and mashes fermented on a practical scale contain a surplus of nitrogenous nutriment; so that this is still far from being exhausted by the time cell-reproduction has come to a standstill in consequence of the gradual change for the worse in the other conditions of nutrition, especially by the increased alcohol content (*see* chapter lii.). Hence a larger or smaller quantity of useful nitrogenous nutriment remains in the fermented product. As we have been informed by J. von Liebig (II.), Graham, A. W. Hoffmann, and Redwood in 1853 found that pale English worts containing 0.217 per cent. of nitrogen furnished beers containing 0.134 per cent. Similar investigations—also with infusion worts in an English brewery—were made by H. Grimmer (I.), with the result that, of the nitrogen (0.132–0.138 per cent.) in the original wort, about one-fourth (24–26 per cent.) was found to have been taken up by the yeast, the greater portion (one-half to two-thirds) being absorbed during the first twenty to twenty-four hours after pitching. This was confirmed by C. F. Hyde (I.). Substantially the same observation was made by Delbrück (I.) in the case of pressed yeast in 1879, so that the nitrogen consumption curve rises very sharply. One pressed yeast and three different samples of low-fermentation beer yeast (containing 8.24 and 8.94 to 9.54 per cent. of nitrogen in the dry residue), which were allowed by Hayduck (V., VI.) to develop under identical conditions in malt-extract solution containing initially 0.0876 per cent. of nitrogen, absorbed 43 and 30–39 per cent. of this foodstuff for the structural purposes of the cell. That the residual nitrogenous substances

in young beer are capable of affording nutrition for the further development of yeast has been repeatedly shown, *inter alia*, by F. Hyde (I.) and Hayduck (V., VI.), though it would be erroneous to assume that the whole of these nitrogenous substances are suitable for this purpose, or that any one of them is accessible to any race of yeast. Unfortunately, the observations available on this important question are but few, *e.g.* those communicated by Delbrück (V.). The appearance of yeasty haze in lager beer (*see* p. 186, vol. ii.) and in stored wine may probably be traced by further investigation to wort or must proteids, which the yeast concerned in primary fermentation has been unable to consume, but which during storage has furnished structural material for the development of some other race of yeast that has crept in in the meantime. Wine must also contains an excess of nitrogenous nutriment for the yeast, and left unconsumed by the latter. H. Müller-Thurgau (II.) took a Geisenheimer Riesling must of 1888 vintage, which he subjected to six fermentations in succession, removing the resulting yeast and alcohol and adding fresh sugar each time, the final result being a wine containing 0.051 grm. of nitrogen per 100 c.c., as against 0.100 grm. in the must, whilst the removed yeast contained 0.049 grm. In accordance with the gradual diminution of nitrogen in the maturing wine, the successive deposits of yeast separated from the supernatant wine also exhibit a diminished nitrogen content. Thus in a case examined by A. Czéh and H. Müller-Thurgau (I.), the first deposit of yeast furnished 6.19 per cent., and the fourth only 4.3 per cent. The course of fermentation in the must is also influenced by the method of treating the vines. Müller-Thurgau (II.) reported on a case of stormy fermentation in must which, in consequence of the heavy manuring of the vineyard, contained not less than 0.12 per cent. of nitrogen. The residue of nitrogenous constituents in the new wine, after the removal of the primary yeast, enables the secondary fermentation to be carried through.

Apple and pear musts are frequently poor in nitrogenous yeast food, and this is generally the case with berry musts, especially bilberry must; and for this reason they ferment very sluggishly and incompletely. These defects may be remedied—as was first advocated by Nessler and tried by H. Müller-Thurgau—by an addition of 20 grms. of sal ammoniac per hectolitre (about 3 oz. per 100 gallons). Similar results, but at higher cost, can be obtained—as was done by R. Otto (I.)—by the use of ammonium tartrate or even asparagin.

It would be incorrect to suppose that the total quantity of nitrogenous matters present in wines or beers already existed as such in the must, wort, or mash. On the contrary, a portion, varying considerably according to the conditions of fermentation, originates in the metabolism of the yeast employed, and is excreted

by the latter; if soluble, it remains in the liquid, but if transformed into the insoluble condition it will be found in the sedimental yeast. The quantity of the soluble matters excreted from the cells increases with the temperature, other conditions being equal. The experiments of E. Hantke (I.) on this point showed, for example, that beer produced from a wort containing 5.59 per cent. of nitrogenous substances, by means of one and the same type of yeast, contained 4.42 per cent. of these substances when fermented in the cool cellar of the brewery, and 5.10 per cent. when the fermentation was completed in the laboratory (at 66° F.). The characteristic disagreeable flavour produced in beer by fermentation at an unduly high temperature is due to this increased formation of metabolic flavouring products, which will be the more noticeable in proportion as the other (flavouring) extract constituents is smaller. For this reason beers of this class (especially Pilsen) must be fermented at a lower temperature than those of the Bavarian type.

Finally, it should be mentioned that the fact of the migration of nitrogenous substances from the yeast to the nutrient medium renders illusory the results of all investigations in which it is sought to ascertain the quantity of nitrogenous matter assimilated by yeast from the difference in the nitrogen content of the medium at the beginning and end of the experiment. Consequently, this question also needs reinvestigation by the application of more delicate methods of experiment.

CHAPTER LI.

CULTIVATION AND REPRODUCTION OF YEAST.

§ 264.—Hansen's Method of Single-Cell Culture.

In samples of yeast as they reach the laboratory from natural sources or from the fermentation industries, the cells are not infrequently in an enfeebled condition, and therefore need to be reinvigorated before they can be sowed in the nutrient gelatin employed for making pure cultures. Thus in the case of breweries, for instance, the usual practice adopted for sending yeast samples to a pure-culture laboratory through the post is to place a drop of the thick balm, about the size of a pea at the most, on a sterilised filter-paper, and thus free it from water to a sufficient extent to enable it to be sent, enclosed in several layers of the paper, in an envelope. On reaching the laboratory this desiccated drop is placed in wort, where the cells are revived and regain their full power. The same thing is done in the case of wine lees. The reinvigorated sample is then subdivided, in the manner described below, in order to obtain cultures that are indubitably grown from a single cell, and are therefore termed "single-cell cultures."

The insecurity of the dilution method has already been pointed out in vol i. (p. 125); and for the purpose of obtaining pure yeast cultures, this method was improved upon by E. Chr. Hansen (II.) in 1879. Hansen had observed that when several cells were present in a culture vessel they settled down separately, when properly stirred, and being devoid of locomotive power, each developed into a colony by itself. In such cases those in which only a single colony developed were alone suitable for the purposes of pure culture. The first six species of Saccharomycetes introduced into the literature by Hansen, namely *S. cerevisiæ I.*, *S. Pastorianus I–III.*, *S. ellipsoideus I.* and *II.*, were obtained in this manner. At a later date (1883) Hansen (XII.) made use of the liquefiable solid medium, 10 per cent. wort-gelatin. J. Chr. Holm (IV.), in a critical investigation of the resulting priority controversy, established the fact that this was done by Hansen independently, and especially so with respect to the method of R. Koch.

As already stated in vol. i. (p. 132), it is only capable of fur-

nishing true pure cultures when the experimenter has succeeded, by shaking, in distributing the sample sown in the nutrient gelatin so uniformly that the cells are all embedded separately in the solidified gelatin stratum. Despite the opinion of G. Topf (I.) to the contrary, this condition is not always attainable. By means of an artificial mixture of beer yeast with *Sacch. apiculatus* —which is recognisable from the peculiar shape of its cells— Hansen (XII.) showed that about 2 per cent. of the colonies on the resultant plate cultures were impure, *i.e.*, contained cells of both species. In a special experiment with a series of pure yeast, partly alone and partly in artificially prepared mixtures, J. C. Holm (IV.) showed that from 108 to 135 cells formed the basis of 100 colonies obtained on nutrient gelatin plates by the Koch method. Still more unfavourable are the conditions in cases occurring in laboratory practice, where natural mixtures have to be separated in which the mutual connection of different species is, for various reasons, of more frequent occurrence and more intimate. Thus P. Miquel (IX.) found, in a case of air analysis, that out of 442 colonies—of which 385 consisted of bacteria— only 136 contained a single species, whilst 87 contained two, 75 three, and 87 four or more species, and Lafar (I.) has shown that mixed colonies, formed of yeast and bacteria together, also occur. Hence, in view of the small dimensions of the cells, the only way to remedy the unreliability of the plate culture method in the case of bacteria is by making repeated cultures, as recommended in vol. i., chapter xi. With yeast, on the other hand, it is much easier to overcome the defects of the method and obtain cultures undoubtedly arising from a single cell, the cells being large enough (usually 5–10 μ) to enable a low-power objective to be used, the longer focus obviating the risk of contact with the gelatin layer under examination. Consequently the freshly moulded plate, inoculated with the sample to be subdivided, can be examined with a low power (40–60) for the purpose of discovering cells that, in addition to being alone, are far enough from their neighbours to ensure the isolation of the resulting colonies and enable re-inoculations to be made from these without incurring the risk of including members from other colonies in the transfer. The position of these suitable cells in the gelatin stratum is noted down at once, so that they may be readily identified later and used for cultures that shall be indisputably descended from a single cell and therefore pure cultures in the strictest sense of the term.

At present we can only deal briefly with the technique of the preparation of single-cell cultures, the reader being referred for more complete details and modifications of the method to the special works on the subject, notably the instructions given by E. C. Hansen (XV. and XXXV.) himself, and A. Klöcker's book (VII.) detailing the experience gained in this branch at the

Carlsburg laboratory. A small portion of the sample (previously reinvigorated, if necessary) is placed in sterilised water—or preferably in a 0.5 per cent. solution of common salt—and shaken up so as to separate the agglomerations of yeast and distribute the cells as uniformly as possible. The number of cells in a single drop of the diluted mass having been examined under the microscope, sufficient is transferred to a flask, already charged with 20–100 c.c. of liquefied wort-gelatin (or must-gelatin) to ensure that a tiny drop of this latter contains only a very few cells. To prepare a miniature plate culture, a large cover-glass is sterilised, by passing it through a spirit flame, and one side of the same is then coated with a layer (about 0.2 mm. thick) of the inoculated gelatin, by means of a small platinum loop, in such a manner as to leave an outer ring of clear glass several millimetres wide. The cover-glass is then laid on the vaseline-coated ring (*c*) of a sterilised Böttcher cell (Fig. 159), the bottom of which has already been coated with a minute quantity of sterilised water (*d*). If this moist cell be laid horizontally on a suitable foundation and exposed to a medium room temperature (15°C.), the gelatin film (*b*) on the inside will quickly set evenly. A hollow-ground microscope slide may also be used in place of the Böttcher cell. It is advisable to make several of these plate cultures and not merely a single one. As soon as the gelatin film has set, the cell is examined under the microscope, preferably one fitted with a nose-piece to enable the two requisite objectives of different power (*e.g.*, Zeiss Nos. A and D) to be rapidly exchanged. The low power (about 40–60) is used for systematically examining the gelatin film all over, to find suitable cells that are isolated and far enough away from all others. The higher power (about 250–400) is then used to make sure, and the exact position of the selected cells is noted down. The various methods and appliances for fixing the position of the cells in single-cell culture have been critically reviewed by H. Will (XXIV.) on the basis of his wide experience. The author finds the simplest and most convenient method—and much less troublesome to the beginner than the object-marker—is that of marking the position of the cell direct on the glass (the low power being used so that the objective is about 1 cm. away from the slide) by means of two fine dots of ink or preferably black varnish, applied to the top surface of the cover-glass by means of a fine drawing-pen or a pointed inoculating hook. About half a dozen or more cells are marked off in this way, a necessary precaution, because it often happens that one or

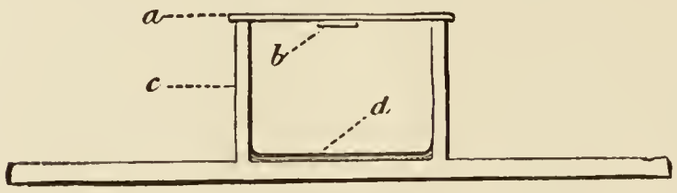


Fig. 159.

Böttcher Cell in vertical section. Slightly reduced. (*After Hansen.*)

more of those selected remains barren, either because it was already dead at the commencement of the test or else was too feeble to develop in the strong (10 per cent.) gelatin medium. The task of seeking out the cells is troublesome, even for the experienced worker. It may be greatly lightened by using cover-glasses cross-etched in squares of about 2 mm. side, as recommended by Will, and preferably marked with etched figures as advised by Alfred Jörgensen. This is also desirable on account of the ease with which it enables the position of the cells to be entered in the note-book, and facilitates keeping a record of the observations. When the marking is finished, the Böttcher cells are placed in a

Fig. 160.
Chamberland Flask.
About one-fifth natural size.
(*After Hansen.*)

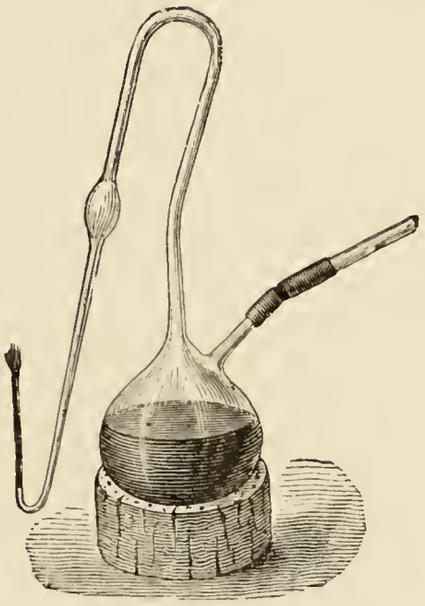


Fig. 161.—Pasteur Flask, with Hansen's improved modification by widening the swan-neck tube. About one-fifth natural size. (*After Hansen.*)

large double basin and left for about twenty hours in the thermostat at about 20° C., by the end of which time the cells that are capable of development will be actively forming colonies. After another twenty-four hours the colonies will be visible to the unaided eye, as small white dots. The selected colonies are again examined under the low power, for the presence of dangerous neighbours, and inoculations are made from each colony into flasks already charged with sterilised nutrient medium (wort, wine, must, &c.), another examination being afterwards made to make sure that the selected colonies alone have been drawn upon and that the adjacent ones are intact. When an inoculating hook is used, it will be found more convenient to transfer the cells to a Freudenreich or Chamberland flask (Fig. 160), and then, after further reproduction, inoculate from this with a loop into a Pasteur flask (Fig. 161), with a capacity of about 250 c.c. and charged with about 100 c.c. of wort. On the other hand, the inoculation can be conveniently effected into the

latter culture vessel direct when use is made of a platinum wire, about 1 cm. long and 0.5 mm. thick, held with a forceps and sterilised in the flame just before using.

Another result—either subsidiary or as the main object—obtainable by means of the above method of reinvigoration, is a purification of the sample. In this way Müller-Thurgau (XX.), for instance, in cultivating yeast destined for the fermentation of red wine or perry musts (rich in tannin) and therefore required to be able to stand the presence of large quantities of that substance, allowed the sediment forming the raw material to reproduce several times in red must or tart perry must. In this way he effected a selection affording greater prospects of ultimate success in the cultures afterwards prepared by the single-cell method.

P. Lindner (XXI. and XV.) applied the name "small drop" culture to a modification of Hansen's single-cell method. He diluted the dissected sample to such an extent with wort that a small drop of same contained only a single cell, and then, by means of a very fine sterilised drawing-pen, made a number of small drops or fine streaks on a sterilised cover-glass, which was next fixed over a Böttcher cell or hollowed slide, by means of vaseline, &c., and examined under the microscope, each small drop found to contain only one cell being marked. The resulting colonies are afterwards transferred to another medium (wort-gelatin, wort). The shrinkage which takes place in cells placed in the highly plasmolytic nutrient gelatin is here avoided, and consequently the proportion of cells that will not develop is reduced. Moreover the cells are more readily detected than is the case with solid media, owing to the greater difference between the refractive power of the cells on the one hand and the medium on the other. F. Schönfeld (II.) found it useful to combine the externals of this method with the essential features of the Hansen single-culture method, by treating a little of the sample (sludge or sedimental yeast) with wort-gelatin and transferring small drops and streaks of this mixture to a cover-glass which is afterwards fastened down on a hollowed slide.

Re-inoculation from a single-cell culture into a fresh nutrient medium gives an absolutely pure culture. The labour devoted to the latter has then a different purpose, more especially to determine whether it is adapted to the object in view, to select the most suitable from a series of pure cultures, and finally to reproduce a sufficient quantity for use in practice.

A few remarks may be made, for the information of beginners, on the best method of keeping pure cultures in the laboratory. The most convenient, when feasible, is to leave the yeast covered by the liquid in which it has been grown. This is only practicable, however, when the operator has sufficient leisure or inducement (in the way of orders for supplies of yeast) to re-transfer the

cultures to fresh medium at frequent intervals. If this be neglected, and the cells are left for a long time in the spent liquid, they will be injured by the products of metabolism and fermentation. The length of the intervals within which this re-inoculation must be performed in order to prevent the danger aforesaid, depends on the species of yeast as well as on the constitution of the nutrient medium. Thus, H. Müller-Thurgau (III.) states that he was able to leave certain wine yeasts in their respective wines for ten months without appreciable injury. On the other hand, the author found that a spirit yeast, Race II., suffered a transitory, but decided, weakening (especially in reproductive power) by remaining only three months in the fermented wort. The live herbarium in the laboratory of a fermentation physiologist, that is to say, the collection of cultures of micro-organisms, usually contains a very large number of yeasts. These include certain kinds—especially yeasts for the production of wines and berry wines, then the so-called wild yeast, and others—for whose re-inoculation there is little or no outside demand. Nevertheless the re-inoculations take a good deal of time and labour when—as is desirable *per se*—the collection is a large one numerically. In order to reduce the work to a minimum the yeast should be transferred to the medium in which it is known to keep longest without suffering a change of character. Beer wort is not suitable for the prolonged storage of brewery yeasts; but, on the other hand, the aqueous 10 per cent. solution of saccharose tried and recommended by E. C. Hansen (XXXV.) usually behaves well. It is generally kept in Freudenreich flasks or in the Hansen modification shown in Fig. 162, these being filled about half full. To minimise the inevitable evaporation, J. C. Holm (III.) employs a modified form of cap or hood. The amount of sediment of the culture to be preserved must not be more than imparts a slight turbidity to the liquid, the reproduction of the sowing and the chemical alteration of the liquid being then very slight. The object in view is not the reproduction, but the preservation of the cells, and it is therefore necessary to prevent as far as possible the distribution of the store of nitrogenous food introduced by the cells and consumed for their own maintenance during storage, or a weakened progeny will certainly result. Moreover, the re-inoculations should be kept in the dark and at a temperature not far exceeding 15° C. Under these conditions nearly all the species (about 50 in number) examined in this connection by Hansen

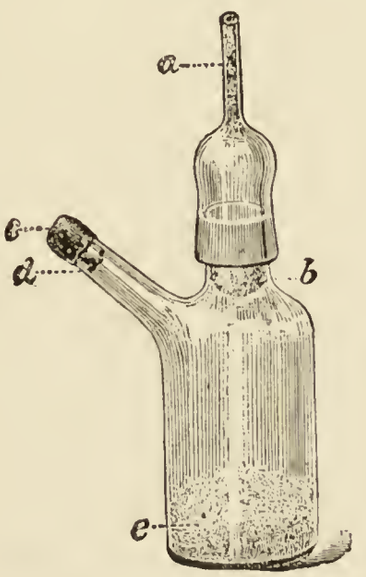


Fig. 162. Freudenreich-Hansen Flask. About half natural size. (*After Hansen.*)

(XXIV., V.) retained their vitality for many years (up to seventeen). *Sacch. Ludwigii* proved less hardy, some of the samples dying within one-two years, though others lasted six years; and Carlsburg bottom yeast No. 2 behaved in a similar manner. To these exceptions must be added the *Sacch. theobromæ* isolated by A. Preyer (I.) from putrefying cocoa, and also—according to J. C. Holm (II.)—a few species of *Schizosaccharomyces*, one of which perished within a year in saccharose solution, though found to be still living in wort after two and a half years. Beer wort, on the other hand, proved unreliable with all the others tested for this purpose. True, in not a few instances, living cells were found in it after ten or even eleven years, but just as frequently none could be detected at the end of a single year. This liquid is subject to considerable variations in constitution which cannot be influenced or ascertained by the experimenter. In countries where the summer is hot, provision must be made at that season for artificially cooling the cultures (in an ice-chest of special construction); otherwise, as was observed by H. Will (XIX.) in Munich, and by A. Kukla (I.) in Prague, degeneration and death will occur even with saccharose solution. In consequence of unfavourable experiences, A. Jörgensen (IX.) recommends that streak cultures on wort gelatin should not be employed for storage purposes. The tendency to and results of the formation of films on ageing cultures have been already referred to on p. 120, vol. ii.

The colour of beer wort is ameliorated by the fermenting yeast, not solely (as is known to every brewer) during primary fermentation, but also, and to a far greater extent, when the fermented beer is left for a long time in contact with the sedimental yeast, as is the case when yeast cultures are stored in wort. A gradual and extensive decoloration of the nutrient medium occurs (*see* p. 126, vol. ii.); so much so, in fact, that the colour may be changed from brown to pale straw-yellow. The experiments conducted on this point by H. Will (XXV. and XXII.) showed that the wild yeasts generally are more powerful decolorants than the culture yeasts.

When a pure culture has to be transferred from one laboratory to another, which possesses the necessary equipment for further reproduction, a small quantity of the culture—even a couple of vigorous cells will suffice—is placed on a little cotton-wool and inserted in a previously sterilised Freudenreich or Freudenreich-Hansen flask. The cotton should not have been freed from fat, and will then be hygroscopic enough to supply the yeast with the requisite minimum of moisture to prolong its vitality. The Hansen modification of the Freudenreich flask (Fig. 162) differs from the original type by the provision of a slanting tube at the side to facilitate connection with a Pasteur flask, both for introducing a drop of the transferred culture on to the cotton (*e*) and also for inoculating it into a fresh nutrient solution. A coating

of sealing-wax (*c*) makes a tight joint over the stopper of rolled asbestos-paper (*d*). The plug of cotton (*b*) in the neck of the flask prevents over-rapid and excessive desiccation of the contained cells, and at the same time excludes any extraneous germs that may have slipped through the plug (*a*) in the cap tube. This method of storage on cotton is of special value, and in fact indispensable, when perfectly pure cultures are to be sent to tropical countries and will be exposed to high air temperatures for some time *en route*. Streak cultures on gelatin or agar-agar, which are occasionally used for transport over short distances in the temperate zone, are out of the question in such cases.

§ 265.—Conditions of Cell Reproduction.

Of the two possible methods of cell reproduction in yeast, namely, budding (the vegetable form) and ascosporulation, the second may be disregarded so far as the practice of yeast culture is concerned, on account of the greatly restricted increase it furnishes. The method of budding, with which alone we shall now have to deal, has already been treated of in § 245, though only from the standpoint of the morphologist, the chief point being the variability in the form and aggregation of the cells resulting from vegetative reproduction. In the present paragraph this information will be supplemented by a review of the external conditions of cell reproduction, the requisite constitution of the nutrient medium having been already dealt with in the two previous chapters. Before, however, entering upon a discussion of this question, it will be necessary to treat of the limitation of three definitions which here come into application.

The term "reproductive capacity" (occasionally also known as "coefficient of reproduction") relates to the total number of cells in the yeast crop obtainable under any given conditions (*i.e.*, independently of time) per unit of the original sowing. This unit may be taken on the basis of weight, in which event a reproductive capacity of 20, for instance, implies that 20 grms. of yeast crop have been obtained for every 1 grm. of yeast sown. The crop is usually obtained in the form of a sediment (*see* vol. ii. p. 114), and is weighed either in the pressed condition (with a variable content of water according to the degree of pressure) or else after drying. The latter method is the most reliable, especially in experiments on the consumption of nutrient substances, provided—as we know (vol. ii. p. 179) is not usually the case—the deposit consists of yeast cells exclusively. For this reason statements, based on unit weight, relating to the ratio of increase are of merely low and conditional value. Far greater comparative reliability attaches to determinations made with the cell-counter (vol. i. p. 124), and referred to the unit of cells in the sowing.

"Reproductive power" (also spoken of as "the velocity of

reproduction" or "reproductive energy") indicates the number of cells produced from the unit of sowing in unit time. With regard to the determination of this value, the remarks just made also apply.

By the "period of generation" is generally understood the interval of time necessary for the production of a fully developed daughter cell from the parent cell by budding. If F. Baseneau's formula (I.) (vol. i. p. 59) is used for this determination, instead of the method of direct observation of the individual cell, it must be remembered that this formula does not strictly apply, except under the condition that the duration of generation has been the same for all cells in the culture throughout the whole experiment, and that each newly formed daughter cell has immediately begun to act as a parent cell. Since this latter condition in particular is not usually fulfilled in its entirety, the value furnished by the calculation is higher than the truth.

The influence of temperature on cell reproduction by budding deserves very close consideration, because the use in practice of nutrient media which are mostly of almost constant and unchangeable constitution, renders it possible to control the progress of reproduction by graduating the influence in question. The limits of temperature within which cells will bud in beer wort have been determined by E. C. Hansen (XXXII.) for eleven different *Saccharomycetes*. The upper limits were: for *Sacch. Past. I.*, 34° C.; for *Sacch. membranæfaciens*, *S. Ludwigii*, and Wortmann's wine yeast, Johannisberg II., 37° C.; for *S. cerevisiæ I.*, *S. Past. II.* and *S. ellips. II.*, 40° C.; for *S. ellips. I.*, 40–41° C.; and for *S. Marxianus*, 46–47° C.; whilst the lower limits were as follow: *S. cerevisiæ I.* and *S. membranæfaciens*, 3° to 1° C., *S. anomalus*, 1° C. to 0.5° C., and for the other eight species 0.5° C. A comparison of these figures with those in the table opposite p. 136, vol. ii., will confirm the remark on pp. 129, 130, that the limits of temperature are somewhat wider in the case of budding than for sporulation, provided the former occurs in wort. If, on the other hand, the cells be allowed to bud in water, the higher limit of temperature is slightly reduced, whilst the minimum is raised. *Saccharomycopsis guttulatus* will only bud at 37° C.

The influence of temperature on the period of generation was first closely investigated by Rasmus Pedersen (II.), after a few observations on the subject had been communicated by Pasteur and others. Working with a low-fermentation beer yeast in unhopped wort (16.2° Balling), Pedersen found that the duration of generation in the first 24 hours of development was 20 hours at 4° C., 10½ hours at 13.5° C., 6½ hours at 23° C., 5.8 hours at 28° C., and 9 hours at 34° C., no budding occurring at 38° C. Consequently, the optimum temperature giving the shortest period of generation in these experiments was between 28° and 34° C. At a later date a more comprehensive test was performed by

D. P. Hoyer (I.) by fixing a small number of cells, in a state of watery suspension, on a thin film of solidified wort-gelatin applied to the under side of the cover-glass of a Böttcher cell. The preparations, which could be examined and counted direct, were maintained at the desired temperature for a certain time, the cells produced in the interval being then counted and the period of generation calculated by means of the formula already mentioned. The following results were obtained at 13° C.: *Sacch. Pastorianus I.*, Hansen, 6 h. 6 min.; *S. Past. II.*, 8 h. 45 min.; *S. Past. III.*, 8 h. 39 min.; *S. ellipsoideus I.*, 9 h. 4 min.; *S. ellips. II.*, 8 h. 49 min.; *S. anomalus*, 5 h. 12 min.; *S. Ludwigii*, 8 h. 10 min.; *S. membranæfaciens*, 7 h. 1 min.; Saaz yeast, 7 h. 48 min.; Frohberg yeast, 7 h. 21 min.; *Sacch. apiculatus*, 4 h. 45 min., &c. The period of generation at 25° C. was, for *S. Past. II.*, 5 h. 12 min.; *S. Past. III.*, 6 h. 8 min.; *S. ellips. I.*, 6 h. 12 min.; *S. ellips. II.*, 6 h. 9 min.; *S. membranæfaciens*, 5 h. 13 min.; Saaz yeast, 4 h. 23 min.; Frohberg yeast, 4 h. 18 min. In the same proportion as new cells are formed, the medium is impoverished of the necessary structural materials and enriched with metabolic products inimical to development. Both these influences grow quicker when the nutrient medium is kept warm, and then soon become so powerful that the velocity of reproduction falls below the value previously obtained from cultures that have been kept much cooler, while at the same time the period of generation is correspondingly increased. Thus in Pedersen's experiments, cited above, the period of generation on the second day, though 20 hours at 4° C. and 16.7 hours at 13.5° C. was increased to 65.5 hours at 23° C. Further details will be given on this point in that portion of the next chapter which deals with the influence of alcohol on the life of yeast. In the case of temperatures near the limits beyond which budding ceases, the velocity of reproduction is so low as to be regarded as nil from the practical standpoint, H. Müller-Thurgau (XIX.) having reported, for instance, that the wine yeasts examined by him ceased to reproduce at 40° C. In climates where the temperature at the time of the vintage approaches this limit—the South of France, for example, according to Kayser and Barba (I.)—the must has to be artificially cooled, or the yeast cells present will reproduce so slowly that only a sluggish fermentation will be set up, incapable of suppressing concomitant injurious organisms. In northern vineyards, on the other hand, the autumn temperature not infrequently approaches the lower limit for yeast reproduction; and in that event the fermentation of the must is greatly retarded and slow.

In breweries using low-fermentation yeast the wort is pitched at 5°–7.5° C., and during primary fermentation the temperature is not allowed to exceed 9° C. for beer of the Bohemian type, 9.5° C. for Vienna beer, or 10.5° C. for Bavarian beer; so that

the temperature throughout remains considerably below the optimum reproduction temperature. The usual amount of pitching yeast, namely, about one part by volume of thick barm per 2000 of wort, causes fermentation to start quickly; but when, from any cause, the brewer is restricted to the use of a smaller proportion of yeast, he does not allow this to act on the whole mash at once, but first puts it through a reproduction process in a portion of the wort that has been cooled to 15°–20° C., and is maintained at that temperature. Reproduction ensues rapidly and abundantly, so that in a few hours the culture, which is beginning to throw up a "head," can be used for pitching the rest of the wort at the usual temperature.

Particulars on the influence of temperature on the reproductive capacity of yeast have also been published by A. L. Stern (III.), who, cultivating Burton yeast in a mineral salt nutrient solution treated with dextrose and asparagin, obtained the maximum weight of yeast crop at a temperature between 21° and 25° C.

The influence of the constitution of the nutrient medium on the reproductive capacity has been established by a series of observations. With regard to the source of nitrogen, F. Hess (I.) was able to prove the superiority of yeast water over mineral salt solutions containing sufficient asparagin or peptone to furnish an approximately equivalent quantity of nitrogen (9.0, 8.2, and 8.5 mgrms. per 100 c.c.) and sugar, the following results having been obtained in each 100 c.c. of the liquid during 28 days, the number of cells being referred to each cell in the original sowing (20 cells per c.c.):

Source of Nitrogen.	Reproductive Capacity of		
	Saaz.	Frohberg.	Logos yeast.
Asparagin	536	601	645
Peptone	704	1580	2037
Yeast water	1529	1659	3608

A similar result was obtained by C. Soldan (I.); and the reports of P. Thomas (I.) and A. L. Stern (III.) have already been mentioned in vol. ii. on p. 214. A certain influence is also exerted by the nature of the source of carbon, as has been shown by Soldan, the Saaz, Frohberg and Logos yeasts exhibiting the highest reproductive capacity when maltose was added, as source of carbon, to nutrient media consisting of mineral-salt solutions containing asparagin or yeast water. Dextrose furnished a medium crop, and saccharose gave the lowest.

The influence of the concentration of the medium on the repro-

ductive capacity is stated by R. Pedersen (II.) to be small. More exhaustive experiments were instituted in 1887 by J. Archleb (I.), pressed yeast (1 grm. per litre) being treated with malt extract ranging in strength from 1 to 25 per cent., the largest yeast crop was furnished by the 14 per cent. solution. In the case of a Burton yeast grown by A. J. Brown (VII.) at 20° C. in hopped ale worts of various strengths, gave no increase in crop when the concentration exceeded about 15 per cent. Balling. A. L. Stern (III.) who grew Burton yeast in two sets of experiments in which the asparagin content (3 grms. and 1.5 grms. respectively) was constant, whilst the dextrose varied between 0 and 30 per cent., obtained the maximum yeast crop with 15 per cent. of sugar in the one case and 12.5–15 per cent. in the other. Again, the experiments of Emil Bauer (III.) show that any excess of nitrogenous nutriment (whether in the form of yeast extract or of the autodigestion products of yeast) beyond the amount absolutely necessary has no influence on the crop.

Nutrient solutions which are very rich in sugar, and therefore have a strongly plasmolytic action on the cells, retard or even entirely prevent reproduction, a circumstance that is utilised in cookery for the preservation of fruit. No generally applicable data can be given respecting the proportion of sugar necessary to cause plasmolysis, because the corresponding influence of other ingredients in the solution also comes into play. In the experiments of E. Laurent (VI.) with a number of beer and wine yeasts, the appreciable reproduction ceased in the cultures in which the nutrient solution (a decoction of malt culms) contained about 60 grms. of saccharose, invert sugar, dextrose or maltose per 100 c.c. Greater powers of resistance in this respect are possessed by *Sacch. Zopfii;* and the yeast isolated by E. Dubourg (II.) from sweet Sauterne proved capaple of acting in an 80 per cent. solution of invert sugar. A. Mayer (VI.) claimed that the plasmolytic action of a 30 per cent. sugar solution could be counteracted by a small percentage of Seignette salt, the addition of which soon caused abundant reproduction and powerful fermentation in a previously quiescent nutrient medium; but this was disproved by M. Hayduck and M. Delbrück (I.). Greater powers of withstanding the influence of high percentages of sugar and other ingredients in the nutrient medium are possessed by the yeasts concerned in the (spontaneous) fermentation of Danzig Jopen beer (chiefly exported to England, under the name spruce beer or black beer). This was demonstrated by P. Lindner (XIV.), who isolated two new species of yeast, *Sacch. farinosus* and *Sacch. Bailii*, from fermenting Jopen wort of the initial concentration 53–54 per cent. Balling.

The relative permeability of the cell membrane also affects the reproductive power. This permeability varies, not merely in the different races of yeast, but among individuals of the same race,

and is determined by numerous factors, more particularly the age of the cells and the conditions of its earlier life. Hence both the fecundity and reproductive power differ, under identical external conditions, with the species (or race) of the yeast. On this point P. Lindner (XX. and XIV.) in 1889 instituted comparative experiments with twenty-two beer yeasts and fifteen white beer, spirit and pressed yeasts. The former, sown in 650 c.c. of hopped wort (11.95° Balling), furnished from 4.3 to 12 grms. of crop, weighed after pressing, whilst the yield from the second group, sown in 1350 c.c. of wort, was 9.3 to 19.5 grms. The highest yield was obtained from the white-beer yeasts, the pressed yeasts coming second. Further contributions on this matter have been published by F. Schönfeld (I.), C. Soldan (I.), F. Hess (I.), Roderich Meissner (I.), G. Korff (I.), W. Knecht (I.), and others.

The influence of the age of the sowing on the reproductive capacity and reproductive power was examined by M. Elliesen (I.) in the case of Frohberg and Logos yeasts. Some of his results are given below:

Age of Cells in the Sowing.	Reproductive Capacity in 42 days at 6°–8° C.		Reproductive Power in 8 days at 6°–8° C.	
	Frohberg.	Logos.	Frohberg.	Logos.
24 hours . .	2880	6400	144	320
3 weeks . .	800	3200	40	160
8 weeks . .	1200	720	60	36

With increasing age the vitality and reproductive power of the cells are weakened. Whilst the gradual thickening of the cell membrane is a good defence against adverse influences on the part of a nutrient medium that is changed for the worse, it equally presents obstacles which must be overcome by the stimulating ingredients of a fresh medium before the sowing can reproduce itself and lay up new material. The far higher reproductive capacity of the day-old sowing in the above table is therefore not surprising.

An important point in fermentation technology is the influence of the extent of the sowing on the amount of crop that can be produced in a nutrient medium of given quantity and composition. This applies to the brewer and vintner, as well as to the yeast manufacturer. The first reliable determinations obtained under practical working conditions were those of Thausing (I.), who instituted parallel experiments in four Austrian breweries (A to D), the wort being pitched with 0.33, 0.5 and 0.66 litre of thick low-

fermentation barm per hectolitre respectively. The results are expressed in the following table, which shows, in the first place, the total crop from each sowing, and then the actual increase (crop *minus* sowing):

Sowing of thick Barm per Hectolitre of Wort. Litre.	Resulting Crop of thick Barm. Litres.				Increase—thick Barm. Litres.			
	A	B	C	D	A	B	C	D
0.33	1.63	1.99	1.74	1.49	1.30	1.66	1.41	1.16
0.50	1.61	1.95	1.95	1.50	1.10	1.45	1.45	1.00
0.66	1.66	1.83	1.79	1.53	1.00	1.21	1.13	0.87

It is thus evident that while an increase in the amount of the sowing has little effect on the total crop, it causes an appreciable diminution of the increase. This fact has been repeatedly confirmed: for instance, by O. Rienke (II.) in 1889 for low-fermentation beer yeast; by A. J. Brown (VII. and IV.) in 1890 and 1892 for Burton top-fermentation yeast; in 1897 by Thausing himself in new experiments; and in the same year by A. Reichard and A. Riehl (II.) at the low-fermentation brewery at Lutterbach (Elsass), and by the Bierbrouwery d'Oranjeboom (I.) at Rotterdam.

The latter also confirmed Thausing's discovery that the accuracy of the results is unaffected by the height of the temperature, it being immaterial whether the fermentation was conducted at a low temperature (starting at 3.8° C. and rising to 9.4° C., to afterwards recede to 6° C.), or in the warm, commencing at 10.1° C., rising to above 15.8° C., and afterwards declining to 10° C., or finally carried on at the usual level of 8° C., 8.8° C., and 11° C. In low-fermentation breweries it is customary to pitch the wort with $\frac{1}{2}$ per cent. by volume of thick barm, which increases about eightfold during fermentation. The resulting deposit of yeast at the bottom of the fermentation vessel consists of three layers: bottom, middle or core, and top, the middle one being carefully separated from the other two and alone used for pitching subsequent brews. It forms about 60 per cent. of the total deposit, but suffers diminution during the washing process to which it is subjected, the final yield being only about twice the original amount of the pitching yeast.

§ 266. Consumption of Oxygen for Cell Reproduction and Respiration.

The question whether strictly anaerobic species of yeast (*see* vol. i. p. 181) exist, and therefore whether cell reproduction can proceed, without restriction, in the entire absence of free

oxygen, cannot yet be finally settled. J. Behrens (VIII.) reports that he found a comparatively large number of anaerobic yeasts on hop cones, and Traube (II.) had previously stated that yeast reproduction may proceed, in certain circumstances, in the absence of oxygen. On the other hand, Brefeld (XIII.) considered he had proved the absolute necessity for the presence of oxygen. G. Korff (I.) observed a considerable degree of reproduction in cultures in yeast water treated with 10 per cent. of saccharose and traversed uninterruptedly by a current of hydrogen, the yeast sowing employed having been grown under similar conditions. At the end of fourteen days the amount of crop obtained per original cell was : Saaz yeast, 876 cells ; Frohberg yeast, 1346 cells; and Logos yeast, 1160 cells. According to P. Barker (I.) a *Saccharomyces* isolated from ginger exhibited no signs of incipient growth when oxygen was completely excluded.

The more closely we criticise the reliability of the methods and appliances hitherto used for obtaining really anaerobic conditions in cultures, the smaller the trust we place in the results of the experiments made in this connection, and the greater becomes the doubt whether this or that anaerobic is one in the strict sense of the term or merely an aerobe requiring only a very small quantity of oxygen. We need only remember how difficult it is to completely eliminate oxygen from the gas (hydrogen, nitrogen, or carbon dioxide) employed to displace the air and traverse the cultures ; and the non-fulfilment of this preliminary condition will certainly influence the results when we are dealing with organisms that are very sensitive toward oxygen and are stimulated by very small quantities of that gas. Now yeast is an organism of this kind, being, as Brefeld (XIII.) observed, satisfied with a tension corresponding to the presence of 1 part of oxygen in 6000 parts of carbon dioxide. Traube's counterproof with the assistance of sulphindigotic acid cannot be accepted unconditionally ; and the fact that yeasts will reproduce during the temporary exclusion of oxygen is insufficient as proof, since in such cases the yeasts consume the previously accumulated store of that element. Moreover, cultures in which the vessel is provided with a seal of dilute sulphuric acid during fermentation cannot be regarded as having developed in the absence of oxygen. E. C. Hansen (XXXII.) states briefly that budding occurred in his experiments with nitrogen freed from oxygen ; but there is a decided difference between this and sporulation, which takes place only in presence of an abundant supply of oxygen.

As a matter of fact, the growth and reproduction of all the yeasts hitherto examined proceed more freely in aerated cultures. The first results that can be classed as reliable (because obtained with the counting cell) were obtained in 1879 by E. C. Hansen (XXX.), who showed that a certain beer yeast when grown in wort at 12°–14° C. increased 11.2-fold in 60 hours

without aeration, and 15.8-fold with admission of air. In a second experiment, at 13°–15° C., the crop was 9 and 27.3 cells respectively per original single cell. Other workers afterwards obtained similar results. The assertion of N. von Chudiakow (I.) that oxygen is indispensable for yeast reproduction only in the case of imperfectly nutritive media, does not seem to rest on a sufficient foundation. Other conditions being equal, the degree of stimulation imparted to cell reproduction by aeration depends on the race of the yeast—a fact observed by G. Korff (I.), M. Delbrück (I.) and several other workers. Nevertheless, as Prior (II.) has shown, the greater fecundity observed in aerated cultures is attributable not merely to the excess of available free oxygen, but also to the circumstance—which should not be underestimated—that the gas traversing the nutrient medium frees the latter from various products (volatile acids) of yeast metabolism that retard reproduction.

Yeast has the power of absorbing free oxygen from the environment, utilising it in completing internal chemical changes, and then excreting the most part in the form of carbon dioxide. The closer examination of this process of respiration is illuminative. The quantity of oxygen taken up by the yeast cell has been determined by P. Schützenberger and E. Quinquaud (I.), in an experiment wherein pressed yeast (containing 26 per cent. of dry matter) was distributed in aerated water, the figures per 1 grm. yeast in 1 hour being 0.1 c.c. at 9° C., 0.4 c.c. at 11° C., 1.2 c.c. at 22° C., 2.1 c.c. at 33° C., 2.1 c.c. at 40° C., 2.4 c.c. at 50° C., and 0.0 c.c. at 60° C. These workers assert that no further absorption of oxygen takes place in media which, like arterial blood, are capable of cedeing 200–230 c.c. of oxygen per litre, instead of the 6–7 c.c. present in the water used, but this is true only when the experiment is conducted under the conditions employed by them. By working under different conditions, A. Harden and S. Rowland (I.) found that 1 grm. of pressed yeast can take up an average of 3.54 c.c. of oxygen per hour. Moreover, the consumption of oxygen would probably reach a high figure if the cells were abundantly supplied with respirable substances, instead of, as in this case, being compelled to feed on one another. In fact, it has been found by Giltay and Aberson (I.) that yeast grown in a medium containing sugar consumed more and more of that substance in proportion as the degree of aeration was increased and the mixture of air and oxygen was richer in the latter constituent, as much as 21 per cent. of the total sugar (utilised for cell construction, respiration, and fermentation) being consumed in this way. The higher final attenuation of wort that has been strongly aerated during fermentation is partly due to the increased respiration.

Few of the investigations made in connection with the dependence of yeast respiration on external conditions are reliable.

Since the discovery that alcoholic fermentation by yeast is a purely enzymatic action (*see* chapter lxiii.), and not directly and inseparably connected with the life of the cell, it has become necessary to bear in mind the two-fold character of the sources from which the liberation of carbon dioxide may proceed, and therefore to consider respiration separately from fermentation. Strictly speaking, the only way in which the influencing of respiration can be reliably tested is by employing conditions in which the nature of the yeast and medium precludes the possibility of any alcoholic fermentation—a stipulation which at once disqualifies a whole series of investigations on the "carbon balance" of the fermentation process, the stimulation of the latter by aeration, &c. Little is known as to the intermediate stages of the combustion of the substances undergoing respiration. *Saccharomyces Hansenii* isolated by W. Zopff (XIII.) from American cotton-seed meal — a yeast incapable of inciting alcoholic fermentation—forms large quantities of oxalic acid as the final oxidation product (instead of carbon dioxide) of the sugars (glucose, galactose, saccharose, maltose, lactose, mannitol, dulcitol, and glycerol) added to the nutrient medium. Several workers, including E. Prior (II.) have found that copious aeration increases the quantity of acids formed in yeast cultures; but it is not yet certain how far these are to be regarded as products of the purely chemical action of oxygen on the constituents of the medium or as the result of hyperstimulation of yeast metabolism. According to the comparative experiments of G. Korff (I.) with Saaz, Frohberg and Logos yeasts, the aerated cultures contain a higher proportion of fixed acids, whilst those traversed by a current of hydrogen furnish a higher yield of volatile acids. In this connection we may recall the remarks made on p. 126, vol. ii. with reference to the divergent chemico-physiological behaviour of the cells of sedimental and film yeasts. The nature of the influence of external conditions on the coefficient of respiration of yeasts (*see* p. 79, vol. ii.) has not yet been sufficiently investigated. Under favourable circumstances the amount of heat liberated by respiration may give rise to a considerable increase in temperature, and in a case observed by Effront (VIII.), in which 2 kilos. of pressed yeast were crumbled down and exposed to the air (20° C.) as a layer 37 cm. in depth, this amounted to 36° C. in three hours.

The utility of aerating the nutrient medium as regards the development of the yeast to be grown therein has long been recognised in practice. During the early stages of rousing (aeration), whilst it is still very hot, the wort absorbs oxygen freely, fixing it by chemical combination and retaining it partly in the form of carbon dioxide. Afterwards, when the temperature has fallen, a considerable amount of the gas is also retained physically (in solution). The quantities were found by Pasteur (III.), in

one experiment, to amount to 41.7 and 7 c.c. respectively per litre of wort; but a considerably lower figure (5.7 c.c.) was obtained in the latter case by P. Petit (III.). A. Petersen (II.) detected 2–4 c.c. of oxygen per litre in the worts of the Alt-Carlsburg brewery; and, finally, C. Bleisch and R. Schweitzer (I.), in investigating the connection between oxygen absorption and the temperature, chemical composition, gravity motion of the wort, determined the amount of the physically dissolved oxygen in wort of 14.4 and 14° Balling, as 2.4 c.c. per litre at 62.5° C. and 4.4 c.c. at 5° C., the chemically combined portion, on the other hand, being 53 c.c. at 85° C. and 4 c.c. at 45° C. The decomposing action of oxygen, whilst very mild at medium temperature (15° C.), is nevertheless appreciable when continued for a long time. In this connection reference may be made to the occurrence of formic acid (associated with carbon dioxide) observed by Rayman and Kruis (I.) in old, sterile wort exposed to the air.

The aeration method of yeast manufacture (*see* vol. ii. p. 184) is based on the observation that cell reproduction is stimulated by powerful aeration, and originated in Sweden, though the first incentive was given by H. Hayduck's experiments (V.), subsequently followed up by M. Delbrück (VI.). Under the stimulating influence of oxygen the yield of pressed yeast furnished by this method amounts to 25–30 per cent. of the weight of cereal substances in the mash, as compared with about 12 per cent. in the old method which is now being gradually superseded.

In the laboratory cultivation of yeast for technical purposes, the aeration of the nutrient medium must not be omitted; for it has been shown by Hansen, and confirmed by A. Jörgensen (X.) that beer yeast grown in imperfectly aerated wort gives unsatisfactory results when used in practice, the "break" of the wort especially being defective.

The influence exerted by oxygen on the progress of alcoholic fermentation will be dealt with in chapter lxiv.

CHAPTER LII.

THE EFFECT OF CERTAIN TECHNICALLY IMPORTANT CHEMICAL INFLUENCES ON YEAST.

§ 267. Copper and its Salts.

In the previous three chapters we have set forth the formal conditions relating to the nutrition and reproduction of yeast; and we have now to deal with certain important adverse influences to which this organism is exposed either in nature or in fermentation on a practical scale. The effect of physical agencies may be disregarded at present, since they are treated of elsewhere, and we confine ourselves for the moment to chemical influences, beginning with those of copper as the first to which the yeast is generally exposed during reproduction or fermentation.

The behaviour of yeast cells toward copper and copper salts is of particular interest to the vintager. As the reader is aware, the ravages of *Peronospora viticola* are combated by sprinkling the vines with "bouillie Bordelaise" (introduced by A. Millardet of Bordeaux), an approximately 3 per cent. solution of copper sulphate in which—as has been shown by Berlese and Sostegni (I.)—the copper is converted into hydroxide (or basic double salt) by the addition of an equivalent amount of calcium hydroxide. Experience has proved that this remedy produces the desired effect, the conidia of the fungus being destroyed, and the leaves and fruit of the vine preserved; but at the same time the copper exerts a toxic influence on the natural yeasts present on the grapes, and since the various races of yeast probably differ in their susceptibility to this action of copper, it may be supposed that the treatment of the vines will result in an alteration in the flora of the grapes, and that its influence will extend as far as the must vat—more especially when sprinkling has been performed late in the season. Thus, A. Rommier (I.) has observed that must from late-sprinkled grapes gave no sign of fermentation, even under favourable conditions of temperature; whilst in other cases the only living cells detected were those of *Saccharomyces apiculatus* (probably less sensitive to copper), so that the fermentation remained incomplete. This observation led Rommier to investigate the influence of copper on yeast cells, with the result that even an addition of 25 mgrms. of Cu (= 98 mgrms.

of crystallised copper sulphate) per litre of must was found to retard the commencement of fermentation. On the other hand, P. Pichi (I.) stated in 1891 that the addition of less than 150 mgrms. of copper per litre of must had no adverse influence on the development and fermentative action of the species of wine yeast examined by him. Both workers, however, omitted to bear in mind Polacci's observation that the copper sulphate added to wine must combines with the tartrate present to form potassium sulphate and copper tartrate, which latter, being insoluble, is precipitated and thrown out of action. Observing this precaution, F. Krüger (I.) in 1894 found, in the case of a pure culture Johannisberg yeast, that the maximum amount of copper sulphate—present in solution and therefore active—that the yeast would stand without appreciable injury was equivalent to 44–45 mgrms. of copper per litre. When increased above this limit it gradually diminished the fermentative power, though the reproductive faculty and fermentative capacity were not so quickly destroyed. In the experiments conducted on low-fermentation beer yeasts by H. Will (I.), a large number of cells, after immersion in a 5 per cent. solution of copper sulphate for twenty-four hours still proved capable of fermenting the sugar solution to which they were transferred.

The assumption that the above figures obtained by Krüger may be of general application was controverted by the important discovery, made by E. Biernacki (I.) in 1891, to the effect that the amount of antiseptic (here copper sulphate) necessary to retard the fermentative activity of yeast varies with the amount of the sowing. This has been confirmed by other workers; for example, by H. Mann (I.), H. Pottevin (I.) and A. Amand (I.) in connection with copper sulphate; by Mann in the case of iron sulphate, lead acetate and corrosive sublimate; by C. Wehmer (XV.) with potassium and sodium arsenite, and by Müller-Thurgau (XVII.) in the case of sulphurous acid. Nevertheless, though the idea of obtaining unconditionally accurate limit values in this way must be abandoned, it is still highly desirable to make further investigations, especially on the comparative sensitiveness of different pure yeasts and more particularly as a contribution to the solution of the important question of the influence of the copper treatment on the modification of the yeast flora of the vine.

It should be mentioned—as might be foreseen from particulars already given in vol. i. p. 118, when copper sulphate is added in far smaller quantities than the above limits, it no longer restricts, but actually stimulates the fermentative activity of yeast. This occurs, according to Biernacki, when the dilution reaches the proportion of 1 part of copper sulphate per 600,000 parts of nutrient solution.

Apart from very exceptional cases, the vintager has no need to

fear that any disturbance of vinous fermentation will be caused by the copper sprinkled on the grapes. According to Tschirch (II.), the results of E. Mach's researches—confirmed by the investigations of M. Hoffmann (V.) on Portugese wine—only about one-tenth of the copper on the grapes finds its way into the must, all the rest remaining on the skins. Moreover, it seems from Polacci's researches that only a small fraction of that tenth actually becomes operative, so that but little damage can be caused, and Chuard has shown that this soluble remainder is mostly precipitated as malate and tartrate as the percentage of alcohol increases during fermentation, some portion being also converted into sulphide by the sulphuretted hydrogen (*see* vol. ii. p. 200) produced by the yeast cells. Consequently the clarified wine after separation from the yeast will only contain a few milligrammes of copper per litre, even when made from grapes that have been extensively sprinkled with copper. Of course a correspondingly larger amount will be present in the deposited yeast.

From the researches of H. Mann (I.) and H. Pottevin (I.) it may be concluded that the yeast converts a portion of the copper sulphate in the nutrient solution into copper phosphates ($Cu_2H_2P_2O_8$ and $Cu_3P_2O_8$), whilst another portion is retained by the cells. When a yeast thus enriched with copper is transferred to a colourless nutrient solution, the observations of H. Will (I.) show that the metal (*i.e.*, an unidentified compound of same) passes into the liquid, which acquires a blue tinge. The combination is probably between the metal and substances of the nature of those already referred to as yeast gum on p. 176, vol. ii.; and for this reason also, the figures already quoted above with regard to the toxic action of copper sulphate cannot be regarded as unconditionally accurate.

When pure yeast is grown in copper vessels for use in practical fermentation, the inner side of the vessel in contact with the fermenting liquid must always be well tinned. Otherwise the copper will be corroded, and the yeast crop will contain appreciable quantities of the metal, which is undesirable for several reasons. Thus, H. Seyffert (I.) detected 0.27–0.64 per cent. of CuO in the ash of pure yeast (*see* vol. ii. p. 196) grown in an apparatus that was defective in this respect. This quantity probably consisted in part of insoluble copper salts formed during fermentation and deposited, and partly of cupriferous constituents of the yeast cells. The tin used for plating the apparatus should be as low as possible in lead, since, as observed by Prior (IV.), this latter metal injures yeast considerably.

§ 268. Behaviour of Yeast Cells toward Alcohol.

From the standpoint of the œcological theory of fermentation, the alcohol produced by yeast should be regarded as a weapon

capable of hindering the appearance of other fungoid competitors in saccharine nutrient media. However, when accumulated in the medium during the progress of fermentation, it also restricts the further development and action of its producer. In this case, as with yeast poisons in general, the first result is the cessation of cell reproduction, a larger quantity of alcohol being necessary to arrest fermentation, and a still further quantity to kill the cells. On this point again it is impossible to expect absolutely applicable figures, because, as in other cases, the quantity of alcohol requisite for producing a given effect, even with one and the same species of yeast, is determined by external conditions (*e.g.*, composition of the nutrient medium) that cannot be closely gauged.

The restriction of cell productionis effected by a quantity of alcohol that is smaller in proportion as its increase in the medium is gradual, so that the cells have been subjected to its adverse influence through many generations. Thus, according to M. Hayduck (VII.) the reproduction of yeast cells in fermenting distillery wash becomes sluggish when the alcohol content reaches 2 per cent. by volume, and ceases when 6 per cent. is attained. This end point coincides approximately with the appearance of ebullient fermentation, a phenomenon well known to the practical man, and one that ordinarily appears about thirty hours after pitching. From this point onward, reproduction proceeds very slowly if at all, and therefore the maker of pressed yeast gathers his crop during that preliminary stage, since the main point with him is the amount of yeast produced, and the provision of suitable conditions for reproduction his chief care, the amount of alcohol formed being a minor consideration. On the other hand, the conditions are reversed in distilleries, the object there being to minimise cell reproduction, since this goes on at the cost of the sugar in the wash and therefore of the production of alcohol. In low-fermentation breweries also the end of the cell-reproduction period is indicated by the characteristic appearance of a "head" on the surface of the wort. During this initial period the alcohol content of wort reaches 2–2.2 per cent., as was determined by Mohr (II.) and confirmed by F. Schönfeld (I.).

When it is desired to prevent the development of active yeast cells in a liquid that is as yet free from alcohol—for example, to preserve wine must in an unfermented condition—the quantity of alcohol to be added, in order to produce this effect, must be larger than the 6 per cent. referred to above, and according to Hayduck (III.) and E. Laurent (VI.), at least 10 per cent. is necessary. And even this limit must be raised considerably, for H. Müller-Thurgau (III.) not only confirmed the observation of earlier workers that the various races of yeast differ in their degree of sensitiveness toward alcohol, but also discovered races that are capable of active reproduction in presence of

12–12.5 per cent. of alcohol in the nutrient medium. This discovery, which was made chiefly in connection with German and Swiss wine yeasts, was afterwards extended and confirmed by the researches of C. Forti (I.) on Italian wine yeasts. In investigating the preparation of the rice spirit, Awamori, in the Loochoo Islands, near Formosa, Inui (I.) described a yeast, *Sacch. Awamori*, which plays an active part in the process, and whose development is not crippled until the medium contains 13 per cent. of alcohol, 20 per cent. being required to arrest it completely. The Saké yeast examined by K. Yabé (II.) continues to grow until the alcohol content of the medium attains 24 per cent. A far lower power of resistance is exhibited by two red budding fungi discovered by Yabe (IV.) in the air of Japan and on the surface of rice straw, to which he gave the hardly appropriate names, *Saccharomyces Japonicus* and *S. Keiskeana*. The development of both these organisms is checked by 7 per cent. of alcohol in the medium. The budding fungi described by Richard Meissner (II.), which are incapable of forming alcohol and therefore do not belong to the yeasts, though they are of technical importance on account of their power of turning must and wine ropy (*see* p. 177), cease to reproduce and to form mucus when the medium contains over 5 per cent. (vol.) of alcohol.

Under otherwise equal conditions a larger quantity of alcohol is necessary to check fermentative activity than to stop reproduction. Thus a pressed yeast examined by Hayduck (IV.) was able to set up active fermentation in a saccharose nutrient salt solution containing 7 per cent. of alcohol, though not to reproduce therein. According to the researches of Brefeld (XV.), the addition of 17.3 per cent. of alcohol to the nutrient solution is necessary for preventing fermentation, though in Hayduck's experiments (III.) this result ensued in presence of 15 per cent. of alcohol. U. Peglion (II.) found a yeast still actively engaged in the secondary fermentation of an Italian wine containing 14.3 per cent. of alcohol. Further reports on the influencing of alcoholic fermentation by the presence of alcohol will be found in chapter lxiii., and the same point in connection with *Mycoderma* is discussed in chapter lx.

Temperature is the first of the external conditions exercising a determinating influence on the amount of alcohol required to restrict fermentation. This influence is progressive as the temperature rises within the limits that can be taken into consideration. In the first place, the rise in temperature is accompanied by a greater physical permeability of the cell membrane for the osmotic conveyance of alcohol into the cell; and, secondly, by the physiological mobility of the molecular groups of the plasma, and consequently the sensitiveness of this substance to external influences. For definite information on this point we are indebted to H. Müller-Thurgau (XIV.), who found that, under otherwise

identical conditions as regards the composition of the nutrient solution and the race of yeast, fermentation was arrested

At 36° C.	as soon as	the	alcohol	content	reached	3.8	per cent. by weight.
27°	,,	,,	,,	,,	7·5	,,	,,
18°	,,	,,	,,	,,	8.8	,,	,,
9°	,,	,,	,,	,,	9·5	,,	,,

Practical brewers would seem to have long recognised the existence of some relation between temperature and the degree of sensitiveness of yeast toward alcohol, since they laid it down as an axiom that, in order to obtain an active sedimental yeast as well as a good beer, the temperature in the fermenting tun should be allowed to rise at the start (when the amount of alcohol present is small) and be afterwards gradually reduced.

The composition of the nutrient solution also influences the sensitiveness of the yeast toward alcohol, and therefore affects the amount of alcohol that can be produced in the fermenting liquid. Unfortunately, there is little reliable information available on this point, though the researches of H. Müller-Thurgau (XIV.) have shown that, under otherwise identical conditions, the retarding effect of alcohol increases with the amount of sugar in the medium. This fact (the cause of which is still undetermined) is utilised in certain fermentation industries, notably in molasses distilleries. In countries where the excise duties are levied, partly or entirely, on the dimensions of the mash tun or fermenting vessel, the interests of the distiller lead him to work with mash of the highest possible concentration. To overcome the difficulty arising from the increasing sensitiveness of the yeast to alcohol in these strong mashes, prolonged tentative experiment has led to the mash being pitched at the highest gravity found to be compatible with regular fermentation, and replenished with concentrated mash in proportion as the sugar is consumed. In distilleries where raw grain (maize, &c.) or potatoes are used this method is impracticable, owing to reasons which need not be discussed here; but in breweries it is not infrequently resorted to, especially when the amount of pitching yeast is insufficient for a brew and must be increased quickly. In the strong musts intended for the production of choice wines, and fermented without this artifice, fermentation comes to a standstill before the whole of the sugar is consumed, and the resulting wines, though completely fermented, are sweet.

Yeasts in general are capable of a certain degree of habituation in respect of alcohol, so that they can be gradually accustomed to work in a nutrient solution containing a larger proportion of alcohol than was previously sufficient to arrest their activity. However, as was shown by E. Laurent (VI.), for a series of beer and wine yeasts, this is possible only within comparatively narrow limits.

H. Müller-Thurgau (XIV.) has shown that yeast cells in a nutrient medium in which the alcohol produced by their own activity gradually reaches menacing proportions, assume the condition of resting cells (*see* p. 118, vol. ii.) which remain at the bottom of the liquid, and are incapable of setting up fermentation when transferred to a fresh, non-alcoholic nutrient solution. Under these latter conditions, however, they produce in a short time daughter cells, which effect the fermentation of the proffered sugar. The prevention of this formation of resting cells is the main cause of the beneficial effect resulting from the practice of stirring up the yeast with a stick, &c., when fermentation grows sluggish in the must. When fermentation has reached the stage at which the wine begins to clarify by the deposition of the yeast, the consumption of sugar and formation of alcohol are effected almost entirely by the sedimental yeast collected at the bottom of the vessel. This alcohol, however, diffuses with considerable difficulty in the already strongly alcoholic liquid, its buoyancy being insufficient to overcome the frictional resistance. Hence a stratum, rich in alcohol, is formed immediately above the sedimental yeast, the upper cells of which are converted, under its influence, into the resting condition, and constitute a wall separating the active yeast from the still saccharine liquid, so that fermentation is arrested. The object of the stirring is to alter this state of things and remix the yeast with the liquid. The researches of J. Wortmann (VII.) on the yeast content of bottled wines confirm the above recorded observation of Müller-Thurgau, the presence of budding fungi (yeasts and torulæ), capable of reproduction, having been detected in twenty-eight out of fifty-four samples of wine of guaranteed age in bottle.

The discovery and use of yeasts possessing high powers of resistance and low sensitiveness toward alcohol has proved especially useful to makers of wine and fruit wine; in the first place for artificially incited secondary fermentation, then for re-fermentation, and, finally, for the production of champagne (*see* p. 188, vol. ii.). Similar interest attaches to the preparation of Saké, the Japanese rice wine, which, according to A. Schrohe (II.), usually contains 14 per cent. (by weight) of alcohol, and occasionally even 18 per cent.

The foregoing particulars relate exclusively to ethyl alcohol. With regard to the influence exerted on the fermentation activity of yeast by the homologous allies of this alcohol, certain experiments were instituted, first by P. Regnard (I.) with an unspecified yeast in 1889, and then in 1897 by K. Yabé (III.) with the Saké yeast mentioned on p. 240. The former used for each 10 grms. of yeast, 250 c.c. of an 8 per cent. solution of grape sugar, *i.e.*, a not particularly favourable nutrient medium. No fermentation ensued in the cultures when treated with the following additions (per cent. by volume):

	Methyl-A.	Ethyl-A.	Propyl-A.	Butyl-A.
Regnard . .	20	15	10	2.5
Yabé . .	—	—	—	—

	Isobutyl-A.	Amyl-A.	Hexyl-A.	Capryl-A.
Regnard . .	—	1.0	0.2	0.1
Yabé . .	3.0	1.0	—	0.5

Hence the toxic properties of these alcohols increase with the number of carbon atoms in the molecule.

§ 269. Inorganic Acids and Salts.

Though several observations have been published already with regard to the influence of carbon dioxide on the yeast cell, the importance of this hitherto imperfectly appreciated question to fermentation technology renders further investigation highly desirable. On repeating in 1887 the experiments first performed by C. Prandtl (I.) in 1865 on the cultivation of beer yeast in open and sealed tubes respectively, G. Foth (I.) observed a smaller increase of the sowing under the latter conditions; but it is doubtful in how far this is due to the presence of carbon dioxide or to the lack of oxygen. After L. Lindet (III.) had made further experiments, in 1889, without any decisive result, H. Ortloff (I.) in 1900 recorded a similar adverse effect on reproduction in pure cultures of *Sacch. cerevisiæ I.*, Hansen, *Sacch. Pastorianus I., II., III., Sacch. ellipsoideus I.* and *II.*, Saaz yeast, Frohberg yeast and Logos yeast, traversed by a current of (presumably oxygen-free) carbon dioxide throughout the whole period of reproduction. With regard to wine yeasts, H. Müller-Thurgau (II. and III.) had already shown in 1889 that reproduction was checked by a high content of carbon dioxide in the wine freshly inoculated with that yeast. In the experiments of Lopriore (II.) with the cells of a hanging-drop culture of pure yeast, it was found that budding continued in a few cells during the first 4–6 hours of passing oxygen-free carbon dioxide through the Böttcher cell, but not afterwards. The divergent sensitiveness of the various species and races had already been observed by Foth (II.), who found *Sacch. Pastorianus I.* to be more resistant than Carlsburg bottom yeast No. 1; and this divergence is also deducible from Ortloff's results. A high percentage of the gas in question also diminishes the fermentative activity, judged as a whole, in the culture, this being confirmed by both Foth and Ortloff. As pointed out, however, by E. C. Hansen (XXXVI.) and J. C. Holm (I.), such a result admits of two opposite interpretations; for though the yield of alcohol per cell of yeast crop may work out lower in the cultures treated with carbon dioxide, and thus lead to the conclusion that an adverse influence has been exerted by that gas, the exact opposite may have occurred in

reality, and the fermentative activity of the effective cells may have been even stimulated, especially when a number of cells in the crop became inoperative at a very early stage. Little is known with regard to the effect on the capacity of the individual cells. When working in a nutrient medium contained in a hermetically sealed vessel of sufficient strength (*e.g.*, a champagne bottle), the yeast and its fermentative activity are soon brought under the influence of carbon dioxide at high pressure, and will suffer injury, not through the effect of the high gas pressure *per se*, toward which yeast is not very sensitive, but owing to the increased concentration of the acid in the fermenting liquid. It is desirable that the pressure at which fermentation is finally suppressed in closed vessels should be more accurately determined, the limit not having been reached at 12.6 atmospheres in the experiments made by C. G. Matthews (III.) with Burton yeast in beer wort. Conversely, the artificial removal of pressure from the fermenting liquid, by drawing off the carbon dioxide (and other volatile injurious products) accelerates fermentation and heightens the attenuation (*see* chapter lxiv.), a point already observed by Boussingault (I.), followed up subsequently by Prior (II) and others, and finally evolved by Grauaug and Kranz (I.), by Nathan (in his Hansen apparatus), and by Pfaudler into a vacuum fermentation process which has been reported on by L. Aubry (III.).

The most important points in connection with the influencing of yeast by sulphur dioxide, whether in the gaseous state or in the form of acid salts—especially calcium bisulphite—have already been discussed on pp. 108, 109, vol. i.

Though the comparatively slight action of arsenious acid and its potassium and sodium salts on yeast—investigated by C. Wehmer (VII.) and C. Knoesel (I.)—is of no importanee in practical fermentation, it has proved useful in the theoretical study of the enzyme of alcoholic fermentation (*see* chapter lxiii.).

Hydrochloric acid, sulphuric acid, hydrcfluoric acid, and certain other fluorine compounds have a relatively stronger effect on most bacteria than on yeasts, so that, by selecting an appropriate degree of concentration, the latter organisms can be protected against the former (*see* vol. i. p. 248). C. Knoesel (I.) has shown that an acid nutrient medium is not unconditionally essential to yeasts; but, within certain limits, the presence of free acids stimulates both reproduction and fermentative activity.

That boric acid has little adverse influence on yeast was observed by J. Mattern (I.) and E. Biernacki (I.); and in H. Will's experiments (I.) it proved unable to kill the whole of the cells, even after an exposure of 20 minutes. The same has been found in respect of calcium borate, and also—contrary to a previous report by Wernke (I.)—of borax, so that use can be made of

another property of this substance, as already mentioned on pp. 179, 180, vol. ii.

Though corrosive sublimate has been shown by WERNKE (I.) and WEHMER (VII.) to have but a separatively slight toxic effect on yeast, its otherwise strongly poisonous properties render it unsuitable for use in practical fermentation. Other substances also devoid of practical importance are: bismuth nitrate, zinc sulphate, zinc chloride, ferrous sulphate, ferrous chloride, manganous chloride, potassium permanganate, aluminium sulphate, aluminium chloride, and potassium alum, the influence of which on yeast was studied by H. WILL (I.).

As shown by J. DUMAS (V.) and subsequently more closely investigated by U. GAYON and E. DUBOURG (IV.), a considerable amount of cell juice, rich in nitrogen, can be extracted from yeast cells by exposure to the influence of a saturated solution of a suitable salt, such as sodium acetate, phosphate or sulphate, potassium acetate, oxalate, tartrate or iodide, magnesium sulphate, calcium chloride, &c. A. BÉCHAMP (VIII. and XI.) obtained a still higher yield by kneading pressed yeast with the powdered, dry salts, an almost immediate liquefaction of the pasty mixture occurring in most cases. Saccharose is also suitable, when used in the proportion of two parts to three of yeast, and the same applies to gum arabic, &c. The auto-fermentation frequently occurring under these conditions was investigated by C. J. LINTNER (III.), in a series of experiments to which further reference will be made in chapter lxv.

§ 270. Organic Stimulants and Poisons.

The mutual relations between yeasts and the organic acids of the aliphatic series are varied. Some of them, succinic acid, for instance, occur as decomposition products of the cell substance (*see* chapters lxiv. and lxvi.). In other cases similar acids play the part of a source of carbon, and therefore supply material for the process of metabolism, as already mentioned on p. 205, vol. ii.; whilst in still other instances, with which we shall now deal, they excite interest on account of their stimulant or poisonous action. Yeasts are very susceptible to butyric acid, and must therefore be protected from the danger with which they are menaced by this acid in distillery work (*see* vol. i. p. 245). The general rule that yeasts of different species are variously influenced by any given stimulant has found practical utilisation in connection with tartaric acid. On the occasion of his critical examination of Pasteur's method of purifying the pitching yeast used in the brewery, E. C. HANSEN (XXXIV.) found that the beer yeasts, *Sacch. cerevisiæ I.*, Hansen, Carlsburg bottom yeast No. 2, &c., are more susceptible to tartaric acid than the wild yeasts (*Sacch. Pastorianus I.* and *III.*, *Sacch. ellipsoideus II.*); so that in a mixture of the two

classes the relative proportion changes in favour of the wild yeasts when the culture is grown in a 10 per cent. solution of saccharose containing 4 per cent. of tartaric acid. The progressive diminution of the culture yeasts and increased percentage of wild yeasts obtained by successive re-inoculations in such a liquid, forms an excellent means of detecting the latter in critical cases. The Saké yeast examined by K. Yabé (III.) will not develop at all in this solution. The influence of acetic acid was first investigated, in the case of pure cultures, by Lafar (II.), who found that the fifteen species of wine yeast examined differed considerably in point of sensitiveness. Roderich Meissner (I.) afterwards found the same result in the case of Saaz, Frohberg and Logos yeasts, which, however, cannot stand nearly so much of this acid as the wine yeasts in question, the first two losing their fermentative capacity almost completely in presence of 0.25 per cent., and the third with 0.375 per cent., whereas all the fifteen wine yeasts continued to ferment in presence of 0.78 per cent. of acid, and three of them even with 1 per cent. H. Müller-Thurgau (XXI.) found that the restrictive influence of this acid on development can be ameliorated by aerating the nutrient medium (must). According to Duclaux (I.), the presence of 0.4 grm. of formic acid per litre will retard the development of cells of various kinds of beer yeast sown in wort, reproduction ceasing entirely when the addition is doubled. Oxalic acid is found by O. Loew (IX.) to destroy the fermentative power of yeast in twenty-four hours, when forming 1 per cent. of the solution; and H. Will (I.) states that the same result ensues with 10 per cent. in five minutes. In the case of succinic acid, M. Hayduck (VIII.) stated that even 0.59 per cent. does not hinder fermentation by yeast; and E. Kayser (X.) showed that this quantity is consumed by the organism. The same worker (IX.) also found variable degrees of sensitiveness to malic acid and citric acid on the part of different yeasts. In the comparative tests made by J. Behrens (VIII.) with Carlsburg bottom yeast No. 1 in unhopped beer wort, an addition of 0.2–0.4 per cent. of citric acid retarded the maximum development of fermentative capacity to some extent, without diminishing the total effect.

The behaviour of yeasts toward hop resins—a chemical bibliography of which has been compiled by G. Barth (I.)—has not yet been sufficiently investigated. Three of these resins (*see* vol. i. p. 110) were isolated from hops by M. Hayduck (IX., X., XII., I.), who showed that no appreciable antiseptic effect is produced by hop tannin or by the ethereal oil which imparts to hops their characteristic aroma. On the other hand, according to this worker, the two soft resins decidedly retard the progress of fermentation; but L. Aubry (III.) proved that the final attenuation in hopped wort is higher than in unhopped wort, a result confirmed by J. Behrens (VIII.), though F. W. Richardson (I.)

observed the contrary. Further attention should be devoted to Hayduck's observation that the amount of nitrogen compounds absorbed by yeast from wort increases with the quantity of hops employed, bearing in mind a statement made in this connection by Behrens (VIII.). With regard to the part played by hop resins in the formation of "head" on the fermenting wort, compare p. 183, vol. ii.

Many yeasts are rather sensitive to the tannins in wine must and certain fruit musts, as was observed by A. Rosenstiehl (I.). This is a well-known fact among vintagers, and special measures are adopted in consequence.

The nature of the resins and ethereal oils rendering the fermentation of juniper-berry juice a difficult operation is still unknown. G. Kassner (I.) published a note on this point.

According to Wernke (I.), oil of mustard ($C_3H_5.NCS$) in the proportion of 1 : 16,700 is fatal to yeast.

The maltol ($C_6H_6O_3$) first discovered by J. Brand (III.) in caramel-colour malt, and regarded by Kiliani and Bazlen (I.) as a methylpyromeconic acid, is stated by H. Will (XXVI.) to have but a feeble toxic effect on yeast and to be devoid of influence in practice, for though 0.1 per cent. will delay yeast reproduction, the amount present in wort is far below that proportion. Even the furfural ($C_5H_4O_2$) produced during the curing of malt (*see* p. 207, vol. ii.), from which it passes into the wort—though rarely found in beer—is stated by H. Will (XXVII.) to have but little effect on yeast, though the different yeasts are variously affected and all are killed by an addition of 0.5 per cent. It has not yet been definitely settled whether and to what extent this or other products of the curing of malt are responsible for the admitted fact that dark worts furnish a lower attenuation than those from pale malts. Researches on this point were undertaken by M. Irmisch (I.) and F. Niemeyer.

Among the compounds of the aromatic series, Wernke (I.) states that benzene (C_6H_6), toluene ($C_6H_5.CH_3$), and xylene ($C_6H_4(CH_3)_2$) are fatal to yeast in the proportions of 1 : 200, 1 : 300, and 1 : 800 respectively. The action of carbolic acid or phenol ($C_6H_5.OH$) on yeasts was studied by Lemaire, W. Bucholz (I.), H. Hoffmann (V.), and H. Fleck (I.), and afterwards more carefully by C. Knoesel (I.), who found that an addition of about 0.5 per cent. of phenol, at room temperature, kills the cells. According to Biernacki (I.) the introduction of a second and third hydroxyl group into phenol lowers its toxic properties, so that resorcin ($C_6H_4.(OH)_2$) is only half as powerful, and pyrogallol ($C_6H_3(OH)_3$) has only one-third the strength of phenol. K. Yabé (V.) confirmed this observation, and found it also applicable to pyrocatechin, hydrokinone, and phloroglucin. Benzoic acid, even in the small proportions in which it is soluble in aqueous liquids, has a somewhat powerful effect on yeasts, according to the dis-

covery made by H. Will (I.) and (at a later date) by C. Wehmer (VII.), and confirmed by H. Fleck (I.) This is utilised in the practice of preserving fruit (*see* vol. i. p. 108). Comparative tests made by Wehmer (XV.) on Frohberg yeast with benzoic acid and its three monoxy-derivatives, showed that 0.1 per cent. of the first-named and also of its ortho-derivative (salicylic acid) entirely prevent the development of yeast, though the m- and p-oxybenzoic acids in the same concentration have no appreciable influence. According to G. Heinzelmann (VI.), 0.1 grm. of salicylic acid per litre has a stimulating rather than an injurious action (*see* vol. i. p. 104), whereas 0.37 per cent. is fatal to yeast; and similar results were obtained by H. Will (I.) The behaviour of yeast toward cinnamic acid was reported on by Fleck (I.). Saccharin, which was found by Burkard and Seifert (I.) to have little effect on yeast, is stated by Macheleidt (I.) to prevent fermentation in hopped wort when used in the proportion of 1 per cent. No appreciable influence in this connection could be detected by G. Bryilants (I.) with 0.7 per cent. of phenolphthalein. The behaviour of yeasts toward certain alkaloids was investigated by Cl. Fermi and E. Pomponi (I.).

SECTION XIV.

LIFE HISTORY AND VARIABILITY OF THE SACCHAROMYCETES. CLASSIFICATION OF THE SACCHAROMYCETES AND SCHIZOSACCHAROMYCETES.

By ALBERT KLÖCKER, Copenhagen.

CHAPTER LIII.

THE LIFE HISTORY OF SACCHAROMYCETES IN NATURE.

§ 271. Fundamental Researches on the Life History of Saccharomycetes.

The first of the yeast fungi whose cycle of existence was traced in nature was the *Saccharomyces apiculatus*, more fully described in chapter lxi., the researches having been carried out by Hansen. This small, lemon-shaped asporogenic alcohol yeast, which is widespread in nature and generally known, received its name, *Sacch. apiculatus*, from Reess. In the following lines the term Saccharomycetes, whether used in a general sense or qualified by the prefix "true," will be applied to alcohol yeasts capable of producing endospores as well as of budding. As the researches in connection with *Sacch. apiculatus* are typical of those concerned with the life history of the true Saccharomycetes, a brief introductory *résumé* of them will now be given.

In the years 1880–1881 Hansen (XXII. and IX.) published the results of his investigations on the career of the yeast in question. From these it appears that the chief habitat and breeding-place of this fungus in the summer and autumn are damaged, sweet, juicy fruits, in the juice of which it reproduces in great abundance, whilst in winter and spring it inhabits the soil underneath fruit trees and bushes. From the fruit it finds its way into the soil, either through the dropping of the fruit or the swilling action of rain, as well as in the excrement of the numerous insects that infest and devour the sweet, juicy fruit inhabited by the cells. With regard to the means by which the minute cells are trans-

ported from the soil to the fruit, Hansen ascribes the principal *rôle* to the wind; though, both in his first and subsequent treatises he remarks on the great importance of rain, insects and other animals in this connection, the transference of cells from one fruit to another being attributed to insect agency. A powerful storm of rain may splash up the wet soil, accompanied by the yeast cells, on to the fruit of low-lying plants, such as strawberries. Whilst the action of insects as conveyers of infection is restricted to a short period in the year—not only in the vicinity of Copenhagen, where Hansen's researches were carried on, but also throughout the greater part of Europe—the wind continues to act all the year round, enormous numbers of the cells being carried up in clouds of dust and deposited on the fruit. The researches also show that this life history is the normal one for the yeast in question, and that the cells soon die when deposited on unripe fruit, owing—as was ascertained by Hansen (X.)—to the fact that the fungus has a very low power of resisting drought and the action of the sun's rays.

We will now turn to the question of the life history of the true *Saccharomycetes*. When Hansen commenced his investigations in this direction there had already been published a series of researches by Brefeld and Pasteur running contrary to the ideas which formed his starting-point.

Brefeld (XVII.) formed the opinion that yeast cells not only reproduced in the alimentary canal of the animal organism, but that their chief breeding-place and habitat was the excrement of herbivorous animals. Hansen showed that this is incorrect.

From the time of the first researches on the fermentation of wine, it was a recognised fact that ripe—and especially damaged—grapes are rich in yeast cells at gathering time, and it naturally followed that the yeast cells would find their way into the soil with the fallen grapes or when swilled off by rain. Pasteur (XXX. and XVIII.), however, concluded from his experiments that the cells could not live long in the soil, and that the latter therefore could not form their winter habitat, though he gave no hint as to what actually constitutes the latter.

Hansen (LVII.) adopted two methods in his researches: partly the analysis of samples of soil and other natural substrata, including the dust in the air, and partly the sowing of certain species in the soil under natural conditions. The results showed that true Saccharomycetes are to be found in the soil and the air at all periods of the year, but most abundantly when the sweet, juicy fruits are ripe. The inoculation experiments were performed with *Sacch. cerevisiæ*, *Sacch. ellipsoideus* and *Sacch. Pastorianus*, the yeasts being sown in sterilised soil, placed in flower-pots embedded in the ground out of doors. Here it was proved beyond dispute that the cells live from one fruit harvest to another; and at the same time the observation was made (LVIII.) that the cells

are capable of producing endospores on the surface of the ground. On the basis of these experiments Hansen was able, in his second communication in 1882, to demonstrate that the life history of the true *Saccharomycetes* is substantially identical with that of *Sacch. apiculatus*. The chief breeding-place is on sweet, juicy fruit, the soil constituting the winter habitat, whilst the principal methods of transport are the wind, rain, insects and other small animals.

The inoculation experiments were repeated subsequently by Hansen (LIX. and XLIII.) both with *Sacch. apiculatus* and with, in part, the same *Saccharomycetes* (viz., *Sacch. ellipsoideus*, *Sacch. Pastorianus*, Carlsburg bottom yeast No. 1 and a top-fermentation beer yeast), but in this case the flower-pots were replaced by Chamberland earthenware pipes, in order to ascertain whether the yeasts could survive for several years in the soil. The earthenware pipes were used in order to protect the cells, as far as possible, from infection and the ravages of animals from the surrounding earth. In the result it was found that the yeasts in question are able to live for more than three years in soil.

Both at that time and subsequently, the question of pleomorphism in these fungi was actively discussed, especially in consequence of the researches of Brefeld, and Pasteur's theory of the development of Saccharomycetes from brown *Dematium* cells must be classed in the same catagory. It was considered possible that still living original forms of these yeast fungi might be discovered, and probably of such a character as to point to a very different life history to that established by the researches on *Sacch. apiculatus*. Hansen himself, by referring to this possibility in several of his later publications, not only led to the following up of this line of research by several workers, but also to the institution of experiments by others with a view to upsetting the theory he had commenced to establish in connection with the life history of yeasts.

The repetition of Hansen's experiments, especially in connection with the analysis of vineyard soils, became a matter of practical and theoretical importance ; and in 1889 a series of investigations in this direction was undertaken by H. Müller-Thurgau (XXIX.). This worker, also, found that the chief breeding-place of the Saccharomycetes is on fruit, and that the cells of wine yeasts can be discovered in soil all the year round. His experiments were made in a vineyard at Geisenheim, and he was the first to ascertain how deep yeast cells can be embedded in the earth and continue to live, namely, 8–12 inches on the average, none being found as deep as 16 inches. In the summer time the number of yeasts cells on the surface is smaller than at a depth of a few centimetres. At the outset, Müller-Thurgau held the opinion that insects formed the chief means of transporting the yeast cells, and he declined to admit the importance of wind in this connection. Hence the only point on which he was in complete accord

with Hansen was that, also in vineyards, the soil forms the normal winter habitat of the Saccharomycetes, and the latter find their way thence to the breeding-place, namely, the grapes. Later on, however, he recognised (LVIII.) that both wind and rain are important means of transport. His experiments with sowing wine yeasts in earthenware pipes showed that the fungi can live in the soil from one autumn to another. Müller-Thurgau also states that *Sacch. ellipsoideus* has a very low power of resisting drought, and therefore soon dies when on the surface of grapes exposed to very dry weather and intense sunlight—an observation also confirmed by Martinand (II.).

A few years later a thorough investigation of vineyard soil was undertaken by Wortmann (VI.), with samples taken from the same plot at intervals of 14 days during two years. The largest number of yeast cells was found in November and December; and must inoculated with the samples of soil quickly began to ferment. During January, February and March the number of yeast cells diminished; and during the spring and summer—the latter especially—the proportion became more and more unfavourable, and the yeasts disappeared from a progressively larger number of the samples. The most unfavourable results were obtained in late summer: August and September; but from the beginning of the grape harvest the conditions improved almost immediately. Wortmann's conclusion was to the effect that the yeast cells become enfeebled during their sojourn in the soil, most of them dying off; and that the continuation of the species is confined to the few cells that survive the winter and are fortunate enough to find themselves on a damaged grape. As will be evident, especially from what follows, this does not entirely coincide with Hansen's experiments, according to which the conditions in the soil are not so unfavourable for the *Saccharomycetes*. With regard to the means of transport, he thinks that Hansen gives undue credit to the wind, his own experiments tending to show that the chief part is played by wasps.

Whilst both Wortmann and Müller-Thurgau agree in the main with Hansen's theory on the life history of the *Saccharomycetes*, the workers named below express a different view on certain points, several of them stating that in hot climates, Italy in particular, the soil is not the chief habitat of the yeast cells.

Boutroux (IX., X.) regards the nectar of flowers, insects, and unripe fruit as constituting the habitat of yeast fungi from the end of winter to the fruit season, and states that the cells are conveyed from flower to flower and from fruit to fruit by insects. It must, however, be remembered that he makes no distinction between *Saccharomyces* and *Torula*, but applies the former name to all yeast cells capable of inciting fermentation. Probably, therefore, the cells found by him were not always *Saccharomycetes*, most of them being certainly forms of *Torula*, which are widely

met with in nature. Neither Hansen (LIX.) nor Beijerinck (XXVIII.) was able to confirm Boutroux's communication. Another remarkable result obtained by this worker (III.) was that insects play a more important part than wind in the conveyance of yeast cells that are unable to invert saccharose, the converse being the case with yeasts capable of inversion.

Rommier (I.) is of opinion that *Sacch. apiculatus* passes the winter in honeycomb; but neither these nor any other yeast cells have been discovered in comb by Boutroux, Hansen, Beijerinck, or Klöcker. Of course it is possible that a few isolated yeast cells or other micro-organisms may be found occasionally in any situation; but we are now concerned solely with large quantities and constancy of occurrence.

Berlese (IV.) asserted that the digestive canal of insects forms the true winter resort of the *Saccharomycetes*, and that Hansen was in error when he located this resort in the soil; and he claimed that the cells pass the winter in flies (especially in Italy). This statement may, however, be disregarded, since the insects do not themselves pass through the winter in the perfect state (imago) in Europe, except in the most southerly districts (and therefore not at all in the largest part of Italy); in addition to which he only succeeded in finding a single fly containing *Saccharomyces* among 150 examined. He also states that the cells pass the winter in ant-runs in hollow trees and woodwork—a circumstance of no importance even if true, because these habitats are so rare in comparison with the area presented by the soil that even if every ant's dwelling contained *Saccharomycetes* their number would be insignificant in comparison with those found in the soil. Moreover, Klöcker carried on a large number of experiments on the behaviour of insects toward *Saccharomycetes*, and found that insects are devoid of importance as a winter habitat, at least in Europe north of the Alps.

That *Saccharomycetes* are able to pass without injury through the alimentary canal of various animals has been demonstrated by several workers, *e.g.*, by Klöcker and Schiönning (VIII.) in the case of insects and birds; by Berlese (IV.) with insects; and by Cordier (I.) in the case of insects and animals. Consequently the micro-organisms in question may also be disseminated by these means.

§ 272. Later Experience on the Life History of Saccharomycetes.

The starting-point and basis of the foregoing researches on the life history of the true *Saccharomycetes* were constituted by the results of Hansen's experiments on *Sacch. apiculatus*. However, though the Hansen theory sufficed to explain all the observations made with respect to that species, it was, in several cases, not

unconditionally applicable to the true *Saccharomycetes*. Thus, in his researches in the German wine districts, he found that at certain periods the samples of soil from the vineyards contained fewer yeast cells, and also wine-yeast fungi, than similar samples from adjacent meadows. Indeed some 50 c.c. samples of vineyard soil did not contain a single living cell that could be identified as *Sacch. ellipsoideus*. Analogous, though scarcely so extensive, irregularities occurred in certain analyses of orchard and arable soils in the vicinity of Copenhagen. Consequently, HANSEN (LX.) was led to extend his researches and proceed in a different manner, the methods being made more stringent, the number of the analyses greatly increased, and the area of the experiments broadened to comprise an enormous district, from Scandinavia to southern Italy, from the plain to the highest mountain top. The principal new direction taken by these experiments was, however, the investigation of the secondary habitats. The analyses of soils from round Copenhagen showed that true *Saccharomycetes* are to be found everywhere in the soil all the year through, even in places where *Sacch. apiculatus* is only detected occasionally if at all, that is to say, at considerable distances from fruit gardens. Only when the number of analyses had reached sufficient dimensions did it appear that the garden soils are the richest in *Saccharomycetes*, and that the number of the cells diminishes as the distance from these centres increases. For instance, in a series of 200 analyses, true *Saccharomycetes* were found in 67 per cent. of samples of soil taken from under fruit trees and fruit bushes; in 30 per cent. of those from under deciduous and coniferous trees in the vicinity of fruit gardens, and only on 19 per cent. of samples from distant fields. Similar results were obtained in the experiments conducted in mountain districts, *e.g.*, the Hartz Mountains and the Alps. The greater the altitude and the distance from fruit gardens, the less plentifully are *Saccharomyces* cells found in the soil. Hansen's newer analyses show that the parallel also holds good in warmer climates, *e.g.*, Italy.

The reason of the presence of *Saccharomycetes* at considerable distances from fruit gardens and primary breeding-places in general is in part traceable to the fact that their power of producing endospores makes them better fitted than *Sacch. apiculatus* for resisting drought. On the other hand, it is to some extent due to their higher capacity for reproducing in nature in numerous secondary breeding-places, apart from the primary ones (sweet, ripe fruit), the latter, moreover, being located in woods and other places, and not merely restricted to gardens and vineyards. Such secondary breeding-places are formed by the liquid matters of the soil, *i.e.*, organic extracts of animal and vegetable substances, manure, &c. True, the reproduction effected in this way is very small in comparison with that of the primary breeding-places, and

especially so far as *Sacch. apiculatus* is concerned, this species being reproduced under these conditions less extensively than the true *Saccharomycetes*. In addition, the latter will also stand a longer immersion in water than *Sacch. apiculatus*.

Saccharomycetes are found not only on the surface of the ground, but also in the thin layers of soil found above ground on trees, brickwork, stones, &c., where they are protected from drought by a stratum of moss, lichens and algæ. These plants avidly absorb water, and the under layer—which, especially in the case of moss, consists of dead residual matter—readily cedes nutrient substances to the water. In forests of deciduous trees the foliage affords additional protection against drought; but in the open fields different conditions obtain, the *Saccharomycetes* occupying secondary breeding-places there, being exposed to more or less extensive desiccation.

These studies on secondary breeding-places and on the behaviour of the various species towards drought, furnished Hansen with an explanation of the irregularities referred to above. When *Saccharomycetes* cannot be found in the primary breeding-places (sweet, juicy fruit), sun, wind and weather have made their influence felt; and when, on the other hand, they are found abundantly in the open fields, the reason is that they have discovered unusually favourable secondary breeding-places there, and have at the same time been protected from desiccation. The secondary breeding-places are of considerable importance by reason of their extensive distribution. An examination of the behaviour of the species towards temperature also explains in some respects what is taking place in nature, Hansen having found that several species are capable of reproduction when the surrounding temperature is at freezing-point—though under these conditions several months are necessary for the production of a single generation, even when the cells are situated in a favourable nutrient liquid. As a rule reproduction ceases when the temperature falls to 1°–2° C., and a much higher degree is necessary to enable it to proceed with vigour. For this reason the number of yeast cells found in a given spot varies according to the time of year, being greatest in the fruit season, at which season the most favourable conditions are found in respect of temperature, food-supply and moisture. Among the primary breeding-places the soil furnishes its maximum yield in autumn; and it follows from what has been stated above that the secondary breeding-places are active at the same time. Afterwards, the fluctuations in the course of the year are considerable, more especially, as already mentioned, where extensive drying takes place.

The principal result therefore is that the soil is the chief habitat of the *Saccahromycetes* in general (as it is in the case of *Sacch. apiculatus*) all the year round. From this starting-point the cells are transported by the aid of wind, rain, insects and other small

animals, to the primary breeding-places (the sweet, juicy fruits), and thence in turn by the aid of the same factors either to new primary breeding-places, where extensive reproduction occurs, or else to a more modest existence for an inderterminate period in a secondary breeding-place. In the fruit season an important part in the conveyance from one primary breeding-place to another (often over long distances) is played by birds; and, in addition, cells find their way from primary breeding-places to the soil in the excrement of these animals. At present the Hansen theory, especially since his investigations on the secondary breeding-places, affords a natural explanation of all the observations hitherto made.

The importance of these experiments to the practice of brewing consists chiefly in the light they have thrown on the habitats of wild yeasts, and on the way in which these yeasts can find their way into the brewery. Thus it is evident that atmospheric dust at all times of the year may contain cells of true *Saccharomycetes* and also those of wild yeasts. The soil of fruit gardens and vineyards constitutes the chief source of danger, especially during the season of ripe fruit. In the air analyses referred to above it was found that the greatest risk of infection by yeast cells in the brewery is greatest, for Denmark, in the months of August and September. As a rule these cells find their way into the brewery *viâ* the cooler, but they may also enter the fermenting-room direct. Where the conditions allow the cooler to be abolished, this should be done. Nevertheless, the risk of infection has been greatly diminished by the introduction of pure-culture yeast, the more or less enfeebled wild yeasts gaining access to the wort on the cooler being generally suppressed by the pure yeast in the fermenting vessel.

CHAPTER LIV.

VARIABILITY AND HEREDITY IN SACCHAROMYCETES.

§ 273. Temporary Variations.

Both the morphological and physiological characteristics are subject to variation, not only among micro-organisms but also among higher organisms, both vegetable and animal. Just as the study of the *Saccharomycetes* first obtained precision when the discovery of reliable methods of pure culture furnished a sure starting-point for the investigations, so also the era of trustworthy observations on variation dates from the same period.

We are indebted to the researches of Hansen for the foundation of our knowledge of the variations of the *Saccharomycetes*. These researches form two main groups : one comprising such variations as must be considered temporary and dependent on conditions at present unknown, whilst the other relates to variations occurring under known conditions. The experimental researches and the results obtained in connection with this latter group form the most important part of Hansen's labours in this field.

The variations may be classified into temporary and constant from another point of view, temporary variations being those continuing for only a limited time, at the expiration of which they disappear, either spontaneously or under special treatment, whilst the constant variations are those that cannot be restored to their original condition by any treatment.

We shall first discuss the temporary variations, citing different examples. The total number of these variations is naturally enormous.

Hansen's observations in this connection were commenced at the time he introduced pure culture, and they include examples from nearly every branch of the morphology and physiology of the yeast cell, the important being given below. Thus he observed that a yeast from the brewery gave a higher attenuation, defective clarification, a strange flavour, &c., after it had been grown for some time in the laboratory, but regained its original properties on being returned to practical use. This observation has also been made by other workers. Moreover he found (XL.) that when Carlsberg bottom yeast No. 1 was grown on wort gelatin, it

furnished some colonies consisting of oval cells, whilst those in others were elongated and therefore abnormal in shape. Both sets of colonies when grown by themselves produced descendants which retained their characteristic shape for some time, the elongated cells only resuming their normal form after recultivation in wort for a certain period. In the brewery, also, the normal elongated form was retained during several fermentations. The asporogenic form of *Sacch. intermedius* (=*S. Past. II.*) obtained by cultivation at 25° C. on wort gelatin, produced cells which, when grown in wort cultures, furnished vegetations some of which resembled *S. ellipsoideus* and others *S. Pastorianus*, the difference persisting during a large number of cultures, both at the ordinary room temperature and at 25° C.

The clarifying power of beer yeast can be influenced to some extent by the previous method of cultivation (*see also* pp. 157, 188, vol. ii.), as was clearly shown by the experiments of Hansen (XLII.) with Carlsberg bottom yeast Nos. 1 and 2. The cultivation of these two species separately in aerated wort furnished vegetations which clarified satisfactorily in the brewery; but when the same species were grown in unaerated wort, the resulting yeasts did not act normally in practice until they had been put through several fermentations. The No. 1 yeast reverted to its original condition quicker than No. 2, the transitory modification sustained by them both having been greater in the one case than the other. The beer obtained by the fermentation of the unaerated wort was highly opalescent, and, as a rule, little improvement in this respect was effected by prolonged storage, the beer remaining cloudy even after the yeast cells had settled down and the liquid had remained exposed to ordinary room temperature for several days. This was more particularly the case with the beer fermented with the No. 1 yeast. The subject of variation in clarifying power has also been reported on by A. Jörgensen (XII.), who observed that top yeast kept on gelatin clarified more slowly and gave a higher attenuation than when kept in wort. There is no doubt that chemical influences are concerned in this case, as may be concluded from Hansen's observation that *Sacch. Pastorianus I.*, when repeatedly grown for many generations in a solution of saccharose in yeast water at 32° C., loses for a time its property of producing the characteristic disagreeable taste and smell in wort (*see* p. 116, vol. ii.). Continued cultivation in wort, however, soon causes the vegetation to revert to its original state.

These instances of temporary variation may be supplemented by the following, also observed by Hansen (XXXVIII.). The film cells of certain species, and also cells derived from old vegetations grown in saccharose solution, gave, in wort cultures, a loose, curdy deposit, quite different from the ordinary, pasty form; but the original type was restored by repeated cultures in wort. Similar

curdy yeast may be produced when the yeast has been left for some time in a desiccated condition.

In 1886 the same observer (XXXIX.) reported an experiment in which pure-culture bottom yeasts behaved like top yeast, but reverted to the original type after several re-inoculations. It was also found that typical top yeasts can behave like bottom yeasts for several generations, the whole being therefore merely transitory variations. A few instances had also been previously reported (in 1884) by Hansen and Kühle, in which stormy fermentation, with the characteristics of top fermentation, was produced at once in wort by samples of Carlsberg bottom yeast No. 2, which had been kept in the brewery for several weeks, partly in beer, partly in wort, and partly as washed, pressed yeast in an ice-chest. Even in this case, however, the vegetation quickly reverted to the original state. Similar observations have also been recorded in later years, Henneberg (IV.), for instance, having examined at the Berlin Experimental station a typical Dortmund bottom yeast which, after acting satisfactorily for some time in the brewery, at length began to form a head and deposit similar to top-fermentation yeast. Nothing could be ascertained as to the cause of this variation. According to Lindner (XXVIII.), this yeast eventually reverted to its normal state, and was successfully used in practice.

The percentage content of the enzymes present in the cells is also subject to variation, the cause being largely attributable to the method of nutrition. There is, however, no proof available that yeast can lose its capacity of producing enzymes so completely as not to regain it under favourable conditions of growth, nor is there any known instance of a yeast producing, under special treatment, a new enzyme that it previously lacked. The assumption put forward by Dubourg (I.) and other French workers, that, under suitable treatment, a yeast could be induced to form an enzyme that it had not previously produced, has been shown to be totally inaccurate by the experiments of Klöcker (IV.). This statement does not imply the non-existence in nature of species in a state of transition in this respect, but only that all the species hitherto closely examined have proved constant in their behaviour towards sugars (*see* chap. lxv.). Recently it was found by Warschawsky (I.) that *Sacch. cerevisiæ I.* and *Schizosacch. Pombe* produce zymase only when grown on a fermentable nutrient medium, this enzyme not being formed when the medium is unfermentable. He also found that even under the former conditions, *Schizosacch. Pombe* does not produce zymase when the nitrogen in the medium is in the form of ammonium phosphate. On the other hand, zymase is again formed when favourable conditions of cultivation are restored, so that the case is merely one of temporary weakness. Moreover, no one has yet succeeded in depriving an alcohol-forming yeast of that property,

nor has this tendency been found in any case of spontaneous variation.

With regard to the influence of chemical and physical factors on the production of more or less temporary variations, the reader is also referred to chapter xlvi. of the present volume.

§ 274. Hansen's Researches on Asporogenation. The Production of Constant Varieties by Transformation.

Hansen's (XL.) discovery, in 1889, of asporogenation, *i.e.*, the loss of the capacity for producing spores, in *Saccharomycetes*, opened up a new stage in the investigation of variation among these micro-organisms. He observed in the case of *Saccharomycodes Ludwigii* that a number of cells lost the power of forming spores when grown for some time on one and the same nutrient medium, whilst another portion of the cells was considerably weakened in this respect; the remainder, however, remaining unaffected. This variation proved hereditary for some time in wort cultures. The same peculiarity was also observed in other species, *e.g.*, *Sacch. cerevisiæ*, *Sacch. Pastorianus*, *Sacch. intermedius* (= *Sacch. Pastorianus II.*), *Sacch. validus* (= *Sacch. Pastorianus III.*), *Sacch. ellipsoideus I.*, and several bottom-fermentation beer yeasts, when kept on wort gelatin or in wort, a larger or smaller proportion of the cells losing the faculty of producing spores. Beijerinck (XXII. and XXIV.) subsequently found the same behaviour in the case of *Schizosaccharomyces octosporus*. This worker also noted several points of difference between the asporogenic cells and the others, the former producing less trypsin and larger quantities of acid. Another species, which he named *Sacch. orientalis*, also revealed the existence of a relation between sporogenation and proteolysis, inasmuch as the asporogenic colonies in a surface-plate culture did not liquefy the gelatin, whilst the sporogenic cultures did. Moreover, the former cells were quite destitute of glycogen, though the sporogenic cells contained that substance. The loss of sporogenic capacity in *Saccharomycetes* stored in the laboratory is also mentioned by Lindner (XXIX.).

In these cases the variation is partly transitory and partly constant. In certain instances Hansen succeeded in restoring the faculty of sporogenation to asporogenic cells of *Sacch. Ludwigii*, by cultivation in a nutrient medium containing dextrose. In other cases, however, both this and other culture methods proved unavailing, the cells remaining asporogenic. In this connection mention may also be made of Klöcker's observation (III.), that a vegetation of *Sacch. Marxianus*, which produced only a few spores, was considerably strengthened in this respect by cultivation in a medium containing dextrin.

Hansen (XXXVII.), in 1883, showed that spores can stand greater heat than the vegetative cells. This observation was taken by Beijerinck (XXII. and XXIV.) as the starting-point in his endeavours to produce vigorous sporogenation in a culture originally forming only a small number of spores. There is no fixed method for this purpose, the treatment differing with the kind of culture and requiring to be performed tentatively in each case. Beijerinck made the mistake of applying the term regeneration to the result of his experiments, the matter being merely one of selection of the individuals which have not lost their sporogenic power, so that it is incorrect to speak of a lost capacity of the individual.

We will now deal with Hansen's fundamental experiments, in which he produced permanently asporogenic varieties by the action of certain external agencies, in the same year (1889) in which he observed the spontaneous asporogenic varieties of *Sacch. Ludwigii* mentioned above. In the course of his experiments on the limits of temperature for budding and sporulation in *Saccharomycetes*, he observed that the maximum temperature for the former function is always a few degrees higher than that of the latter; whilst the minimum temperature of budding is a few degrees lower than that of sporulation. This law holds good for all true *Saccharomycetes* (*see* pp. 129, 130, vol. ii.).

Hansen tried to ascertain what happens when *Saccharomycetes* are grown at temperatures intermediate between the said two maxima and minima respectively, and whether the result is the same in each case. This latter, however, does not occur, the species undergoing a remarkable change on cultivation at a temperature intermediate between the two maxima, though not in the other event. This change consists in the *Saccharomyces* vegetation completely losing the power of sporulation when cultivated for a number of generations in nutrient liquid at the temperature in question. As might be anticipated, it was also found that the maximum temperature is not exactly the same for all the individuals constituting a vegetation. In experiments for ascertaining the maximum temperature of a species, the result applies to the individuals possessing the highest maximum, though there may be also other individuals present which have a slightly lower maximum temperature. Hence modification experiments may reveal the presence of individuals which apparently can be modified at a temperature below the maximum found for sporulation, whereas in reality these individuals belong to the group exhibiting the lower maximum temperature of the species; and therefore, even in this case, the modification occurs at a temperature intermediate between the maxima for sporulation and budding respectively. Consequently it may be stated, as a general proposition, that the said modification proceeds by cultivation at a temperature bordering on the maximum temperature for sporulation. The

material for these modification experiments is taken from a young and vigorous vegetation, grown in wort under ordinary conditions and at a suitable temperature. A new culture is started with this material in wort, at a temperature between the maxima for sporulation and budding, this temperature varying for different species. After this method of culture has been in progress for a short time, an average portion of the vegetation grown at the high temperature being transferred every day to a new flask of wort at the same temperature, the cultures being shaken up several times a day, asporogenic vegetations are obtained. The number of cultures necessary for producing this result varies according to the species. In this manner Hansen obtained constant asporogenic varieties of all the species that have been proved to belong to the genus *Saccharomyces;* but no modification could be produced by this treatment in the case of the genera *Pichia*, *Willia* and *Saccharomycodes*.

In order to elucidate the progress of this modification, Hansen (XLIV.) afterwards carried out special investigations, the chief result of which may be summed up as follows: The material consisted always of a single cell of the species in question; in some cases a vegetative cell, in others a spore, the material throughout being thoroughly capable of sporulation, and in which the strictest examination failed to reveal a single asporogenic cell. To facilitate the examination of the conditions of sporulation throughout the treatment, plate cultures were prepared by transferring the cells to the surface of wort gelatin by means of a platinum stylus. The mature colonies, when of sufficient size, were transferred direct on to moist gypsum blocks for sporulation, those too small for this treatment being first placed in wort and the sedimental yeast therefrom transferred to the gypsum. The principal experiments were made partly with *Sacch. Past. I.* at 32° C., and partly with Johannisberg 2 wine yeast at 36° C. The following table gives an example of the results obtained from the former yeast during the various stages of the treatment.

In stage	2		1	per cent.	of constantly	asporogenic	cells was found.
,,	4	60	,,	,,	,,	,,	
,,	7	100	,,	,,	,,	,,	

Each stage represents twenty-four hours.

To settle the fundamental question whether this formation of asporogenic varieties is due to selection on transformation, Hansen instituted further investigations with Johannisberg 2 yeast. In the normal vegetation it was absolutely impossible to find a single cell which did not produce sporogenic vegetations when grown under normal conditions. The vegetation employed for starting the experiment was analysed by isolating at least 1000 cells and testing the resulting vegetation for sporulation, an abundance of spores being found in every case. The experiments also showed that the

intermediate, temporarily asporogenic forms appeared as soon as the treatment commenced; and since they were never found in the original material, their origin must therefore be attributed to the treatment applied. Finally, the variation in question is a universal phenomenon, appearing in all cases when the cells are subjected to the treatment described. The results obtained, both from the analysis of the original material and that of the various stages of the treatment, indicate clearly that the variation produced by the treatment is due to transformation or modification. It has been urged against this view that, since all the cells are of equal value, they must all undergo transformation at the same time if the process is really one of modification. This, however, is incorrect, since the cells are far from being of equal value, their condition at the moment of commencing the treatment differing considerably in respect of age, nutrition, &c., and therefore the transformation cannot proceed simultaneously with all the individuals present.

A highly characteristic feature, which was also tested experimentally by Hansen, is that, even when the initial material consists of a single vegetative cell or spore, the following three classes: sporogenic cells, temporarily asporogenic cells, and constantly asporogenic cells, appear during the treatment. From the two former classes it is possible to take single cells which in turn produce members of all three classes. This also proves the change is due to modification, inasmuch as these intermediate forms, which revert to the sporogenic form when excluded from the treatment, become constantly asporogenic only after treatment for a considerable time.

With regard to the conditions necessary for the modification, it might be thought that the chemical composition of the nutrient medium, the vibration produced by shaking the flask, the aeration of the medium, and the temperature would constitute influential factors. The experiments, however, showed that neither vibration nor a medium of definite chemical composition is essential; and that aeration is incapable of bringing about the change in the absence of the high temperature. The nutrient liquid, vibration, and aeration exercise an indirect influence, inasmuch as they more or less facilitate reproduction, but the high temperature forms the most important and absolutely indispensable factor.

In the foregoing experiments Hansen used nutrient liquids for the cultures, but he also tried solid media. In the latter case several species produced constantly asporogenic cells when allowed to remain on wort gelatin at 25° C., or at ordinary temperature, and it may be assumed that chemical factors were here in operation. When grown at 32° and 34° C. on wort-agar gelatin under the same conditions as in the experiments with liquid media, *i.e.*, repeated re-inoculations at short intervals, *Sacch. Pastorianus*

produced constantly asporogenic cells, which was, however, not the case when the culture was left undisturbed. Here, again, the high temperature shows itself the modifying factor.

The oldest asporogenic varieties obtained from the different species have now been in existence for more than sixteen years, and have remained constantly asporogenic although repeatedly cultivated under highly divergent conditions.

It is found to be the rule that loss of sporogenic power is accompanied by loss of the capacity for producing film growths. In some species the variety has been found to possess a greater reproductive capacity than the original form, and possibly this applies to all. The asporogenic varieties also exhibit considerable fluctuations in respect of the production of alcohol. Since, as mentioned on p. 126, vol. ii., the film cells of *Saccharomycetes* are able to decompose alcohol into carbon dioxide and water, a power not shared by the sedimental yeast cells (at least so long as the liquid is of sufficient depth), the quantity of alcohol formed in wort fermented by an asporogenic (and therefore filmless) variety does not become appreciably less when left to stand in a flask (*e.g.*, Pasteur flask) precluding evaporation.

In addition to the instance of an accidentally produced constant variation observed with *Saccharomycodes Ludwigii* (p. 260, vol. ii.), mention may be made of Lepeschkin's (I.) observation of the formation of mycelium by *Schizosaccharomyces Pombe* and *Schiz. mellacei*. No particulars are given by this worker respecting the conditions under which this result was obtained, but he states that the phenomenon remained constant during numerous generations and that it was found impossible to secure reversion to the original form of single cells.

§ 275. Hansen's Experiments with Top and Bottom Yeast.

Special interest attaches to Hansen's (XLV.) latest researches into variations in fermentative habit, namely, the appearance of top-fermentation yeast cells in a typical bottom yeast, and *vice versâ*. As mentioned on p. 260, vol. ii., he had previously observed the faculty of certain bottom yeasts for temporarily producing top-fermentation phenomena after storage at a low temperature. In this connection he instituted some very comprehensive researches with *Sacch. turbidans* (= *Sacch. ellips. II.*), a trace of a vigorous young vegetation being transferred to Freudenreich flasks charged with a thin stratum of wort and kept at 0.5° C. At the end of three and five months the cultures were examined, by sowing an average sample in wort contained in test-glasses. In every instance the fermentation phenomena were decidedly those of top fermentation, so that all or most of the cells had acquired a top-fermentation habit. Test-glasses were used because of the necessity for

employing a deep layer of wort in making comparative observations on top- and bottom-fermentation phenomena. One test performed with 150 cells showed that not a single bottom-yeast cell was present. In order to solve the problem whether the low temperature had produced modification, the vegetation used for the culture at 0.5° C. was subjected to analysis, the result being that, of 100 cells, one-half gave top fermentation, the other bottom fermentation. On a series of flasks, charged with a thin stratum of wort, being inoculated with cells from each category and kept for 3–4 months at 0.5° C., it was found that no reproduction occurred in the flasks containing the bottom-fermentation cells, whereas, on the contrary, the top-fermentation cells exhibited decided reproduction. The cultures treated in this way were next grown in test-tubes, with the result that the bottom cells again gave rise to bottom fermentation, and the top cells to top fermentation, thus demonstrating that no modification, but only selection, had been effected by the experiment. When Hansen described *Sacch. turbidans* in 1883, it was a bottom yeast; and the formation of top cells—the cause of which is unknown—occurred spontaneously during the period of storage in the laboratory. In the course of a year the bottom cells and top cells continued to behave as such respectively through a long series of cultures; and 1000 cells isolated from each class all produced the same type of fermentation as that of the class from which they originated.

Experiments with the typical bottom yeast, Johannisberg 2, showed that the cultures not infrequently contain 70 per cent. of top-yeast cells, the isolated cells in this case also retaining their characteristic fermentative habit through a long series of cultures.

Whilst in the cases cited above a transition occurred from bottom fermentation to top fermentation, the converse change seems more difficult to bring about. In this connection Hansen carried on several experiments with *Sacch. validus* (= *S. Past. III.*), which is certainly a typical top yeast, but he only succeeded in obtaining a few bottom cells—not exceeding 3 per cent.—in one of the cultures. The vegetations from these cells retained their character as bottom yeast through a series of generations, extending over two years, and under conditions favourable to the production of top fermentation phenomena.

In this manner the old question whether the top- and bottom-fermentation yeasts are independent forms or not has been settled by the demonstration that bottom cells can be developed from top cells, and *vice versâ*. This essentially modifies our previous conceptions (*see* p. 124, vol. ii.). The two forms into which the species is subdivided may exist for a long time, side by side in the same nutrient medium, until the growth of one of them is favoured by the environment, as was the case in the experiments with *Sacch. turbidans* at 0·5° C., where the top-fermentation form

increased at the expense of the bottom-cell form until the latter was entirely suppressed.

In contrast with the asporogenic varieties which, as we have already seen, are modifications produced by the influence of a known external factor, high temperature, the appearance of top-yeast cells in a typical bottom yeast must be relegated to the category of variations to which the name mutation was given by de Vries, and comprising all sudden variations due to unknown causes. In most cases the properties of these mutation varieties are hereditary, as we have already learnt in the case of the temporary variations. Thus, the variations in the cell form, the shape and size of the spores, and also the production of mycelium observed by Lepeschkin, must be classed as mutations. The great difference existing between a transformation and a mutation is that the former is produced gradually, the latter suddenly. Both may form the starting-point of new species or races. Throughout the entire vegetable kingdom only very few instances are known where a new variety, capable of transmitting its newly acquired properties permanently to its offspring, has resulted from a transformation brought about by external influences; in fact Hansen's researches on asporogenic varieties constitute the sole experiment performed in this connection.

§ 276. Practical Results of the Researches on Variation. Occurrence in Brewing Practice.

Before bringing this chapter to a close we will examine the practical bearing that the results of the foregoing investigations have on brewing, and also consider the occurrence of variations of yeast type in practice.

By employing an asporogenic variety of pitching yeast in the brewery, the detection of wild yeasts by spore analysis is simplified. It will be remembered (pp. 135, 136, vol. ii.) that this method of examination is based on the fact that, at a certain temperature, sporulation occurs sooner in wild yeasts than in culture yeasts. When, however, an asporogenic yeast is used for pitching, the mere presence of asporogenic *Saccharomyces* cells of any kind will suffice to reveal an extraneous yeast. The fact that an asporogenic yeast will produce just as good beer as the original form has been demonstrated by Hansen, who obtained a good normal beer with an asporogenic variety obtained from Carlsberg bottom yeast No. 2 by the treatment already described. It must not, however, be forgotten that in many instances the behaviour of the asporogenic variety in practice will differ appreciably from that of the original yeast from which it was produced.

Will (XXVIII.) has reported an abnormal fermentation phenomena resulting from the presence of film cells or their descendants in the pitching yeast; and A. Jörgensen (II.) has

stated that film cells may produce a disagreeable flavour. The production of films, and therefore also their disturbing influence, may be avoided by the preparation of an asporogenic variety, which, as has already been shown, is incapable of film formation (*see* p. 127, vol. ii.).

The production of varieties with an increased or diminished power of producing alcohol is also of importance in practice. Hansen (XLIII.) carried out experiments on this point, and, by cultivating Carlsberg bottom yeast No. 1 in eight successive cultures at 32° C. without aeration, obtained a variety which gave 1–2 per cent. of alcohol (by volume) less in wort containing 10 per cent. of saccharose than the standard No. 1 yeast, whilst at the same time it clarified the beer better. He also obtained a variety with increased powers of alcohol production by growing the same species, Carlsberg bottom yeast No. 1, for several months on wort gelatin with frequent renewal of the medium, whereas 13 per cent. (by volume) of alcohol was furnished by a vegetation of the same original stock when grown in wort, under equal conditions as regards time and renewal of the cultures; finally, in a wort containing 25 per cent. of saccharose, the variety obtained by growing in wort gelatin produced 13.6 per cent. of alcohol from the same final medium. By cultivating the spores of another culture yeast, *Sacch. cerevisiæ*, on yeast-water gelatin, he also obtained a variety furnishing more alcohol than the original stock yeast, the increased production in this case being 3 per cent. by volume, as compared with that given by the stock yeast grown in wort throughout. According to Hansen the matter is one of selection rather than modification; but nothing more definite can yet be expressed on the point. It may, however, be mentioned that considerable differences in fermentative capacity are exhibited by the individual cells of one and the same species in pure culture in wort, even when grown under identical conditions; and this applies also to their clarifying power.

The variety obtained by Hansen from Carlsberg bottom yeast No. 1, by the method employed for producing asporogenic varieties, was characterised by diminished attenuation and gave a beer of greater palate fulness than the original stock; but it exhibited the defect of being too slow in action.

There is evidently a wide field open for the practical application of varieties of yeast obtained by treating the original stocks in certain ways on the lines indicated above; and important results are undoubtedly obtainable by continuing these researches. We will now deal briefly with the occurrence of variations in practice, premising that lucid experimental investigations in this connection are still lacking. As already stated, such variations have been observed from the time pure-culture yeast was introduced into practice, but there is little use going into details, since all that is known is based on more or less uncertain observation. Reports

from practical sources, on the variation known as degeneration in pitching yeast, will be found in the columns of the technical press for some years past; and we will now merely cite two communications relating to injurious variations. One cause of yeast degeneration is ascribed by Hayduck (VI.) to the enrichment of the yeast with nitrogen, and he recommends, as a means of regeneration, that the yeast should be allowed to ferment a solution of saccharose before pitching. In the case of a yeast which suddenly began to clarify badly, Seyffert (II.) found that the addition of gypsum to the brewing liquor (well water) restored matters to their normal condition. Sudden disagreeable changes with regard to smell and flavour may also arise in practice, the cause being generally attributed to cultivation at abnormally high temperature, excessive rousing of the wort, &c.; and Will (XXIX.) states that boiling the wort too long in the steriliser may also influence the activity of the yeast. In short, the yeast may be affected by any unusual conditions in brewing; and in this category should be included the experiments of Biernacki, Effront, Hayduck, Heinzelmann and Schulz, with chemical stimulants (*see* vol. i. p. 108). In addition to Hansen, observations on the variation of pitching yeast in practice have also been published by Delbrück, A. Jörgensen, Kukla and Will.

Proof of the temporary character of the variations caused in practice by the influence of the conditions prevailing there is afforded by the fact that the pure-culture system has not only obtained a solid footing in breweries throughout the world, but is also gaining ground daily in other fermentation industries. Certain culture yeasts are particularly constant, others again show a tendency towards variation. Carlsberg bottom yeast No. 1 belongs to the former class, a pure culture of this yeast having retained its character, apart from temporary fluctuations, for more than five years in the fermentation cylinder of the pure-culture apparatus at the New Carlsberg brewery. Various authors have reported on special constancy in culture yeasts, the researches of Irmisch, A. Jörgensen and P. Lindner being worthy of note in this connection.

As we have seen, the practical application of the pure-culture system consists not merely in the preparation of pure cultures of a given species or race, but also in a selection of the vegetations furnished by individual cells. In this way the introduction of pure cultures in the brewery is accompanied by an attempt at race improvement, the requirements being confined not merely to the preservation, by the race or species, of all its properties that are of value to the brewery, but extending to the selection of individuals exhibiting variations of special value for the brewery in question —that is to say, possessing the good properties in an increased degree and with the undesirable qualities eliminated. Of course these results cannot be more than partially accomplished even in

the most favourable circumstances. The race improvement in such cases consists in a repeated selection of the best individuals; but the results of the experiments made by Hansen and others in this direction show that it is impossible to lay down any definite rules, tentative experiments being essential. In some cases disappointment will follow, the results failing to come out as desired, owing to uncontrollable circumstances. The matter is entirely different from the mere production of asporogenic races and the like, where the conditions are known and under control. Finally, it must be borne in mind that when the material for the experiments in race improvement are taking from the contents of the fermentation vessel in the brewery, one cannot be certain that any genetic connection exists between the race so taken and the original pitching yeast, for they are not necessarily descended from one and the same ancestor even though exhibiting the same botanical characteristics.

Similar communications on race improvements have also been published in connection with wine yeasts, though again without any definite statement of method; and indeed some of these reports, from practical sources, even fail to mention the species of yeast originally employed.

CHAPTER LV.

CLASSIFICATION OF THE FAMILIES SACCHAROMYCETACEÆ AND SCHIZOSACCHAROMYCETACEÆ.

§ 277. Introduction. Division of the Family, Saccharomycetaceæ.

The fungi to be classified in the present chapter comprise two families, the *Saccharomycetaceæ* and the *Schizosaccharomycetaceæ*, though they were formerly grouped together as a single family under the former title. According to the principles of classification established for the *Saccharomycetaceæ* by E. C. Hansen (XLIX.) in 1904, however, the *Schizosaccharomycetaceæ* form a separate family, and are therefore dealt with by themselves later on (§ 281).[1]

As already explained on pp. 100, 101, vol. ii., the *Saccharomycetaceæ*, which form the subject of §§ 277–280, belong to the *Ascomycetes* class, of which they constitute the lowest family. On the other hand, the position of the *Schizosaccharomycetaceæ* in the botanical system cannot yet be definitely fixed. They appear to form an intermediate link between the *Ascomycetes* and the *Schizomycetes;* but, for practical reasons, they are ranged in this chapter beside the *Saccharomycetaceæ*.

Before proceeding to a systematic description of the species belonging to the two families in question, we will glance briefly at the earlier attempts at classification, and then state the principles which have been utilised in the present rearrangement.

It is difficult to find in any other department of botany greater confusion than existed in the classification of the *Saccharomycetes*, chiefly on account of the fact that so many workers unacquainted with botany have been engaged in the investigation of fermentative organisms.

The first worker to establish endosporulation as the characteristic feature of the genus *Saccharomyces* was Reess (*see* p. 108,

[1] On p. 101, vol. ii., the species belonging to these two families were treated as one family, according to the state of knowledge at the time (1901), and for the same reason only three genera, *Monospora*, *Saccharomyces* and *Schizosaccharomyces* were mentioned. The classification is now amended in the light of recent research, and hence the divergence from the statements made on the pages mentioned.

vol. ii.), who described the following seven species of this genus: *Sacch. cerevisiæ*, Meyen (*see* p. 114, vol. ii.); *Sacch. ellipsoideus*, Reess (*see* p. 114, vol. ii.); *Sacch. conglomeratus*, Reess; *Sacch. exiguus*, Reess; *Sacch. Pastorianus*, Reess (*see* p. 116, vol. ii.); *Sacch. mycoderma*, Reess; and *Sacch. apiculatus*, Reess. Of these, however, only one, viz., *Sacch. apiculatus*, has been since identified with certainty. Reess did not act consistently in this matter, since, as he himself pointed out, this species does not produce endospores, and therefore should not have been placed with the *Saccharomycetes*, which he expressly declared to be characterised by endosporulation. He describes this budding fungus (for which *see* chap. lxi.) as consisting of "lemon-shaped cells produced by budding, and provided with short apices at each pole; average width 2–3 μ, length 6–8 μ; sometimes elongated as short filaments. New buds are formed solely at the apices of the parent cells, and usually detach themselves at once, rarely remaining joined, in short lengths or branching. Ascosporulation not detected with certainty, and assignment to the *Saccharomycetes* consequently doubtful."

With regard to *Sacch. mycoderma*, Reess probably based his description on a mixture of *Mycoderma cerevisiæ* or *M. vini* and a species of *Pichia*, since he expressly states that the species produces spores, which (*see* chap. lx.) the *Mycoderma* do not. The five remaining species mentioned by Reess were characterised almost exclusively from the form of their cells, thus rendering their identification impossible.

Most of his contemporaries followed Reess, except C. O. Harz, who rejected all Reess's species but *Sacch. mycoderma*, on the ground that they were only different forms of beer yeast due to altered nutrition.

Hansen's researches on classification are closely interwoven with his work on the biological and physiological sides, and he proceeded consistently from the outset with the assumption that only such yeasts as produce endospores can belong to the *Saccharomycetes*. This conception, the correctness of which was demonstrated in the course of the investigations, was generally accepted and adopted with but few exceptions, chiefly physicians who followed Schlendrian and called all yeasts *Saccharomyces*, whether they form spores or not. A few other workers also took the same view, Saccardo (II.), for instance, continuing in 1889 to confound *Saccharomyces* and non-*Saccharomyces*, a plan also followed by J. Schroeter in his work on the Cryptogam Flora of Silesia (1893).

The characteristics established as the basis of classification by Hansen, and employed in the present work, may now be briefly described. Among the morphological characteristics he assigns an inferior position to cell form, owing to the extent to which this is affected by external influences, most species exhibiting a

large number of cell forms (*see* p. 116, vol. ii.). In fact, it is only under definite conditions of culture that the cell form can be utilised as a characteristic of species. Unfortunately, there are still botanists who call all large rounded cells *Sacch. cerevisiæ*, all small oval ones *Sacch. ellipsoideus*, and all elongated cells *Sacch. Pastorianus*, and thus keep to the same standpoint as Reess. The shape of the spores and the production of films are important generic characteristics, and in some cases of species as well. Physiological characteristics are of great importance in classification, especially the critical temperatures of budding, film production, and sporulation (*see* table opposite p. 136, vol. ii.); and also the behaviour of the species toward different sugars (*see* chaps. lxiv. and lxv.), large quantities of yeast and pure sugars being essential for this purpose. In this instance a macroscopical examination is necessary, microscopical tests not affording sufficient accuracy. The yeast is sown in yeast-water containing 5 to 15 per cent. of the sugar in question, and the production of alcohol is tested for. Finally, Hansen employed as a means of differentiation the macroscopic appearance of the vegetations on different solid nutrient media. His methods differ essentially from those of Reess by being entirely of an experimental character, and it follows therefore that the value of the results for purposes of comparison depends on the experiments being carried out under identical conditions.

Lindner employs the appearance of the giant colonies as a specific characteristic; and Will has also done good work in the study of these forms.

The fermentative habit (as top or bottom yeast) has no longer the same importance as a means of classification that it formerly enjoyed, on account of Hansen's recent investigations in this connection (*see* p. 264, vol. ii.).

Even many of the species put forward as new during the past few years are described in such an imperfect manner that they cannot be included in our classification, the reason in many cases being that the newly discovered species have shown the description of the older ones to be insufficient. An example of this kind is afforded by H. Lindner's so-called *Sacch. hyalosporus*, which is characterised by the production of bead spores. This peculiarity, however, is shared by several other species, and consequently a more complete description is necessary before the species can be identified. Other workers, again, have named species without describing them; and these we are therefore compelled to omit, confining our list to such species that have been so fully described as to render identification feasible. Unless specific mention is made to the contrary, the description in each case is that furnished by the discoverer of the species. The source of information is quoted in each case.

A few preliminary notes of explanation will facilitate due

comprehension of our subjoined *résumé* of the division of the *Saccharomycetaceæ* into genera in accordance with the principles established by Hansen (XLIX.) in 1904. Previous to his researches on the fungi in question, nothing had been done beyond the establishment of the *Saccharomycetes* as a separate genus, the division into species being of an unreliable nature; and Hansen was the first to place these investigations on an experimental basis. Examination of the various species discovered in the course of years then revealed the desirability and possibility of elevating the existing genus *Saccharomyces* to the dignity of a family (*Saccharomycetaceæ*), which Hansen divided into eight genera. In the case of two of these, viz., *Monospora* and *Nematospora*, which are briefly described in § 280, some doubt exists as to whether they really belong to the *Saccharomycetaceæ*. The remaining six genera, which, on the other hand, are recognised as true *Saccharomycetaceæ*, can be separated into two main groups.

The first principal group differs from the second, inasmuch as sowings in nutrient liquids furnish sedimental yeast exclusively at the outset, the production of films occurring only at a much later period, if at all. The film is more or less strongly mucinous, the only exception being *Saccharomyces capsularius*, which gives a film resembling that of *Oidium*. It is probable that more accurate observation will reveal the presence of isolated islands of yeast (*see* pp. 120, 121, vol. ii.), even in those species at present considered to lack the power of producing films. The endospores of the species belonging to this first group are globular, oval or reniform, smooth and provided with one or two membranes. The spores germinate either by gemmation or the production of a promycelium. The great majority of the species incite alcoholic fermentation. Hansen divides this group into four genera, one being the newly defined genus *Saccharomyces*, described in § 278, whilst the other three are called *Zygosaccharomyces*, *Saccharomycodes*, and *Saccharomycopsis*, and are dealt with in § 279. It need only be mentioned that the genus *Saccharomycodes* was established for the organism previously known as *Saccharomyces Ludwigii*, and a similar species described by Behrens. In its new form, Hansen's genus *Saccharomyces* comprises a large number of species, and is divided into six sub-groups based on the behaviour of the species towards sugars. The hitherto imperfectly characterised genera *Hansenia* and *Torulaspora* are referred to briefly at the end of § 278. The nomenclature of the species included in the newly defined species *Saccharomyces* has been altered considerably, a number of names, hitherto current in Mycology and also used in nearly all the previous chapters of the present work, having been replaced by new ones. Thus, for example, the organism previously known as *Sacch. cerevisiæ I.*, Hansen, is shortened in the new classification to *Sacch. cerevisiæ*; *Sacch. Pastorianus I.* becomes *Sacch. Pastorianus*; *Sacch. Pastorianus III.* is changed to *Sacch. validus*, and so on.

Further particulars on this synonym are given at the beginning of the description of the individual species.

The second principal group of the true *Saccharomycetaceæ* is composed of the genera *Pichia* and *Willia*, and is characterised by the production of a film on the surface of the nutrient solution immediately after the same has been inoculated. Hansen had already discovered a representative of each of these new genera, and at that time called them *Sacch. membranæfaciens* and *Sacch. anomalus* respectively. Similar species were afterwards discovered and described by other workers, Pichi, for instance, identifying some which may be ranked with *Sacch. membranæfaciens*, whilst Will and his pupils found others of the type of *Sacch. anomalus*. Consequently, Hansen named his new genera *Pichia* and *Willia*, in honour of these workers.

In conclusion we give the following

Analytical Summary of the Genera of the Saccharomycetaceæ Family.

The *Saccharomycetaceæ* exhibit the following general characteristics: Monocellular, sporogenic budding fungi. Typical mycelium is formed only by a few species, but all produce yeast cells abundantly. Each cell is a potential sporogenic cell. The spores are monocellular. The number of spores in each parent cell (Ascus) is usually 1–4, seldom as high as 12.

(1) Spores oval, round, pileate or lemon-shaped, with or without projecting rim, *see* 2.

Spores acicular or spindle-shaped, *see* 7.

The cells form sedimental yeast immediately in saccharine nutrient liquids, films being produced only later (if at all), *see* 3.

(2) The cells produce a film at once on the surface of saccharine nutrient liquids; the film appears dry, owing to included air bubbles, *see* 6.

(3) Spore with single membrane, *see* 4.

Spore with two membranes. . . . . *Saccharomycopsis.*

The cells fuse together. . . . . *Zygosaccharomyces.*

(4) No fusion of the cells occurs, *see* 5.

The spores germinate by ordinary gemmation . *Saccharomyces.*

(5) A promycelium is developed in the germination of the spores, and from this budding proceeds with incomplete separation . . . . . *Saccharomycodes.*

(6) Spores round or hemispherical, or irregular and angular. No fermentation . . . . . *Pichia.*

Spores pileate or lemon-shaped with projecting rim *Willia.*

(7) Spores acicular. Parasitic on water-fleas . *Monospora.*

Spores spindle-shaped, almost filamentous, with a long flagellum; parasitic on hazel-nuts . . . *Nematospora.*

§ 278.—The Genus Saccharomyces, with the Genera Hansenia and Torulaspora.

The cells of the species belonging to the genus *Saccharomyces* (E. C. Hansen) produce simple membrane spores which gemmate

by germination. In addition to yeast cells a few of them produce a membrane with well-defined septæ.

The first sub-group of this genus comprises the species capable of fermenting dextrose, saccharose and maltose, but not lactose. It includes the following species:

Saccharomyces cerevisiæ, E. C. Hansen. Synonyms: *Sacch. cerevisiæ I.*, E. C. Hansen (XII., XVI., XLVI. and XLVIII.) = *Sacch. cerevisiæ*, E. C. Hansen (XLIX.) = *Sacch. cerevisiæ* (partim), Mayer (I.) = *Torula cerevisiæ* (partim), Turpin (I.) = *Cryptococcus fermentum* (partim), Kützing (I.) = *Hormiscium cerevisiæ* (partim), Bail (III.) = *Sacch. cerevisiæ* (partim), Reess (I.) This species has been drawn by Hansen (XII., XVI., XVII. and XXXII.), also in Figs. 127, 142, 144, and 145 of the present work. The cells of the sedimental yeast are usually large and round; and those of the film vegetation at 6°–15° C. are mostly of the same kind, with but few exceptions. The limits of the budding temperature in wort are 40° C. and 1°–3° C. The dimensions of the spores vary between 2.5 and 6 μ, the number in each cell being usually 1–4, rarely 5. The limits of sporulation temperature on gypsum blocks are 37°–37.5° C., and 9°–11° C., the optimum temperature being 30° C. For the production of films on wort these limits are 33°–34° C. and 6°–7° C. The species generally appears as a powerful top-fermentation beer yeast, and was isolated by Hansen (I.) from the pitching yeast of an Edinburgh brewery. Subsequently the same worker detected it in a London brewery. It is one of the many forms previously grouped under the name *Sacch. cerevisiæ*.

Only a small number of the races and species utilised in the brewing industry have been described in the literature, and even then without systematic names, being generally called after the locality or the owner of the brewery where they were discovered, or again bearing merely the number with which they were labelled in the collection (herbarium) of the investigator. The following six may be cited as examples:

Carlsberg bottom yeast, No. 1, E. C. Hansen. One of Hansen's (XLIV.) drawings is reproduced in Fig. 130. The cells are usually oval or pointed. Spores are produced with the greatest difficulty, being found in very small number even after a considerable time (5–6 days at 25° C.). In the brewery (*see* p. 187, vol. ii.) this yeast gives imperfect clarification, but high attenuation, and the beer is excellent, with good keeping qualities.

Carlsberg bottom yeast, No. 2, E. C. Hansen. The cells, which are illustrated in Fig. 131, after a drawing by Hansen, are more uniform in shape than the preceding species, and also produce spores rather more readily. The beer obtained with this yeast does not keep so well, but clarifies better.

Stock 2, *H. Will* (XXX.). Will's drawing of this species is reproduced in Fig. 139. The cells are round or oval. The limits

of sporulation temperature on gypsum blocks are 31° C. and 11° C., the optimum temperature being 25°–26° C. For the production of films on wort these limits are 28°–31° C. and 7°–10° C. This species is a high-attenuation bottom yeast.

Stock 6, *H. Will* (XXX.). The cells are round or oval. Limits of sporulation temperature on gypsum blocks, 31° C. and 11° C., optimum 28° C. For the production of films on wort the limits are 25°–31° C. and 7°–10° C. A bottom yeast with medium attenuation.

Stock 7, *H. Will* (XXX.). Cells round or oval; giant cells of regular occurrence. Limits of sporulation temperature on gypsum blocks, 30° C. and 13° C., optimum 25°–26° C.; for the production of films on wort the limits are 25°–28° C. and 4°–7° C. The species is a low-attenuation bottom yeast.

Stock 93, *H. Will* (XXX.), is illustrated, from a drawing by Will, in Fig. 137. Cells round or oval. Limits of sporulation temperature on gypsum blocks, 30° C. and 10° C., optimum 28° C. Limits of temperature for the production of films on wort, 30°–31° C. and 4°–7° C. A high-attenuation bottom yeast.

Although imperfectly described from the standpoint of botanical classification, mention may be made of three other beer yeasts, which bulk largely in discussions between fermentation technologists, and in treatises by fermentation physiologists, and are also mentioned frequently in the present Handbook, namely, *Saaz yeast*, *Frohberg yeast*, and *Logos yeast*. The former two were isolated by Lindner (XXXI.) at the Institute for Fermentation Industries, Berlin: one for the pitching yeast used at the municipal brewery in Saaz (Bohemia), the other from the yeast from Frohberg's brewery at Grimma (Saxony). Both have been carefully investigated by Delbrück (IX.), Irmisch (II.), Lindner (XXXI.), Reinke (IV.), and others. Logos yeast was isolated by H. van Laer and Denamur (I.) from the pitching yeast employed at Logos and Co.'s brewery in Rio de Janeiro (Brazil). Its origin is unknown, but was probably the sugar-cane. On chemico-physiological grounds A. Bau (VI.) proposed to divide the old collective name *Sacch. cerevisiæ* into four types: *Sacch. cerevisiæ Frohberg*, top fermentation; *Sacch. cerevisiæ Saaz*, top fermentation; *Sacch. cerevisiæ Saaz*, bottom fermentation; and *Sacch. cerevisiæ Frohberg*, bottom fermentation. In this manner the names Saaz and Frohberg originally applied to two different species of yeast are used to denote types. Various other top-fermentation beer yeasts have also been described by H. van Laer, A Jörgensen, Greg, &c.

The top yeasts also include the well-known distillery yeasts Race XI. and Race XII. (the latter also cultivated in the manufacture of pressed yeast), both of which were isolated at the Institute of Fermentation Industries, Berlin. Compare p. 113, vol. ii., and Henneberg (I.).

Saccharomyces Pastorianus, E. C. Hansen. Synonyms: *Sacch. Pastorianus I.*, E. C. Hansen (XII., XVI., XLVI., and XLVIII.) = *Sacch. Pastorianus*, E. C. Hansen (XLIX.) = *Sacch. Pastorianus* (partim), Reess (I.). This species was illustrated by Hansen (XII. and XVI.); *see* Fig. 129. The vegetation in wort consists chiefly of sausage-shaped cells, though round and oval cells are also present. The limits of budding temperature in wort are 34° C. and 0.5° C. The spores measure 1.5–3.5 μ in diameter, their dimensions seldom reaching 5 μ. Most frequently they number 1–4, but occasionally, in very long cells, 5–10. The limits of sporulation temperature on gypsum blocks lie between 29.5°–31.5° C., and 0.5°–4° C. (optimum 27.5° C.); and the same limits in respect of film formation on worts are 26°–28° C. and 3°–5° C. The species is a bottom yeast, and was first discovered in the atmospheric dust in a Copenhagen brewery, and afterwards in damaged beer. It is a dangerous pest in the brewery, being capable of imparting a disagreeable smell and strongly bitter taste to the beer (*see* p. 116, vol. ii.). As a rule it also retards clarification. On the other hand, according to Mach and Portele (III.), it produces good wines.

Saccharomyces intermedius, E. C. Hansen. Synonyms: *Sacch. Pastorianus II.*, E. C. Hansen (XII., XVI., XLVI., XLVIII.) = *Sacch. intermedius*, E. C. Hansen (XLIX.) = *Sacch. Pastorianus* (partim), Reess (I.). This species has been drawn by Hansen (XII. and XVI.), and is illustrated in Figs. 133 and 136. The cells are of the same form as those of the preceding species, but rather larger. Sporulation occurs in wort between the limits of 40° and 0.5° C., and the spores generally measure 2–5 μ, less frequently 4–5 μ. The limits of sporulation temperature on gypsum blocks are 27°–29° C. and 0.5°–4° C., the optimum being 25° C. In the case of film formation on wort, these limits are 26°–28° C. and 3°–5° C. The cells of the young film, at 13°–15° C., differ from the corresponding cells of the next species in being round or oval, whereas under the same conditions many of the cells of *Sacch. validus* are sausage-shaped. At the end of sixteen days the streak cultures of this species on yeast-water gelatin at 15° C. exhibit smooth edges, in which respect again they differ from *Sacch. validus*. The species is a weak top yeast, and was discovered in the air of a brewery in Copenhagen.

Saccharomyces validus, E. C. Hansen. Synonyms: *Sacch. Pastorianus III.*, E. C. Hansen (XII., XVI., XLVI., and XLVIII.) = *Sacch. validus*, E. C. Hansen (XLIX) = *Sacch. Pastorianus* (partim), Reess (I.). The cells have been illustrated by Hansen (XII. and XVI.), and also in Figs. 134 and 135. The cells grown in wort have the same shape as the two foregoing species, and their limits of budding temperature in that medium are 39°–40° C. and 0.5° C. The spores measure 2–4 μ in diameter, rarely 3.5–4 μ. Limits of sporulation temperature on gypsum blocks, 27°–29° C.

and 4°–8.5° C.; optimum, 25° C. Temperature limits of film formation on wort, 26°–28° C. and 3°–5° C. The cells of the young film grown at 13°–15° C. differ from the corresponding cells of *Sacch. intermedius*, inasmuch as many of them are very long and sausage-shaped; those of the last-named species being, on the other hand, frequently round or oval. The streak cultures on yeast-water gelatin at 15° C. differ, at the end of sixteen days, from those of the preceding species in being decidedly bearded at the edges. The species is usually a top yeast, and is injurious to beer, in which it produces yeasty haze (*see* p. 122, vol. ii.). Under certain conditions, however, a small addition of this species to the pitching yeast may clarify opalescent beer, probably by eliminating, in secondary fermentation, the substances causing the opalescence. The species was discovered in bottom-fermentation Copenhagen beer suffering from yeasty haze.

Saccharomyces ellipsoideus, E. C. Hansen. Synonyms: *Sacch. ellipsoideus I.*, E. C. Hansen (XII., XVI., XLVI., and XLVIII.) = *Sacch. ellipsoideus*, E. C. Hansen (IX.) = *Sacch. ellipsoideus* (partim), Reess (I.) The species has been illustrated by Hansen (XII. and XVI.), and also in Figs. 128 and 132. The cells are ellipsoidal, though they may also be sausage-shaped. The limits of budding temperature in wort are 40°–41° C. and 0.5° C. The spores are 3–4 μ, seldom 3.5–4 μ in diameter. Limits of sporulation temperature on gypsum blocks, 30.5°–32.5° C. and 4°–7.5° C.; optimum, 25° C. Limits of film-formation temperature, 33°–34° C. and 6°–7° C. The cells of the young film, grown at 13°–15° C. differ from those of *Sacch. turbidans* (which are round and oval) by consisting largely of long, sausage-shaped forms. At the end of eleven to fourteen days the streak cultures on wort gelatin at 25° C. exhibit a peculiar reticulated structure, differentiating them from the preceding species and *Sacch. turbidans*. This species is generally a bottom yeast. It was discovered on the surface of ripe grapes in the Vosges district, and is one of the numerous species that play an active part in the fermentation of wine.

A number of wine and fruit-wine yeasts allied to *Sacch. ellipsoideus* have been isolated and described by Aderhold, Hotter, Kayser, Lindner, Marx, Müller-Thurgau, Nastjukow, Osterwalder Seifert, Wortmann, and others. One of the best known species is:

Johannisberg II., Wortmann (XVI.), which has been drawn by Aderhold (I.). According to this observer, it is distinguished by copious sporulation, 99–100 per cent. of the cells producing spores on gypsum blocks. Hansen (XLVIII.) gives the limits of budding temperature in wort as 37°–38° C. and 0.5° C., and those of sporulation temperature on gypsum blocks as 33°–34.5° C. and 2°–3° C. The species is usually a bottom yeast.

Saccharomyces turbidans, E. C. Hansen. Synonyms: *Sacch.*

ellipsoideus II., E. C. Hansen (XII., XVI., XLVI, and XLVIII.), *Sacch. turbidans*, E. C. Hansen (XLIX.) = *Sacch. ellipsoideus* (partim), Rees (I.). This species has been drawn by Hansen (XII. and XVI.). The cell form is generally similar to that of the preceding species. Limits of budding temperature in wort, 40° C. and 0.5° C. The spores are 2–5 μ, seldom 4–5 μ in diameter. Limits of sporulation temperature on gypsum blocks, 33°–35° C. and 4°–8° C.; optimum 29° C. Limits of film formation temperature 36°–38° C. and 3°–5° C. The cells of the young film grown at 13°–15° C. differ from those of *Sacch. ellipsoideus* in being chiefly round and oval. The species occurs as both top and bottom yeast, and is an injurious organism causing yeasty haze in bottom-fermentation breweries. It was discovered, with *Sacch. validus*, in beers affected with yeasty haze (*see* p. 115, vol. ii.).

Saccharomyces Willianus, Saccardo. Synonyms: *Saccharomyces I.* of Will, Bay (II.) = *Sacch. Willianus*, Saccardo (II.). The species was first described and drawn by Will (VIII.), but merely as "yeast No. 11." The cells are ovoid, and the spores measure 1.5–5 μ in diameter, usually 3.5 μ. Not more than 4 spores have been discovered in a cell. Limits of sporulation temperature on gypsum blocks, 39°–41° C. and 4°–9° C.; optimum, 34° C. Limits of film formation temperature on wort, 39°–41° C. and 4° C. The species produces disagreeable flavour and haze in beer.

Saccharomyces Bayanus, Saccardo. Synonyms: *Saccharomyces II.* of Will, Bay (II.). *Sacch. Bayanus*, Saccardo (II.). This species was first described by Will (VIII.), but merely as a "yeast causing beer haze." The cell form is pointed ovoid, turbinate or spindle-shaped, 7–11 μ in length and 5–6 μ in breadth. In old films the length of the cells reaches 30 μ and the breadth 2–4 μ. From two to four spores are produced, their dimensions being 2–4 μ. The limits of sporulation temperature on gypsum blocks are 30°–32° C. and 0.5°–3° C., the optimum being 23.5°–24° C. This species produces both haze and a sweetish metallic and disagreeably aromatic taste in beer, as well as an unpleasant bitter, astringent after-taste. At the same time the beer acquires a peculiar aromatic smell, like rotten fruit.

Saccharomyces ilicis, Grönlund, has been drawn by Grönlund (I.) The cells are mostly globular. The limits of sporulation temperature on gypsum blocks are 36°–38° C. and 8°–9.5° C., the optimum being 32° C. The streak cultures on wort-gelatin have a mealy appearance. The species was discovered on the fruit of *Ilex aquifolium*, and is a bottom yeast, producing 2.78 per cent. of alcohol (by volume) in wort, to which it imparts a disagreeable bitter taste.

Saccharomyces aquifolii, Grönlund (II.), forms cells analogous

to those of *Sacch. ilicis*. The limits of sporulation temperature on gypsum blocks are 27.5°–31° C. and 8°–10 5° C.; optimum 27° C. The streak cultures on wort-gelatin have a shiny appearance. The species is a top yeast, and probably a culture yeast. It produces 3.71 per cent. of alcohol in wort, and imparts a sweetish flavour, with bitter after-taste, to the beer. The fruit of *Ilex aquifolium* is the natural habitat

Saccharomyces Jordermanii, Went and Prinsen Geerligs. Drawings of this species have been made by Went and Prinsen Geerligs (I.). The cells are rounded, pear- or onion-shaped, angular or elongated forms being found occasionally. The number of spores is usually four. No film is produced, but only a yeast ring in old cultures. The species is said to produce 9–10 per cent. of alcohol, and was discovered in the "Ragi" employed in the manufacture of Javanese arrack (*see* p. 92, vol. ii), the product obtained being of very fine quality, devoid of fusel oil.

Saccharomyces pyriformis, Marshall Ward, has been drawn by Ward (II.), and is illustrated in Fig. 97. The cells are generally ellipsoidal or oval, occasionally globular, and measure 5–9 μ in diameter. Four spores are usually produced in a cell, the time of formation on gypsum blocks at 25° C. being twenty-four hours. A film composed of pear-shaped cells, with interspersed sausage-shaped cells, is formed in three weeks on nutrient solutions. The limits of budding temperature are 35° C. and 10° C. The species is a bottom yeast and was discovered in England, in ginger-beer (*see* vol. i. p. 256).

Saccharomyces mali, Risler, Kayser (I.) was drawn by this last-named worker. The cells are generally globular and measure 4–6 μ. The sedimental yeast is very firm. This species does not produce film. The spores develop in ninety-six hours at 15° C. The species is a bottom yeast, found in cider.

Saccharomyces Saké, Yabe (I.), was first described by Kozai (III.) without being named. The cells are generally globular and 6–12 μ in diameter. Giant cells are present in old cultures. Spores are developed on gypsum blocks, in thirty-six hours at 41° C., fourteen hours at 30°–32° C., and fifteen days at 3°–4° C. The number of spores in each cell rarely exceeds 1–3. This species was discovered by Kozai on Koji, and has been successfully employed, as a pure culture, in the peparation of "Saké."

The second sub-group comprises such species as ferment dextrose and saccharose, but not maltose and lactose. It includes:

Saccharomyces Marxianus, E. C. Hansen (XLVI., XLIV. and XLVIII.), which has been illustrated by Hansen (XLIV.). The vegetative cells of this species are small, oval, or ovoid, or else elongated and sausage-shaped, frequently assembling in colonies. Mycelial colonies are formed when the cultures have stood for some time in wort. The limits of budding temperature in wort are 46°–47° C. and 0.5° C. After about three months, wort

cultures develop a tender film, composed partly of short sausage-shaped cells and partly of oval forms. On solid media the species forms a mycelium resembling that of *Monilia candida* in structure. The spores are more or less reniform, occasionally round or oval, and most frequently about 3–5 μ in length. According to Klöcker (I.), the limits of sporulation temperature on gypsum blocks are 32°–34° C., and 4°–8° C., optimum 22°–25° C. Hansen states that only 1–1.3 per cent. of alcohol (by volume) is produced after prolonged sojourn in wort. In a solution of 15 per cent. of saccharose in yeast water, 3.75 per cent. (by vol.) of alcohol were formed in eighteen days at 25° C., and 7 per cent. after thirty-eight days. In yeast water containing 10 and 15 per cent. respectively of dextrose 6.5 and 8 per cent. of alcohol were produced in one month. The species was discovered on grapes by Marx.

Saccharomyces exiguus, E. C. Hansen (XLVI.) Synonym: *Sacch. exiguus* (partim), Reess (I.) This species forms cells similar to those of the last named, but differs therefrom in not forming mycelial colonies in wort, or a mycelium on gelatin. Sporulation is very scanty, and only a mere suggestion of a film is formed even after several months. Up to 6 per cent. (by vol.) of alcohol was formed in yeast water treated with 15 per cent. of saccharose at 25° C., and 8 per cent. of alcohol in a 15 per cent. solution of dextrose at the end of fourteen days. This species has been found repeatedly in the yeast of a pressed yeast manufactory.

Saccharomyces Zopfii, Artari (I.) has been drawn by the last-named worker. The cells are short, broad ellipsoids or globular, and measure 3–6 μ in diameter, occasionally 8 μ. When the species is grown in a solution of dextrose (*see* p. 211, vol. ii.) containing 5–8 per cent. of ammonium sulphate, septa are developed in the cells. The maximum limit of budding temperature in wort is 33°–34° C., the optimum being 28°–29° C. Spores are readily formed both in fluid and on solid media, the number in each cell being usually two, though occasionally one, three or four are produced. They are globular and measure 1.5–3 μ. The maximum sporulation temperature is about 32° C., and ripe spores are found after twenty-one hours at 29° C. The vegetative cells are stated to withstand 130° C. dry heat and 66°–67° C. moist heat for half an hour. The species was discovered in sugar juice at a sugar works in Saxony.

Saccharomyces Bailii, P. Lindner (XIV.) was drawn by the latter worker. The cells are large, of somewhat elongated shape and with tough membrane, and old cultures exhibit amœba-like cells of irregular form. The spores are highly refractive. Film formation does not occur on nutrient solutions, and only occasionally are small islands of yeast found thereon. The streak cultures on wort gelatin are greyish white and lustrous; and the same colour and appearance are exhibited by the giant colonies which

develop slowly on the same medium. No liquefaction of the gelatin occurs. The species was isolated from Dantzig "Jopen" beer (*see* p. 229, vol. ii.).

Saccharomyces Joergensenii, Lasché (I.), was drawn by the last-named worker. The cells are round or oval, measuring 2.5–5.5 μ, and united to short chains, the spores globular, 1–2.5 μ thick and highly refractive, two to three being usually present in a cell, but rarely four. No development of film has been observed, but only a slight yeast ring, composed of round and oval cells. The limits of sporulation temperature on gypsum blocks, after cultivation in dextrose yeast water, are 26°–30° C. and 8°–12° C., with 25° C. as the optimum temperature. The species was discovered in American "Temperance beer," and when used in wort of the gravity 10.19 per cent. Ball., produces 0·89 per cent. (by weight) of alcohol.

The third sub-group comprises the species which ferment dextrose and maltose, but not saccharose and lactose as well. They are:

Saccharomyces Rouxii, Boutroux (IX.), which was drawn by Boutroux. The cells are round or oval, unite in chains, are very regular and measure 4–5 μ in diameter. No film is developed, but only a few yeast islands here and there. The number of spores in a cell is one, two or three, and they are also formed in the cells on the surface of the medium. The volume of alcohol produced does not exceed 5.3 per cent. even in presence of an excess of dextrose. The species is apparently identical with that mentioned by Roux (II.) and found in dextrose. Boutroux discovered it in fermenting fruit juices. Though imperfectly described, the species is mentioned here on account of its interesting behaviour toward sugars.

Saccharomyces Soja, Saito (I.). This species has not yet been fully described, but the deficiency will be repaired shortly. It is distinguished by the circumstance that invertase is formed within the cells, though no fermentation of saccharose occurs. Lævulose, galactose and mannose are attacked, but not raffinose, inulin or di-methyl glucoside. The species was discovered in "Moromi," the mash employed in the preparation of Soja sauce (*see* chap. lvii.).

The species of the fourth sub-group, which ferment dextrose, but not saccharose, maltose or lactose as well, are two in number.

Saccharomyces mali, Duclaux, Kayser (III.), which was drawn by the last-named. The cells are 6–12 μ long and 4–8 μ wide, and form a loose sedimental deposit. A film is produced. Spores make their appearance at the end of eighty-four hours at 15° C. This species is a top yeast, and was discovered in cider, to which it imparts a fine bouquet.

Saccharomyces flava lactis, Krueger (I.). The cells are small, ellipsoidal, about 3.8–4 μ in diameter, and united in chains. The

colonies on gelatin are yellow in colour, and rapidly liquefy the substratum, which they cover with a yellow film. The same appearance is also observed in the sowings on milk and on solutions of lactose. The yellow colouring-matter is formed only in presence of air. The species was discovered in butter, to which it had imparted an abnormal yellow colour and a highly disagreeable smell like stale urine. It is included here on account of the remarkable production of colouring-matter, although only imperfectly described at present.

The species of the fifth sub-group are characterised by their power of fermenting lactose. Hence they belong to the organisms which excite alcoholic fermentation in milk (*see* vol. i. p. 85) and play an important part in the preparation of Kefyr, Koumiss, Mazun, &c. They are but few in number. A species of budding fungus discovered in milk by GROTENFELT (III.) was named by him *Sacch. acidi lactici* (not *S. lactis acidi* as is frequently, but erroneously, written). In respect of this species, and of another previously, described by DUCLAUX (XIV.) and named *Sacch. lactis*, he says that both sporulate on potatoes. Kayser afterwards showed that Duclaux's species cannot produce spores and is therefore a torula; consequently it is also highly probable that Grotenfelt's species is not a *Saccharomyces*. A number of other species also described as *Saccharomyces* are really torulæ (*see* chap. lix.). On the other hand, the following species must be classed as true *Saccharomycetes:* a *Saccharomyces* capable of fermenting lactose, discovered by E. VON FREUDENREICH and O JENSEN (II.) in Emmenthal cheese; two species afterwards isolated from butter by O. JENSEN (II.), and one found by MAZÉ (I.) in cheese. None of them has received a systematic name, and the descriptions are imperfect.

The only species of which a complete description is available and to which a systematic name has been given is the following:

Saccharomyces fragilis, JÖRGENSEN, which has been drawn by that worker (XIII.). The cells are small, oval, and elongated. The spheroidal spores are produced both in fermenting liquids on gelatin, and in gypsum-block cultures, appearing in the latter case after twenty hours at 25° C., and in forty hours at 15° C. Grown in 10 per cent. lactose yeast water at room temperature, the species produces 1 per cent. (by weight) of alcohol in eight days, and 4 per cent. in four months; whilst in wort of the gravity 11 per cent. Balling, it produces about 1 per cent. of alcohol in ten days at room temperature. The species was isolated from Kefyr.

The sixth sub-group of the *Saccharomycetes* is characterised by lacking the faculty of exciting alcoholic fermentation. The only representative known as yet is:

Saccharomyces Hansenii, ZOPF (XIII.). The cells are globular to ellipsoidal and measure 4–11 μ in diameter. Each cell contains

one or more fat globules. The inoculation streaks on wort gelatin form lustrous white colonies; the gelatin is not liquefied. The spores are globular and measure 2–4 μ, and occur singly or in pairs. The species forms oxalic acid in solutions of dextrose, galactose, saccharose, lactose, maltose, dulcitol, glycerol, and mannitol. It was discovered in cotton-seed meal. Owing to the brief description (film formation ?), the position of the species is doubtful.

Closely allied to the genus *Saccharomyces* are the two following genera, *Hansenia* and *Torulaspora*.

In the genus *Hansenia*, P. LINDNER (XXXII.), many of the cells are lemon-shaped, in other respects they exhibit the same characteristics (including sporulation) as the genus *Saccharomyces*. Lindner proposed to apply this generic name to "the *Apiculatus* yeasts" without giving any further indications, on the basis that all the "*Apiculatus* yeasts" produce spores. However, since the species named *Sacch. apiculatus* by Reess is asporogenic, it cannot be classed with this genus. For the present, only that species which is morphologically analogous to *Sacch. apiculatus*, but differs therefrom in being sporogenic, can be included in the genus *Hansenia*. A few species belonging to this genus have been discovered by Beijerinck, Lindner and Röhling, but have not yet been more fully described.

In the genus *Torulaspora*, P. LINDNER (XXII.), the cells are small and globular, with a single large fat globule in each, and resemble the cells of *Torula*. Lindner has not yet enumerated the characteristics of this genus either, except to cite as typical the species *Torulaspora Delbrücki*, LINDNER (XXXII.), formerly described and illustrated by him (XXXI.) under the name *Sacch. Delbrücki*. This species exhibits 1–2 spores in a cell, ferments dextrose and lævulose, and was discovered in English ale.

Although the cell form is the only characteristic as yet specified in connection with the two foregoing genera, they have been included here because of the probability of a sufficient characterisation being established later on. For the present they cannot be differentiated from the genus *Saccharomyces*, the cell form alone being insufficient to serve as a generic characteristic.

§ 279. The Genera Zygosaccharomyces, Saccharomycodes and Saccharomycopsis.

The genus *Zygosaccharomyces*, BARKER (I.), coincides in general with the genus *Saccharomyces*, but differs therefrom in respect of the phenomenon of cell fusion, which precedes sporulation.

Zygosaccharomyces Barkeri, SACCARDO and SYDOW (I.) was first described and drawn by BARKER (I.), but without being invested by him with a systematic specific name. The cells are oval. The limits of budding temperature on wort agar-agar are 37°–38° C. and 10°–13° C. This species develops merely a yeast

ring, but no film. Spores are produced, not only on gypsum blocks, but also on various solid media containing wort, and on damp bread, potatoes, ginger, &c. The limits of sporulation temperature on gypsum blocks are 37°–38° C. and 13° C. The species ferments dextrose, lævulose and saccharose, but not maltose, lactose and dextrin. It was discovered in a vessel containing ginger in Mayer's nutrient solution with saccharose.

Zygosaccharomyces Priorianus, Klöcker, was described provisionally by Klöcker (IV.), without being named. The cells in young wort cultures are of various forms, round, oval or elongated, and firmly attached together so that the sedimental yeast forms a coherent mass. The largest cells are produced at 13°–16° C., which temperature is on the whole highly favourable to their development, whereas at higher temperatures, *e.g.*, above 27° C., many of them are very small, and at lower temperatures elongated (sausage-shaped) cells are frequent. Old cultures often exhibit very highly elongated, mycelial cells. The limits of temperature for macroscopical development in wort are 36°–38° C. and 3°–8° C. The colonies in plate cultures on wort-gelatin at room temperature occasionally resemble *Peziza* or lichens. At high temperatures the surface of the colonies is smooth, but at 18° C. and lower it is greatly wrinkled or convolute, and often yellow in colour. Film formation is rare, but well-defined yeast rings are often observed. The spores are round or oval, and generally 2–4 in a cell. At 16°–18° C. they form in large numbers on the surface of the wort gelatin, on sterilised carrot slices, and on gypsum blocks that have been immersed in wort instead of water. In ordinary gypsum-block cultures, on the other hand, spores are produced with difficulty if at all. The limits of sporulation temperature on gypsum blocks in wort, and on slices of carrot, are 27°–28° C. and 3°-9° C. The species ferments dextrose and maltose, but not saccharose and lactose. It was discovered on the bodies of honey-bees, and a similar or identical species has been found on humble bees.

In the genus *Saccharomycodes*, E. C. Hansen (XLIX.), the spores, which are provided with only a single membrane, germinate into a promycelium, and the new cells, produced from this and the vegetative cells by budding, are incompletely separated, a mycelium with well-defined septa being formed. Up to the present two species are known :

Saccharomycodes Ludwigii, E. C. Hansen. Synonyms : *Ludwig's Saccharomyces*, E. C. Hansen (XLVII.) = *Saccharomyces Ludwigii*, E. C. Hansen (XVII., XLIV. and XLVIII.) = *Saccharomycodes Ludwigii*, E. C. Hansen (XLIX.). The species has been illustrated by Hansen (XVII. and XLIV.), and in Figs. 146 and 150. The cells vary considerably in form, the lemon-shape predominating. The limits of budding temperature in yeast are 37°–38° C. and 1°–3° C. Sporulation occurs not only on gypsum

blocks and on gelatin, but also in nutrient liquids, *e.g.*, a 10 per cent. solution of saccharose. The spores are 3–4 μ in diameter. According to Nielsen (I.), the limits of sporulation temperature on gypsum blocks are 32°–34 C. and 2.5°–7.5° C. Hansen reports that the species ferments dextrose and saccharose, but not maltose. The volume of alcohol produced in dextrose yeast water may attain 10 per cent., but does not exceed 1.2 per cent. in wort. The species was discovered by Hansen and Ludwig in the mucilaginous exudation from oak-trees.

This rare genus also comprises a species discovered and fully described by J. Behrens (VIII.), though left unnamed by him. The author therefore proposes to call it

Saccharomycodes Behrensianus, Klöcker. The cells are large, and round or oval; the spores globular, 4–4.5 μ in diameter, and generally 2–3 in a cell, being formed at the end of twenty-two hours at 18°–20° C. Film formation has not been observed. The giant colonies on 10 per cent. must gelatin exhibit a highly decorative appearance, the dark central, crater-like hollow being surrounded by very delicate concentric striations. The edges of the colonies are pure white, the older middle part being somewhat darker and of a yellow tinge. These giant colonies show numerous cells containing spores. The species ferments dextrose, lævulose and maltose, but not saccharose, lactose and galactose, and was discovered on hops.

In the genus *Saccharomycopsis*, Schiönning (II.), the spores are bi-membranous. During germination the exosporium opens in a different manner in each of the two known species. In other respects the characteristics, so far as they have been ascertained, approximate most nearly to those of the genus *Saccharomyces*.

Saccharomycopsis guttulatus (Robin). Synonyms : *Cryptococcus guttulatus*, Robin (II.); *Saccharomyces guttulatus*, autt.; *Saccharomyces guttulatus*, Wilhelmi (I.); *Saccharomycopsis guttulatus*, Schiönning (II.). A drawing of this species has been given by Wilhelmi and also in Fig. 148. The following description is chiefly derived from Wilhelmi (I.): Cells ellipsoidal, elongated oval with flattened ends, length 6–16 μ, breadth 2–4 μ, with linear or vortical budding. The optimum budding temperature is 35°–37° C. Nothing is known as to the formation of a film. The spores are of elongated oval form, and 1–4 are present in a cell. In germination, the exosporium bursts, with irregular edges, either at the poles or laterally, and gradually contracts to a small residue of indefinite shape. The species thrives on several artificial nutrient media, *e.g.*, on tartaric glycerin agar-agar with an addition of dextrose. It ferments dextrose and saccharose, and was discovered in the alimentary canal of rabbits, less frequently in that of guinea-pigs and in the excrement of these animals.

Saccharomycopsis capsularis, Schönning (II.) has been drawn by this worker. The cells are sometimes ovoid, sometimes sausage-shaped; and typical septated mycelia are also observed. The limits of budding temperature in wort are 38.5° C. and about 0.5° C., the optimum being 25°–28° C. On nutrient liquids the species quickly forms a decidedly white, irregular, shaggy film; but on solid media it develops into a more or less irregular, white, shaggy vegetation, which turns chocolate-brown in old cultures on wort-gelatin agar-agar. The spores are generally of oblate spheroidal form, with a maximum diameter of 3.5–8 μ, and usually 4 in a cell. The limits of sporulation temperature on gypsum blocks are 34.5°–35° C. and 5°–8° C., the optimum being 25°–28° C. In germination, the exosporium opens in the form of two valves, generally of unequal size, and often remaining for some time attached together at one point and adhering to the germinating spore. The exosporium is stained pink by concentrated sulphuric acid and several other concentrated mineral acids. The species thrives in wort, yeast water, on wort gelatin, wort-gelatin agar-agar, yeast-water gelatin, rice, and bread. It ferments dextrose, lævulose and maltose, but not saccharose, lactose and raffinose. It was discovered in the soil of a meadow in the Swiss Alps.

§ 280. The Genera Pichia and Willia. The doubtful Genera Monospora and Nematospora.

The two main groups of the true *Saccharomycetaceæ* (p. 273, vol. ii.), comprise the genera *Pichia* and *Willia*, the species of which produce a film on saccharine nutrient liquids immediately. The film has a dry, dull appearance, due to the inclusion of air bubbles, and exhibit well-defined differences from that produced by the genera described in §§ 278 and 279. The spores are of various shapes, with or without a projecting ledge, and have only a single membrane. Several of the species are characterised by the formation of esters, and a few of them do not excite fermentation.

In the genus *Pichia*, E. C. Hansen (XLIX.), the spores are rounded, hemispherical, or irregular and angular. No fermentation is produced. A strong mycelium is formed. The following eight species (*inter alia*) of this genus are known:

Pichia membranæfaciens, E. C. Hansen. Synonyms: *Saccharomyces membranæfaciens*, E. C. Hansen (XLVI. and XLVIII.) = *Pichia membranæfaciens*, E. C. Hansen (XLIX.). The species has been drawn by Seifert (II.). The film consists of sausage-shaped and elongated oval cells, rich in vacuoles. Limits of budding temperature on wort, 35°–36° C. and 0.5° C. The colonies on wort gelatin are dull grey, often with a reddish tinge, and the medium is liquefied very quickly. The spores are rounded or hemispherical, and are produced in large numbers

both on gypsum blocks and in the films. According to Neilsen (I.) the limits of sporulation temperature on gypsum blocks are 33°–35° C. and 2.5°–7.5° C., the optimum being 30.5°–31° C. Seifert states that the species continues to grow even in presence of 12.2 per cent. (by vol.) of alcohol. It was discovered by Hansen in a mucinous mass exuding from the damaged roots of an elm; and was also found subsequently in impure well-water by Koehler, and in white wines by A. Jörgensen.

Pichia membranæfaciens II. (Pichi). Synomyms: *Saccharomyces membranæfaciens II.* Pichi (I). The species was drawn by Pichi (II). The cells are 5–7 μ long and 3–5 μ broad, or 10–19 μ long and 3–4.5 μ broad. The spores are often round, or slightly compressed or flattened, and measure 2.5–3 μ in diameter. There are usually 3–4 spores in a cell. The asci in the rugose milk-white film are oval, 6–8 μ in length and 3–5 μ in breadth. Few asci are formed on wort at 22°–25° C. This species was found on the leaves of *Euonymus europæus.*

Pichia membranæfaciens III. (Pichi). Synonym: *Saccharomyces membranæfaciens III.*, Pichi (II). A drawing of the species was given by the last-named worker. The cells are 5–7 μ long and 3–4.6 μ broad, the spores 2.5–3.5 μ in diameter. The asci are globular or oval, contain 2–4 spores, and measure 5–8 μ by 3–5 μ. The film produced on wort at 22°–25° C. is uniform, thin and smooth, and contains a large number of asci. This species was produced in "vin des Côtes."

Pichia californica (Seifert). Synonym: *Saccharomyces membranæfaciens, var. californicus* Seifert (I.) The species was drawn by this worker. The cells are mostly oval, occasionally contain a small highly refractive body, and measure 4–8 μ by 3–5 μ. The films are delicate, white and readily sink to the bottom. The spores are globular, 2–4 in a cell and 2–3 μ in diameter, with homogeneous, highly refra tive plasma. Only a few sporogenic cells are found in the films at ordinary room temperature; and sporulation ceases on gypsum blocks at 39°–40° C. and 5°–6° C.; the optimum temperature is 34° C. In wines containing 8 per cent. (by vol.) of alcohol, the maximum temperature at which growth proceeds is 33° C., the minimum being 7°–12° C. and the optimum 28°–30° C.; but in beer wort the limits are wider, he maximum, for instance, being over 39° C. (They were, however, not mentioned by Seifert.) The species, which was discovered in Californian red wine, continues to grow when the volume of alcohol attains 12.2 per cent.

Pichia taurica (Seifert). Synonym: *Saccharomyces membranæfaciens, var. tauricus*, Seifert (I.). In this species, which was drawn by Seifert, the cells are mostly sausage-shaped, elongated, seldom oval, and measure up to 20 μ in length by 4–6 μ in breadth. The films are delicate, readily sink to the bottom, and when kept at room temperature for a short time exhibit an abundance of

sporogenic cells. The spores are oval, 4–6 μ long and 3–4 μ wide, and cease to be produced on gypsum blocks at 34° C. and 4°–6° C. respectively. The optimum sporulation temperature is 27°–30° C. The optimum temperature for growth, in wines containing 8 per cent. (by vol.) of alcohol, is 22° C., the maximum being 28°–30° C. and the minimum 5°–6° C. The species which was discovered in Crimean wine has ceased to grow by the time the volume of alcohol reaches 12.2 per cent.

Pichia tamarindorum (Seifert). Synonym: *Saccharomyces membranæfaciens, var. tamarindorum* Seifert (I.) This worker has made a drawing of the species. The cells are mostly very long, seldom oval or pear-shaped, and often contain a small highly refractive body in the protoplasm. The elongated cells measure up to 26 μ by 2–6 μ, the small oval cells 5–6 μ by 2–3 μ. The films are dense, and of white, dusty appearance, rugose in old cultures. When subjected to vibration, they fall to the bottom as large flakes. The spores are almost hemispherical, about 3 μ high and 4 μ maximum diameter, and they usually contain a small central highly refractive body. In many cases the flat side is slightly arched in the middle, with a small projecting rim. Spores are soon produced in abundance in the films at ordinary room temperature; on gypsum blocks the limits of sporulation temperature are below 34° C. and above 1.5° C., and the optimum temperature is 27°–30° C. Giant colonies on wort gelatin exhibit a peculiar reticulated structure. The species was discovered on tamarind must and a vinous beverage prepared therefrom.

Pichia farinosa (Lindner). Synonyms: *Saccharomyces farinosus*, Lindner (XLIV.) = *Pichia farinosa*, E. C. Hansen (XLIX.) The species was drawn by Lindner (XLIV.). The cells are slender, and old cells in particular are often of angular contour. Spores are abundant in the films, but the latter cease to form at 37° C. The film is bright white in colour, folded like crinkled tissue-paper and looks as though strewn with flour. In old cultures on wort gelatin the medium is liquefied. The species was discovered in Danzig "Jopen" beer (p. 225, vol. ii.), and has also been found by K. Saito (II.) in Japanese Soja sauce.

Pichia Radaisii (Lutz). Synonym: *Saccharomyces Radaisii*, Lutz (I.). The cells of this species are of elongated oval form, 8–8.5 μ long and 3–3.5 μ broad, with a membrane 0.8 μ thick. The spores are round, usually four in a cell and measure 1.5 μ in diameter. On gypsum blocks they are produced in twelve hours at 22°–23° C.; and the maximum sporulation temperature is 25°–28° C. The optimum temperature of film formation is 23° C., all development ceasing at 37°–38° C. This species does not liquefy gelatin: and the colonies on that nutrient medium assume a red colour after a short time. *Pichia Radaisii* was discovered in "Tibi," from which a Mexican beverage is prepared.

In the genus *Willia*, E. C. Hansen (XLIX.), the spores are

pileate or lemon-shaped, with a projecting rim. Most of the species possess considerable ester-forming powers, but a few lack the capacity of exciting fermentation. The genus comprises the following seven species:

Willia anomala, E. C. Hansen. Synonyms: *Saccharomyces anomalus*, E. C. Hansen (XVII. and XLVIII.). *Willia anomala*, E. C. Hansen (XLIX.). The species has been illustrated by Hansen (XVII.), and in Figs. 143 and 147. The microscopic aspect of the cells recalls that of a *Torula*. They are small in size and oval, occasionally sausage-shaped (especially in old cultures). The limits of budding temperature in wort are 37°–38° C. and 0.5°–1° C. At the commencement of fermentation the film is dull grey, the liquid gradually becoming cloudy. After awhile, sporogenic cells can be detected both in the film and in the sedimental yeast. The cells contain 2–4 spores, which are hemispherical with a projecting rim around the basal surface, so that they present a hat-like appearance. The diameter of the basal surface measures 2–3 μ, irrespective of the rim. According to Nielsen (I.) the limits of sporulation temperature on gypsum blocks are 32°–34° C. and 2.5°–7.5° C., the optimum being 30° C. A powerful odour of fruit ester is disengaged during fermentation. Nielsen states that the volume of alcohol and ester produced in wort by this species in eleven days is only 0.9 per cent.; and according to Seifert the ester so formed is the ethyl ester of acetic acid. This worker also states that the species decomposes alcohol to water and carbon dioxide, the acetic ester being also consumed eventually. According to Nielsen, *W. anomala* ferments dextrose, but not maltose or lactose, and very little invertase is produced; but other investigations have shown the production of invertase to be decidedly apparent. The species was first discovered by Hansen in an impure Bavarian beer yeast, and it was afterwards found in English beers, on green malt, bran, marshmallow sap and soil, as well as on plums and other fruit. Klöcker and Schiönning (VI.), Kozai (I.), and Saito (I.), have found it in the Koji used in the preparation of Saké; and, according to Inui (I.), it is also present in the Koji employed for making "Awamori" in the Loochoo Islands. P. Lindner (XXXI.) found the same species in the Armenian beverage, Mazun.

Willia anomala I. (Steuber). Synonym: *Saccharomyces anomalus, var. I.*, Steuber (II.). This species was drawn by its discoverer. The film on wort is initially smooth and chalk-white, but later folded and yellowish. The limits of film-formation temperature are 37°–42° C., and 5°–10° C. The giant colonies on 10 per cent. wort gelatin are yellow in the centre and white, with a silky sheen, at the edge. Giant cells, up to 15 μ, are found in the central portion of the colony, and cells up to 30 μ in length at the edges. The gelatin is liquefied. The

spores are pileate, and are produced both in the film on gelatin and on gypsum-block cultures. The limits of sporulation temperature on the latter cultures are 30°–35° C., and 5°–12° C. The species ferments dextrose, lævulose and saccharose, but not maltose, lactose or galactose. It produces acetic esters and acetic acid, and was discovered in water which had been used for washing yeast.

Willia anomala II. (Steuber). Synonym: *Saccharomyces anomalus, var. II.*, Steuber (II.). A drawing has been made by that worker. The film on wort is smooth and chalk-white at first, afterwards implicate, and turns pink to brownish pink in a short time. The limits of film-formation temperature are 30°–35° C., and 5°–10° C. The giant colonies on wort gelatin soon turn pink to brownish red, and the gelatin is liquefied. An abundant formation of pileate spores is observed, and the limits of temperature for this phenomenon on gypsum blocks are 30°–35° C., and 5°–15° C. With regard to the behaviour of the species toward sugars, Steuber says: "A 10 per cent. solution of saccharose is inverted and fermented completely, though slowly, only 0.45 per cent. of alcohol is produced in a 10 per cent. solution of lævulose. It does not ferment dextrose, lactose, galactose or maltose, merely traces of alcohol (if any) being produced in those solutions. No acetic ether is formed." There appears to be some error in this statement, for if a yeast cannot ferment dextrose, it is also incapable of completely fermenting an inverted saccharose solution.

Willia anomala III. (Steuber). Synonym: *Saccharomyces anomalus, var. III.*, Steuber (II.) Drawn by this worker. The film is white at first, yellowish afterwards. The limits of film-formation temperature are 30°–35° C., and 5°–15° C. The giant colonies on wort gelatin are white and irregular, and liquefaction of the medium is produced. Limits of sporulation temperature on gypsum blocks, 30°–35° C., and 5°–15° C. "In a 10 per cent. solution of lævulose, 0.4 per cent. of alcohol is produced in four weeks. The species does not ferment dextrose, saccharose, lactose, galactose or maltose; nor is any acetic ether ormed."

Willia belgica (Lindner). Synonym: *Saccharomyces anomalus var. belgicus*, Lindner (XXXI.). The species was drawn by the last-named worker. It grows on wort as a creamy, punctated film; the cells are comparatively small, thin-walled and poor in contents. The pileate spores are mostly developed in such abundance that little but the sharp lines of the projecting rims can be seen. The species does not ferment any known sugar, nor does it produce fruit esters. It was discovered in Belgian beer.

Willia Saturnus (Klöcker). Synonym: *Saccharomyces Saturnus*, Klöcker (V.). Drawn by the last-named. The film is white

and rugose, the cells round or oval, seldom elongated, usually 4–6 μ long. The limits of budding temperature on wort are 35°–37° C. and 2°–4° C. The spores are more or less decidedly lemon-shaped, about 3 μ in length, with a projecting peripheral ledge extending from tip to tip, and containing a small central refractive globular body. The limits of sporulation temperature on gypsum blocks are 28°–31.5° C. and 4°–7° C., the optimum being about 25° C. This species ferments dextrose, lævulose, raffinose, and saccharose (the latter after inversion), but not maltose, lactose, or arabinose. An ester (acetic ester ?) is produced during fermentation. The organism was discovered in samples of soil from the Himalayas, and the same or an allied species has been repeatedly found in Danish and Italian soils.

Although, as already mentioned on p. 273, vol. ii., doubt exists as to whether the genera *Monospora* and *Nematospora* really belong to the family *Saccharomycetaceæ*, they will be dealt with in this place.

The genus *Monospora*, Metchnikoff (III.), ought really to be re-named, since this title has already been applied, by Hochstetter, to one of the *Flacourtiaceæ*. In *Monospora*, Metchnikoff, the spore is acicular, and germinates by producing a lateral promycelium, from whence gemmation proceeds. Only a single spore is formed in a cell. The genus contains only one known species, viz., *Monospora cuspidata*, Metchnikoff (III.), which has been drawn by the last-named worker. The cells are an elongated oval. The asci are very long and sausage- or club-shaped, and each ascus produces only a single, acicular spore, pointed at both ends. This species is parasitic in the stomach of the water-flea (*Daphnia*), but since its discovery by Metchnikoff it has not been observed again.

In the genus *Nematospora*, Peglion (III.), the spore is elongated, spindle-shaped, with a long flagellum at one end. Germination proceeds by budding at one or both extremities. Several spores are formed in a cell. Up to the present only one species has been described, namely:

Nematospora Coryli, Peglion (III.), which was drawn by that worker. The cells are elongated, but in old cultures they are round or oval, with a double, lustrous membrane. Budding proceeds from the ends of the cell, as in the case of *Dematium*, but, in nutrient liquids, only a mycelium is formed and no budding occurs. The ascus is sausage-shaped, 65–70 μ long and 6–8 μ broad, and it contains 8 spores, in two bundles of 4 each, disposed along the longitudinal axis. The spores measure 38–40 μ in length, exclusive of the flagellum, which is 35–40 μ long. The thickness of the spores is 2–3 μ. Previous to germination the spores shed the flagellum and become shorter and thicker. The species thrives best, and also sporulates, on sterilised sugar beet,

and will also develop on nutrient meat-broth gelatin, but grows very badly in nutrient liquids. It was discovered in hazel-nuts in Italy.

§ 281. The Family Schizosaccharomycetaceæ.

The species of this family are monocellular fungi, which reproduce by fission, and exhibit endosporogenation. The fission of a cell is preceded by the formation of a septum, which at once commences to divide into two lamellæ from the outside. No budding occurs. Each cell may be sporogenic. The spores are monocellular, and 1–8 are formed in the parent cell. At present the family comprises only a single genus, viz. :

Schizosaccharomyces, P. Lindner (XXX.), the generic characteristics of which are also those of the whole family. In some instances the formation of asci is preceded by fusion. All the three species known at present produce spores which are stained blue by a solution of iodine in potassium iodide (*see* p. 147, vol. ii.). The species excite alcoholic fermentation in various sugar solutions. According to Guilliermond the cells never contain glycogen, in which respect they present a contrast to those of the *Saccharomycetaceæ*.

Schizosaccharomyces Pombe, Lindner (XXX.), has been drawn by the last named. The cells are cylindrical, 5–9 μ long and 4–9 μ broad, but the dimensions fluctuate considerably. As a rule the two ends of each cell differ in appearance, the one being rounded, the other surrounded by a sharply defined ring embracing the newly formed membrane, which already assumes a conical shape. Hammer-shaped cells are not infrequently observed. The cells are shorter in exhausted media. With restricted admission of air many of the cells develop into long tubes, containing numerous septa without, however, undergoing separation, and even when the latter occurs, the cells frequently remain attached at one point, as though hinged. According to Guilliermond (11.), the formation of asci is preceded by the fusion of two cells, which may be sister cells, and he also observed instances in which fusion between three cells took place. Sporulation readily appears, even in hanging wort drops, the spores forming sometimes in seven days. Spores are also found in the sedimental yeast at the close of primary fermentation. 1–4 lustrous spores, measuring about 4 μ, are formed in a cell, and these begin to germinate by swelling up to form an ascus, the spore membrane fusing into the new integument without bursting. As soon as the ascus has attained a length about equal to that of an ordinary vegetative cell, it develops a septum and splits into two halves. No formation of film takes place. At high temperatures the fermentation in beer wort is of top-fermentation character. The species ferments dextrose, maltose, and saccharose, and, in addition, lævulose, inulin,

dextrin, and raffinose, but not *d*-mannose (in which respect it differs from *Schizos. mellacei*). A boiled mash of malt, potato starch, and saccharose was attenuated from 27.7 per cent. Balling to 1.6 per cent., and then contained 15.5 per cent. (by vol.) of alcohol. The species was discovered in Pombe (African millet beer) by Saare, and was isolated by Zeidler. Lindner states that it has been successfully used in a distillery in Argentina.

Schizosaccharomyces octosporus, Beijerinck (XVIII.), has been drawn by Beijerinck (XVIII.), and Schiönning (I.), and is illustrated in Fig. 125. The vegetative cells of this species, grown in beer-wort cultures, are partly cylindrical, partly oval, and measure, according to Schiönning, 4.5–6 μ in breadth and 7–13 μ in length. A yeast ring is formed, as in the case of *Schizos. Pombe*. The asci are of regular oval shape, 14–20.5 μ long by 6–10.5 μ broad, and usually contain 8 spores, though 4 are often found, but rarely 2–7. Sporulation occurs both in nutrient liquids and especially on solid media. According to Seiter (I.) spores are formed on gypsum blocks in six to seven hours at 25° C. In this species the formation of the asci proceeds in three different ways: (1) In the manner observed by Schiönning and described on p. 103, vol. ii., namely, the division of a cell into two daughter cells which fuse together again. (2) By the fusion of two cells not derived from the same parent cell (*Guilliermond*). (3) Without the occurrence of any cell fusion at all (*Guilliermond*). The species does not produce any film, but only a slight yeast ring, and rapidly liquefies wort gelatin. It ferments dextrose, maltose, and lævulose, and according to Lindner, dextrin, raffinose, and *d*-mannose as well, but it is incapable of fermenting saccharose. Schiönning states that it gives rise to bottom-fermentation phenomena in a slight degree in wort (gravity 14 per cent. Ball.), and at the end of three weeks at 25° C., produces 4.6 per cent. (by vol.) of alcohol, increasing in five months to 6.56 per cent. It was discovered by Beijerinck on currants, and by Schiönning on raisins.

Schizosaccharomyces mellacei (A. Jörgensen). Synonym: *Saccharomyces mellacei*, A. Jörgensen (XIII.). The species was drawn by this worker. The cells are 8–12 μ long and 4–6 μ broad, and resemble those of *Schizos. octosporus* and *Schizos. Pombe*. Peculiar, oddly formed cells appear in old cultures. According to Guilliermond (II.), the ascus is formed by the fusion of two cells, frequently sister cells, though in a variety of the species he observed the ascus seemed to be formed without any previous cell fusion. The spores measure about 4 μ in diameter, and are slightly elongated; there are usually 4 in a cell; they are highly refractive. No film is produced, but merely a yeast ring. Lindner states that the species ferments dextrose, maltose, and saccharose, together with lævulose, inulin, dextrin and raffinose. It differs from *Schizos. Pombe* by its greater dimensions, and the

property of fermenting *d*-mannose. In beer wort (gravity 10.5 per cent. Ball.) it gives rise to top-fermentation phenomena, and produces 2.5 per cent. (by weight) of alcohol. An agreeable aroma is disengaged during fermentation. The species was discovered by P. Greig in cane-sugar molasses used in Jamaica for the production of rum.

SECTION XV.

MORPHOLOGY, PHYSIOLOGY AND CLASSIFICATION OF CERTAIN TECHNICALLY IMPORTANT HIGHER ASCOMYCETES AND ALLIED FORMS.

CHAPTER LVI.

MORPHOLOGY AND SUBDIVISION OF THE FAMILY ASPERGILLACEÆ.

By Prof. Dr. CARL WEHMER.

§ 282. Systematic Position and Classification of the Aspergillaceæ.

The systematic position (as *Ascomycetes*) of the *Aspergillaceæ*—a family rendered chemically interesting and technically important by many of its representatives—has already been defined on p. 100 of the present volume. Consequently we have now chiefly to deal briefly with its subdivision.

The *Aspergillaceæ*, which stand next to the *Gymnoasceæ*, but are distinguished from these latter by the possession of carpoasci surrounded by an integument, differ from the majority of *Carpoasceæ* (*Pyrenomycetes*, *Discomycetes*) by the irregular distribution of the asci in the carpoascus, and, on the other hand, from the otherwise similar truffle-like fungi (*Elaphomycetes* and *Terfeziaceæ*) —which mostly produce large subterranean fruit—by the smallness of their carpoasci. The asci in these fruits—which for the most part do not burst open in ripening, but either remain closed or else break up irregularly—develop 2–8 monocellular spores. According to the character of the carpoasci, and more especially in accordance with the structure of the highly divergent conidiophores—which often predominate or are present exclusively —Ed. Fischer (II.) has latterly divided the family into twelve genera. Schröter (I.) in 1893 counted only four, whilst G. Winter (IV.) in 1887 allocated the genera of this family to the sub-order of *Perisporiaceæ* (*see* p. 100, vol. ii.).

Subjoined is a

SYNOPSIS OF THE GENERA OF ASPERGILLACEÆ ACCORDING TO ED. FISCHER (II.).

(*A*) Carpoasci with mostly pseudoparenchymatic Peridium, uniformly filled with Asci.

(*a*) Carpoascus cervicate or with protrusive papillus . . *Microascus*
(*b*) Carpoascus acervicate :
  (*a*) Peridium with spirally coiled appendices . . *Magnusia.*
  (*β*) Peridium with straight hairs or a shaggy coat.
    1 Peridium of more or less carbonaceous nature. *Cephalosthea.*
    2 Peridium membraneous . . . . . *Aphanoascus.*
  (*γ*) Peridium devoid of appendix :
    1. No conidia, merely breeding-cells . . . *Anixiopsis.*
    2. Conidia formed in chains directly on the mycelium, with endogenous spores as secondary organs of fructification . . . . *Thielavia.*
    3. Conidia on conidiophores with terminal swelling, studded with numerous simple or branched sterigmata, in chains . . . *Aspergillus.*
    4. Conidia on sympodial branched conidiophores in chains . . . . . *Allescheria.*
    5. Conidia on branched conidiophores . . *Penicillium.*

(*B*) Carpoasci rounded or pear-shaped, with dense, stratiform peridium. Asci mingled with capillitium threads. The carpoasci undergo dehiscence by opening at the crown or decay of the upper part of the peridium.

(*a*) Asci with dentate projections, spores with equatorial fillet . . . . . *Emericella.*
(*b*) Asci ellipsoidal, with blunt projection at the crown, spores with fine, hairy spines . . . *Amylocarpus.*
(*c*) Carpoascus nodular, stalked, with thick peridium, proceeding from sterile veins separating the asciferous network inside the carpoascus . . *Penicillopsis.*

Not to be confounded with this "natural" family of *Aspergillaceæ* is the group established under that name, as a subdivision of the *Mucedineæ* (*see* p. 7, vol. ii.), solely on the basis of the structure of the conidiophores. To this group applies the following synopsis, differing somewhat from that of LINDAU (II.).

(*A*) Conidiophores invariably distended at the apex, in the form of a bladder or globule :

1. Conidiophore unbranched :
  (*a*) Chains of conidia formed merely at the apex of the sterigma :
    (*a*) Simple unbranched sterigmata . . *Aspergillus Citromyces* (*see* below).
    (*β*) Branched sterigmata, with occasional simple forms . . . . . *Sterigmatocystis.*
  (*b*) Chains of conidia, forming at the apex and below the septum . . . . . *Dimargiris.*
2. Conidiophores with dichotomous branchings . . *Dispira.*

(*B*) Conidiophores without any (regular) distension at apex :

1. Chains of conidia springing from sterigmata at the apex :
   - (*a*) Conidiophores with branches arranged in regular whorls ; conidia barrel-shaped . . *Amblyosporium.*
   - (*b*) Conidiophores without regular whorls, simple or branched. Conidia globular or ellipsoidal :
     - (*a*) Conidia without mucinous matrix :
       1. Conidiophores unbranched, with a terminal tuft of sterigmata, and with or without terminal swelling . *Citromyces.*
       2. Conidiophores always more or less regularly branched, without terminal swelling . . . . . *Penicillium.*
     - (*β*) Conidia united to a terminal head by mucinous matrix . . . . . *Gliocladium.*
2. Chains of conidia, without sterigmata, formed at the apex of the conidiophore . . . . . *Briarea.*

The number of genera coming under consideration for our purpose is limited to four: *Aspergillus*, *Penicillium*, *Citromyces*, and *Allescheria* (= *Eurotiopsis*), the sole distinguishing characteristic of which consists in the shape of the conidiophores, and not in that of the asci. Indeed, this is still unknown in most of the species now in question. Nevertheless, there does not appear to be sufficient justification for excluding these latter species and treating them separately as "fungi imperfecti," any more than there is for separating the *Mucorineæ* which produce zygospores from those in which zygospores have not yet been observed. (In this family also the spore-carriers in many cases form the sole generic characteristic). Consequently, for the time being, we will define these three main genera solely in accordance with the form of the conidiophores, and without reference to the presence or special character of the asci (which would lead to a rearrangement of the grouping), the latter being postponed until more complete knowledge has been gained of the numerous species still outstanding. At present the forms with conidiophores of the *Aspergillus* and *Penicillium* type may be divided into four groups, namely, species with

- (*a*) Soft-skinned carpoasci with continuous development (perithecia): *Aspergillus glaucus, A. fumigatus, Penicillium luteum.*
- (*b*) Tough carpoasci with intermittent development (sclerotia): *Penicillium glaucum, Aspergillus nidulans.*
- (*c*) Sterile sclerotia, no asci being formed : *Aspergillus flavus, A. ochraceus, A. niger, Penicillium italicum.*
- (*d*) Without any organs of the kind : *Aspergillus oryzæ, Penicillium olivaceum*, &c. The majority belong to this class.

Groups (*c*) and (*d*) are only provisional at present, and intermediate forms between the first two are also known to exist (*A. nidulans* approximates to group (*a*)) ; moreover, there is no concordance between the structure and development of the perithecia

and sclerotia of the various species, the differences in some respects being sufficient to necessitate separation. For instance, the carpoascus of *P. luteum* resembles a gymnoascus more than that of *A. glaucus*. Hence the proposal to subdivide the "morphological genus" *Aspergillus* into the genera: *Eurotium* (= (*a*)), *Aspergillus* (= (*b*) and (*c*)) and *Euaspergillus* (= (*d*)), with which would be included the genus *Sterigmatocystis* (*St. nidulans* with carpoasci)—established solely on the basis of conidiophore structure —is unsatisfactory as leaving *Penicillium* out of consideration. Moreover, this proposal does not rest on a proper basis so long as the genus *Penicillium* is left undivided into perithecial, sclerotial and sterile forms; and, finally, the two could be amalgamated by abandoning the conidiophores as the generic characteristic. Contrary to the former disagreement between investigators—compare the works of A. DE BARY (VIII.), VAN TIEGHEM (IV.), WINTER (IV.), and others—there is now, happily, a general desire to include all forms under a uniform name (*Aspergillus*). This has been done by SCHRÖTER (I.), with the sole exception of *Sterigmatocystis*, and also *in toto* by E. FISCHER (II.), who also included the genus *Eurotium*. At present this is the most commendable attitude to assume, and it must be left to the future to show whether the *Aspergillaceæ* can be—as is desirable—classified from the shape of the fruit alone. The existing defect is probably smaller than that which would be caused by separating the groups characterised by their conidiophores, since it would entail the grouping of divergent conidiophores (*Aspergillus, Penicillium*, &c.) in one and the same genus, and thus reducing the conidiophore to the level of a specific characteristic. Perhaps that may prove to be a way out of the difficulty. In the meantime it is clear that, in these genera, the conidiophores connect a number of forms which differ more or less among themselves in the history of their development.

The genus *Aspergillus* (Mich.), Corda (including *Eurotium*, Link and *Sterigmatocystis*, Cramer) possesses conidiophores which, for the most part, stand rigidly upright and tougher than the vegetative hyphæ, 0.2–4 mm. in length (seldom more), carry a terminal swelling, and are usually unbranched and aseptate, *i.e.*, monocellular. The conidial chains spring simultaneously from simple or branched sterigmata, as radial or tufted projections from the swelling. Up to the present, conidiophores are known to exist in only a few species, on which they appear as small coloured, globular capsules or nodules with a delicate single integument, or tougher, stratified skin either with or without a separate husk; the asci (containing 8 spores) either develop at once or after a short period of repose, or again remain sterile a long time. The fruit develops either from one or two special hyphæ, or by the fusion of a number of ordinary hyphæ. The number of species is uncertain, over 100 having been set up, but

barely 20 fully described. The conidial herbage is green, yellow, reddish brown, blackish brown or white.

The genus *Penicillium*, Link, produces conidiophores, which are delicate, barely distinguishable from the ordinary hyphæ, always less than 1 mm. in height, with septate stalk, polycellular, branched alternately or in whorls near the apex, and without terminal swelling. The conidial chains are produced on simple successive sterigmata which, in most cases, form tufts on the ends of the branches. The conidiophores, where such are known to exist, resemble those of *Aspergillus*, being delicate or tough, with or without a cortical envelope, developing continuously or intermittently, or remaining sterile for a time according to the species, and are usually formed by the fusion of two similar hyphæ (*P. glaucum*, Brefeld). The number of species is still uncertain, about 100 having been set up, but only about 12 properly described. The conidia form a herbage, generally green in colour, more rarely white, red, brownish yellow or brown.

The genus *Citromyces*, Wehmer, has delicate conidiophores, like those of *Penicillium*, but unbranched, carrying a tuft of sterigmata with more or less developed terminal swelling, sparsely septated or not at all. The chains of conidia are invariably arranged as projections formed in succession on the swelling or apex of the stalk of the sterigmata, which may be single, tufted or whorled. The herbage is green. Asci are unknown. Two species have been more fully investigated.

The genus *Allescheria*, Saccardo and Sydow (= *Eurotiopsis*, Costantin), has sympodial branched conidiophores, from which chains of oval conidia are formed by constriction, which differ appreciably in other respects from those of the foregoing genera. The carpoasci are globular (perithecia), and the asci contain 8 spores. The herbage is white to reddish or red. Up to now only a single (rare) species is known.

§ 283. The Genus Aspergillus.

In this genus we include all the mould fungi possessing the characteristic *Aspergillus* conidiophore (with globular terminal swelling developing sterigmata), and do not set *Sterigmatocystis* (with branched sterigmata) or *Eurotium* (forming perithecia) apart as separate genera.

The genus, characterised by the shape of the conidiophores, comprises a considerable number of species that are not always easily differentiated, and for whose identification the morphological details of that organ are of importance. In fact these details alone are sufficient to characterise the species in many cases; and this is the point with which we are now concerned, not with the investigation of the obscure conditions of

relationship. With regard to the genus *Aspergillus*, the reader is also referred to the works of Wilhelm (I.), Siebenmann (I.), Tiraboschi (I.), and Wehmer (XVII.), as well as to the recent publications of the French authors, dealing with pathogenic fungi and cited in connection with *A. fumigatus* (p. 316, vol. ii.).

The conidiophore, which, in the mature condition, is far broader and has thicker walls than the vegetative hyphæ, is mostly unbranched and aseptate, springing from a vertical hypha with globular terminal swelling. As a rule, it is clearly separable into stem and globule (*see* Fig. 163), the last-named being covered all over or on the top with a large number of closely set sterigmata of variable length and shape and producing conidia, either direct, or after the formation of secondary sterigmata. The conidia are globular or ellipsoidal, always unicellular, with delicate smooth or finely granular walls, and grow in long, wreathed chains, and in addition to covering the heads with a loosely coherent dust (usually coloured), also impart to the herbage of the mould its specific colour (green, blackish brown, yellow-brown, yellow, &c.). The globule, which is not morphologically constant for the species, and may be spherical, oval or elongated, in which latter case it does not exhibit any sharp line of demarcation from the stem, which it also resembles generally in being colourless, tough-skinned, and occasionally very brittle (*A. minimus*). In the microscopical examination of the head (freed from conidia and lightened in the course of preparation) the most important features are the relative length (in comparison with the globule), and more especially the radial (*A. niger*) or upright (*A. fumigatus*) position of the sterigmata. The number of ultimately developing secondary sterigmata (or sterigmata of any order in comparison with the supporting basidia) varies from 2 to 12 according to the species and other circumstances, and these are all considerably more delicate and shorter than the primary forms. The form of the conidia and the character of the membrane (smooth or rough) may vary in the same species (though chiefly through the influence of the medium or of age), and their dimensions sometimes differ considerably (*A. Tokelau*, *A. oryzæ*, *A. flavus*), even in those from the same head, probably as the result of growth subsequent to constriction. In other cases, however, great regularity is observable on these points (*A. niger*, *A. clavatus*), so that in many instances the dimensions afford a reliable diagnosis. In view of the variable dimensions of the conidiopores noted in one and the same culture, apart from differences in nutrition and temperature, the value of accurate microscropical measurements is after all merely relative, though they cannot be entirely dispensed with and are even capable of affording valuable indications when intelligently applied. While scarcely necessary for the mere differentiation of dwarf and normal growths, the measurements ascertained, nevertheless,

FIG. 163.—Conidiophores of Aspergillus.—Heads, globules and sterigmata of *A. Ostianus* (1), *A. glaucus* (2), *A. fumigatus* (3), *A. varians* (4), *A. claratus* (5), *A. Wentii* (6), *A. sulfureus* (7), *A. nidulans* (8), and *A. candidus* (9). Excretion of granules from stalk and globule in *A. Ostianus* (10). Old sterigmata and globule of *A. candidus* (9, *a–c*). Fragment of globule from *A. giganteus* (high and medium adjustment combined): 11.—Magn. of all the conidiophores approximately equal (about 20–30), except *A. fumigatus* (140) and *A. nidulans* (about 80); of 7*b* about 270, of 10*a* 230, of 11 350. 7 after *Zopf*, 8 after *Eidam*, the rest after *Wehmer*.

serve to define more clearly the object examined. The fact that simple and branched sterigmata are found associated in certain species (*A. spurius*, *A. candidus*, *A. ostianus*) — which, therefore, constitute intermediate types—is not altogether favourable to the subdivision of a separate genus, *Sterigmatocystis*.

Up to the present, ascospores in the form of small globular nodules (Fig. 165) measuring about 60–300 μ in diameter, have only been definitely found in about 5 species (*A. glaucus*, *A. fumigatus*, *A. Rehmii*, *A. nidulans*, *A. pseudoclavatus*), but more will probably be discovered in time, in which event the form of these spores may be utilised as a basis of classification. Meanwhile it seems preferable to postpone the division of the genus *Eurotium* (*A. glaucus*) and to retain the conidiophores as a generic characteristic. In most cases the perithecia are fragile capsules, with thin walls, and yellow, dark red, or even black in colour (*A. glaucus*, *A. pseudoclavatus*, *A. Rehmii*, *A. fumigatus*), which, in the last two instances, is enclosed in a shell formed of specially modified, coloured, thick-walled, swollen hyphæ, but in the others is naked. A shell is also found in *A. nidulans*, but here the ascospore is tougher, the asci (sclerotia), surrounded by a dark, stratified skin, being developed later. Similar naked or sheathed sclerotia, which, however, are sterile, were observed by Wilhelm (1.) in the case of *A. niger*, *A. ochraceus* and *A. flavus*. The development, chiefly through the implication and fusion of morphologically equal hypha, as in *A. Rehmii* and *A. ochraceus*, and also the character of the asci and spores, will be found compared in the description of the various species later. In *A. glaucus* the development proceeds from a single filament, in *A. nidulans* from two. The ascospores are

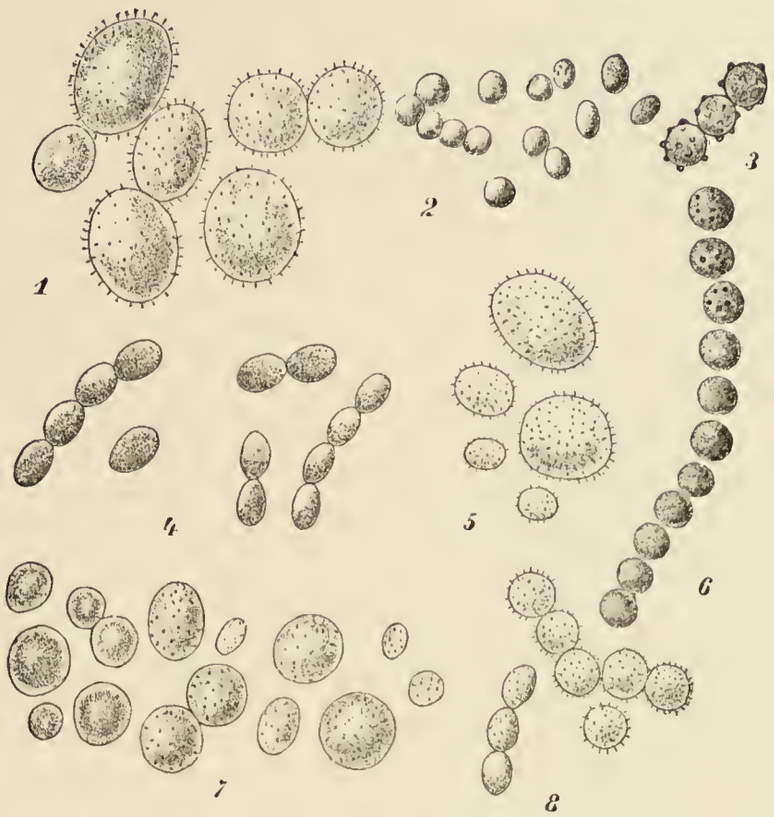


Fig. 164.—Conidia of Aspergillus.

All of approximately equal magnification, showing the differences in form and dimensions. *A. glaucus* (1), *A. fumigatus* (2), *A. niger* (3), *A. clavatus* (4), *A. Tokelau* (5), *A. varians* (6), *A. Oryzæ* (7), *A. Wentii* (8). Magn. about 1000. (Original.)

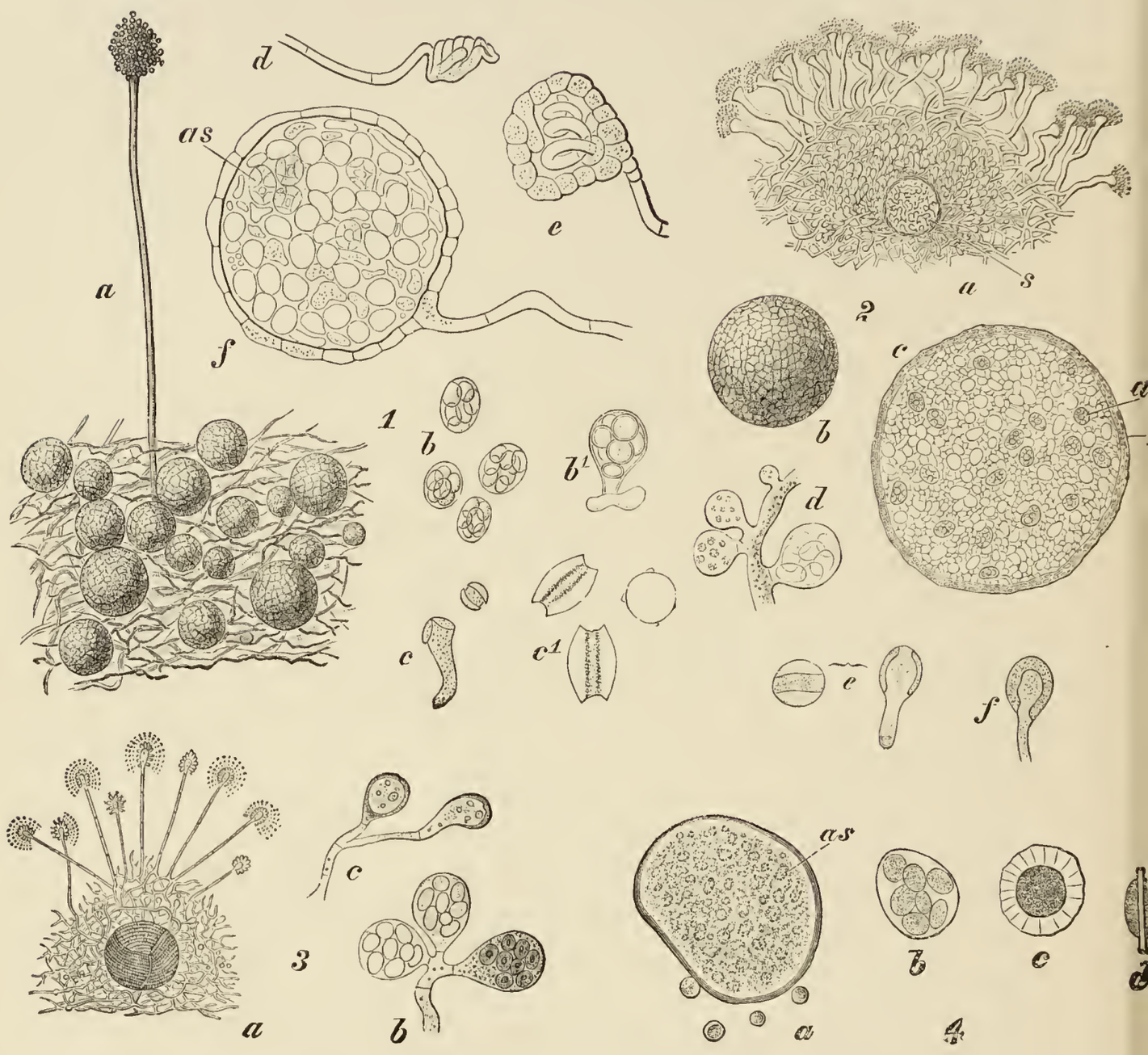


FIG. 165.—Ascospores of four species of Aspergillus.

Division 1.—Perithecia of *A. glaucus* resting freely on the substratum (*a*), also development of the perithecium from the *Eurotium* coil (*d*, *e*); at *f* a ripe perithecium with young asci; *b*, isolated asci; *c'*, ripe spores; *c*, the same germinating. (*c*, *b'*, *d*, *e*, *f*, *after de Bary*; *a*, *b*, *c'*, *after Wehmer.*) Magn. same as Fig. 169.

Division 2.—Sclerotia, with shell, of *A. nidulans* (*a*) prepared in the detached state (*b*); and section, showing sheath and asci (*c*); *d*, young asci; *e*, spores, one with germinating tube; *f*, swollen hypha of shell, with greatly thickened wall. Magn. same as Fig. 176. (*After Eidam.*)

Division 3.—Perithecia with shell, *A. Rehmii*, with separately prepared asci (*b*), and shell hyphæ (*c*). Magn. of *a*, about 100; of *b* and *c*, 1000. (*After Zukal*).

Division 4.—A Perithecium detached from the mycelial envelope of *A. fumigatus*, in section (*a*) containing asci (*as*); *b*, isolated ascus; *c* and *d*, spores with epidermal ridge, viewed from above (*c*) and from the side (*d*). Magn. same as Fig. 171. (*After Grijns*).

therefore very unequal, a proper systematic appreciation of which difference would entail the establishment of different genera, the presence or absence of a separate shell being undoubtedly a generic characteristic. However, as already stated, this must be left out

of consideration at present, unless weighty practical considerations be sacrificed to purely scientific points.

The following summary relates to species of *Aspergillus* which form perithecia or sclerotia :

A. Perithecia (with immediate formation of asci).

1. *A. glaucus*
2. *A. pseudoclavatus* } Perithecium naked without shell.
3. *A. fumigatus*
4. *A. Rehmii* } Perithecium with shell.

B. Sclerotia (asci formed after a while, or still unknown).

1. *A. nidulans*, with retarded formation of asci, and shell.
2. *A. ochraceus*
3. *A. niger*
4. *A. flavus* } Asci not yet observed. With or without simple mycelial sheath.

Eidam applied the name perithecium also to the tough-skinned organs of *A. nidulans*, which develops asci gradually, but also emphasises their intermediate position between the *Eurotium* capsules and the sclerotia.

Undoubtedly a large number of species will have to be struck out of the present list of about 120, the diagnosis made by older workers having been in many cases insufficient for the establishment of a new species. Their general practice was merely to describe and not compare, the latter having been difficult, owing to the scattered literature, previous to the appearance of Saccardo's *Syllogæ*. Probably not more than two or three dozen are really admissible, a circumstance that unfortunately has not been duly considered by modern German workers, Lindau (I.), for instance, mentioning no less than 55 species, of which only 17 are classed as doubtful ; and only a small fraction of these have any interest for the technical mycologist. Nevertheless this genus is more important than the majority, since it comprises not only several species that find industrial application (*A. oryzæ*, *A. Wentii*, *A. luchuensis*), but also others noteworthy on account of chemico-physiological considerations (*A. niger*) and several which are pathogenic toward men and animals (*A. fumigatus*, *A. flavus*, *A. nidulans*), whilst others again (*A. glaucus*, *A. phœnicis*, *A. clavatus*, *A. fumigatus*) are occasionally found in industrial processes, commercial products, food-stuffs, &c. Whether certain species are directly pathogenic toward plants may be left out of consideration, though, according to Pammel, Weems, and Lawson-Scribner (I.), *A. glaucus* and others are the cause of disease in embryo grasses. On the other hand, J. Behrens (XVI.) found *A. glaucus* (= *A. medius*, Meissner) harmless, but *A. niger* dangerous. At all events, the genus *Aspergillus* forms the most interesting, because the most diversified, genus of fungi, except for the *Saccharomycetes*.

In the differentiation of species, the first point to consider is the colour of the herbage (young growth exclusively!); then the size and build of the conidiophores and conidia, and, finally, the physiological characteristics, such as food requirements, optimum temperature, energy of growth, special influences, &c. Moreover, the influence (if any) of the substratum—sugar, albumin, and also gelatin—must be observed in each case. Differences that have not yet been fully appreciated also exist in the behaviour toward gelatin, and in the production of colouring-matters in the mycelium or nutrient solution, &c. Attempts to identify old vegetative growths from the colour of the conidia, which is liable to a speedy change, especially in green species, are not to be recommended, the preparation of a young culture being essential, and other characteristics are liable to alteration as the cultures become aged. Many of the old *soi-disant* species undoubtedly owe their alleged existence to insufficient appreciation of these circumstances.

The conidiophore can in many cases be identified by the unaided eye, since it measures about 1–2 mm. in height (*A. niger*, *A. glaucus*, *A. oryzæ*, *A. clavatus*, *A. candidus*, &c.). Sometimes, under favourable conditions of growth, the length is nearly doubled (*A. Wentii*, *A. ochraceus*, &c.), whilst in adverse circumstances it may be considerably less (0.5–0.25 mm.). Such dwarf conidiophores are of common occurrence in otherwise luxuriant species (*A. oryzæ*, *A. candidus*, *A. glaucus*), accompanied by morphological modifications. Only a single species, *A. giganteus*, far exceeds the average height in its mucor-like conidial vegetation, the slender conidiophores averaging 1–2 cm. in length. Numerous species are characterised by the constant formation of small and very small conidiophores (*A. fumigatus*, *A. nidulans*, *A. minimus*, *A. Rehmii*, *A. spurius*, *A. flavus*), which cannot be detected as such by the unaided eye, except under favourable conditions, their length averaging less than 1 mm., and occasionally falling below 0.5 mm. (*A. fumigatus*, *A. minimus*, *A. Rehmii*), or even as low as 0.1 mm. (*A. fumigatus*), so that the nearly smooth surface growth closely resembles *Penicillium*.

The dimensions of the conidia vary between the limits of about 3 and 10 μ, the latter size being rarely exceeded. Some species invariably produce microspores exclusively, the conidia measuring only about 3μ in diameter (*A. nidulans*, *A. minimus*, *A. fumigatus*, and frequently *A. niger*). The other extreme is reached by the species forming macrospores, with conidia measuring at least 5–6 μ, and often irregular in size (*A. glaucus*, *A. flavus*, *A. oryzæ*, *A. Tokelau*), attaining a diameter of 7–10, and sometimes as much as 15 μ, in the case of *A. glaucus* and *A. Tokelau*. An intermediate position in this respect is occupied by the species (*A. candidus*, *A. clavatus*, *A. Wentii*, *A. giganteus*, &c.) with conidia measuring about 3.5–5 μ, these being preferably classed

with the *Microsporeæ*, the limit being fixed at 5 μ. In contrast to the conidia formed by successive constrictions of the tips of the sterigmata, and sometimes connected by delicate "intermediate cells," the primary sterigmata are formed by protrusions from the surface of the globule, sometimes before the stem has attained its full extension. The aperture of communication in the wall of the globule is but rarely (*A. giganteus*) visible under the microscope as a fine capillary channel (Fig. 163, *c*). The secondary sterigmata, first observed by Berkeley (in 1857) and Cramer (in 1860), are formed in succession from their parent cell.

Malformations are by no means rare in many species (*A. glaucus*, *A. oryzæ*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. fumigatus*, &c.), and have often been described. They include outgrowth of sterigmata into elongated tubes, vegetative hyphæ, and even into dwarf conidiophores; globular swellings of the vegetative hyphæ; irregular branching of the conidiophore apex, the globules being no longer formed; forking of the stem; abnormal branching of otherwise simple sterigmata, &c. It is sufficient here to merely record such (really unimportant) facts in order that they may be appreciated at their true value when observed. The occurrence of septa in the stem (especially in *A. flavus*), and sterigmata should probably be placed in the same category; at least this, and the often observed branching of the conidiophores, seem to be merely sports and not constant characteristics, the conidiophore, as a rule, consisting of an unbranched, unicellular hypha.

It is also worthy of notice that many species flourish best at a high temperature (about 35°–40° C.), *e.g.*, *A. flavus*, *A. niger*, *A. oryzæ*, *A. clavatus*, *A. fumigatus*, *A. nidulans*, *A. Wentii*, a fact which may be utilised in the rapid differentiation of species. Among the kinds known at present, only *A. glaucus* and *A. candidus* exhibit a preference for low temperatures, though even many of the heat-loving species will pull through at very low temperatures (*A. niger* and *A. oryzæ* will grow below 10° C.).

A summary of the species may now be given, this being confined to the better known or more fully described (newer) kinds, omitting the numerous older and often unrecognisable ones. Those of technical importance are indicated by thicker type. It should be noted that the colour of the vegetation is not invariable, being influenced by the substratum, some green or white species, for example, occasionally becoming yellow, whilst, according to Vuillemin, the colour of *A. versicolor* ranges from green to red. The species in group 4 undoubtedly include several synonyms, and also the white (2) and blackish brown (3) kinds require elucidation, so that in reality only the green species can be regarded as anything like properly established.

Summary of the Aspergillus Species grouped according to the Colour of the Conidial Vegetation, the Character of the Sterigmata, and the Existence of Ascospores.

1. Green (grey, bluish green or yellow-green), viz. :
   (*a*) With simple sterigmata. **A. glaucus,** Link, with ascospores (naked perithecia) ; *A. clavatus*, Desmazières ; **A. fumigatus,** Fresenius, ascospores (cased perithecia) ; **A. oryzæ,** (Ahlburg) Cohn ; *A. varians*, Wehmer ; *A. minimus*, Wehmer ; **A. flavus**, Link, with sterile sclerotia ; *A. giganteus*, Wehmer ; *A. cæsiellus*, Saito ; *A. Tokelau*, Wehmer ; *A. penicillopsis* (Hennings), Raciborski.
   (*b*) With branched sterigmata : *A. nidulans*, Eidam, ascospores (ensheathed sclerotia) ; *A. pseudoclavatus*, Puriewitsch, ascospores (naked perithecia) ; *A. variabilis*, Gasperini ; *A. versicolor*, Vuillemin.
2. White, viz. :
   (*a*) With branched sterigmata (associated with simple sterigmata in the case of *A. candidus I.* ; *A. candidus I.*, Wehmer ; *A. albus*, Wilhelm.
   (*b*) With simple sterigmata: *A. candidus* (Link), Saccardo.
3. Blackish brown, viz. :
   (*a*) With branched sterigmata. **A. niger** (Cramer), van Tieghem, with sterile sclerotia ; *A. phœnicis*, Fat. and Delacr. ; *A. strychni*, Lindau ; *A. pulverulenta*, MacAlpine ; *A. atropurpureus*, Zimmermann ; *A. violaceo-fuscus*, Gasperini.
   (*b*) With simple sterigmata. **A. luchuensis**, Inui ; *A. calyptratus*, Oudemans.
4. Brownish yellow, yellow, brown and reddish, viz. :
   (*a*) With simple sterigmata : *A. ostianus*, Wehmer ; **A. Wentii**, Wehmer ; *A. perniciosus*, Inui ; *A. giganteo-sulfureus*, Saito ; *A. citrisporus*, von Höhnel.
   (*b*) With branched sterigmata (occasionally associated with simple ones). *A. sulfureus*, Fresenius ; *A. ochraceus*, Wilhelm (with sterile sclerotia) ; *A. Rehmii*, Zukal (with ensheathed perithecia) ; *A. spurius*, Schröter ; *A. elegans*, Gasperini ; *A. auricomus*, Guéguen (with sterile sclerotia).

§ 284. Aspergillus Species with Simple Sterigmata.

Aspergillus oryzæ, (Ahlburg) Cohn (= *Eurotium oryzæ*, Ahlburg). This species is of practical importance as a saccharifying fungus, and has been cultivated for centuries in Japan for the preparation of the rice mash for Saké, as well as for the production of Soja sauce and Miso. It was first identified (as *Eurotium oryzæ*) by Ahlburg (I.) in 1876, and was renamed *Aspergillus oryzæ* by Cohn (XIII.) in 1883, after which it was examined by Büsgen (IV.) though the full morphological description—by Wehmer (VIII.)—was not given until 1895. The species illustrated in Fig. 166 produces a luxuriant mould vegetation, which is usually yellow-green (rarely yellow), with large, closely set tough conidiophores about 2 mm. high. It grows rapidly on a large variety of liquid and solid media, and is easily cultivated even at room temperature, the optimum temperature being above 30° C. After several weeks, or even months, the colour sometimes

gradually turns brown. The peculiarities of the conidiophores, sterigmata and conidia enables the species to be distinguished with comparative ease from most others, *A. flavus* alone being similar. The clavate or spherical globule, which varies in size and shape, usually exhibits no definite line of demarcation from the smooth or finely granular, pale stem. The sterigmata are radial—or in small conidiophores—confined to the summit and pointing upward; slender, simple, large, yellowish green, spherical conidia (6–7 μ thick, smooth or finely granular), undergoing constriction into chains which rapidly fall asunder; the size and form, however, vary considerably. The green heads may measure over 100 μ across, with globule up to 80 μ in diameter, though often much smaller, appear in all sizes. The sterigmata on well-developed heads measure 12–20 by 4–5 μ, and therefore differ greatly from the short, compact sterigmata of *A. glaucus*. No ascospores or sclerotia have yet been observed; and the same applies to budding cells (the alleged Saké "yeast"), which, though frequently stated to exist, have never yet been described with any precision. Malformations of the conidiophores (forked stem, outgrowth of sterigmata to filaments or delicate conidiophores, and also branching) are not infrequent. This species secretes a very active diastase (*see* § 290); it has been recommended and tried as a malt substitute in Europe as well as in the Orient, KORSCHELT's first report (II.) in this connection (1876) having been succeeded by a number of chemico-physio-

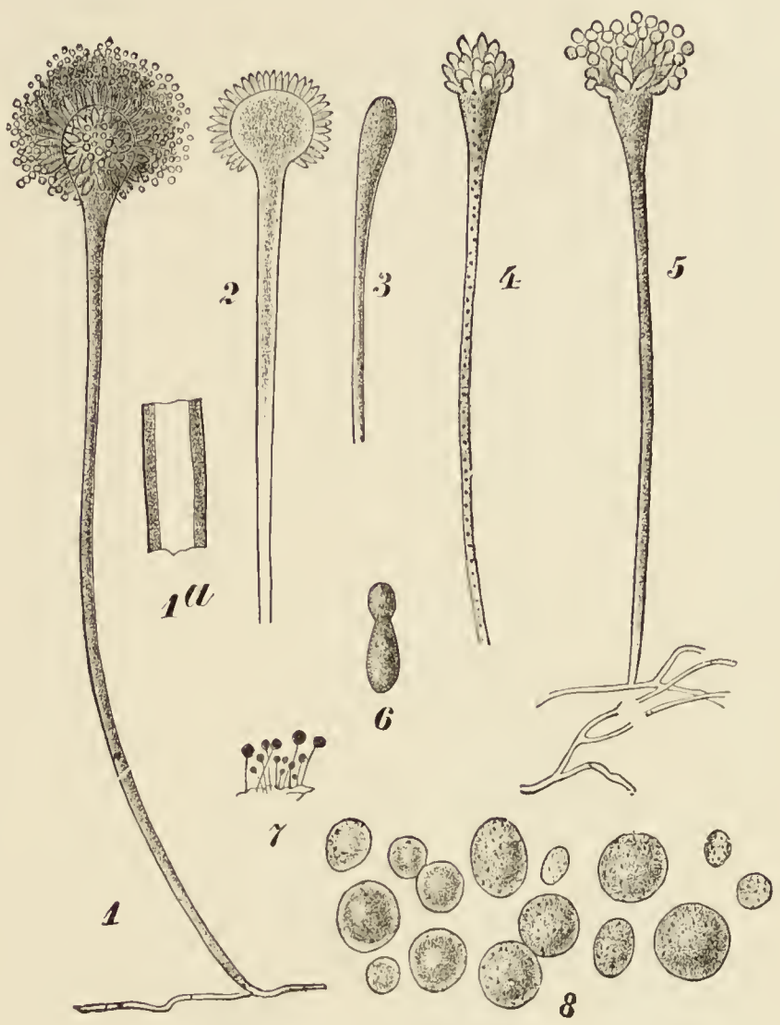


FIG. 166.—Aspergillus oryzæ.

1–2. Conidiophores with clavate and almost spherical globule. 2. In optical section. 3–5. Development of a small conidiophore, distension of the hypha, protrusion of sterigmata and incipient formation of conidia. 1*a*. Optical section of tough stem. 6. Sterigma. 7. Conidial herbage, slightly magnified. 8. Conidia. Approximate magn. of 1–5, 75; of 6, 400; of 8, 900. (*After Wehmer.*)

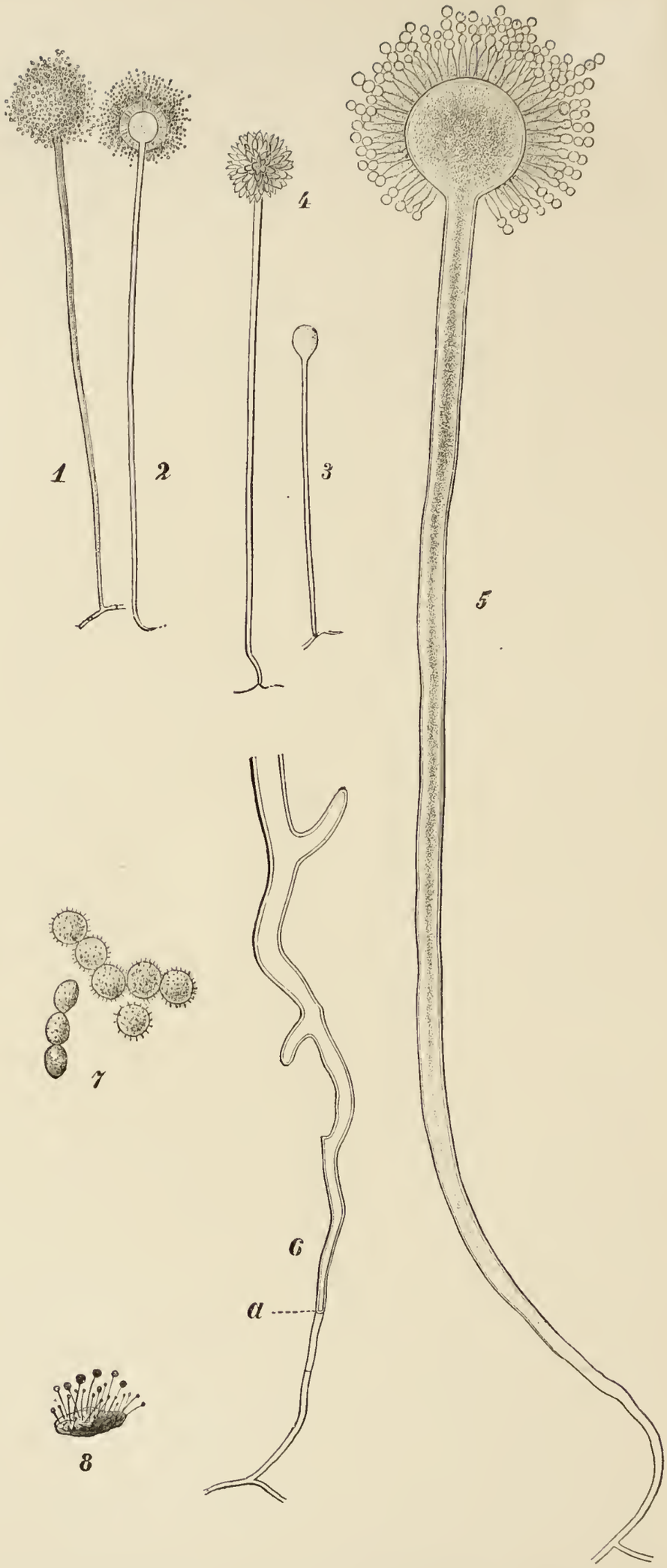


FIG. 167.—Conidiophores (1–5), 2 and 5 in optical section. Figs. 3–5, development of the globule (3) and sterigmata (4). The separation of the conidia is beginning in 5. 6. Base of conidiophore, with lateral protrusions. Conidia (7), young herbage (8). Approximate magn. of 1–5, 20; of 5 and 6, 120; of 7, 900. (*After Wehmer.*)

logical investigations and technical communications on this plant which has been cultivated in Japan from time immemorial—compare Wehmer's compilation (XVII.). According to the latter authority, the conidia remain capable of germinating for years. Hiller (I.) states that the substratum on which the fungus is grown has an influence in this connection, certain nutrient media (wort) being favourable, whilst others (dextrose) are the reverse. Fuller morphological particulars are given by Cohn (XIII.), Büsgen (IV.) and Wehmer (XVII. and VIII.).

Fig. 168.—Aerial mycelium of *Aspergillus Wentii*, with conidiophores, growing rank in a culture flask. About natural size. (*After Wehmer*).

Aspergillus Wentii, Wehmer, was observed by Went in the preparation of Tas Yu (*see* vol. i. p. 323) according to the method practised in Java, and was described by Wehmer (XIX.) in 1896. It appears spontaneously on the boiled Soja beans that have been covered with *Hibiscus* leaves, and effects a loosening and disintegration of the firm tissue of the bean. The species forms a pale coffee-coloured, dense mould vegetation (Fig. 167), with conspicuous conidiophores, about 2–3 mm. in height, their thick brown heads (up to 200 μ in diameter) showing up clearly on the pale, slender, tough-skinned, smooth stalks, and being quite unmistakable for any other species. The decidedly spherical globule (75–90 μ in diameter), sharply contrasting with the stalk, is covered on all sides with a dense growth of slender radial, simple sterigmata (mostly 15 by 4 μ), from which the small coloured, globular to elongated, finely punctated or smooth conidia (about 4–5 μ in diameter) separate by constriction. The mycelium,

which is snow-white, though sometimes red—and in old cultures reddish brown—when grown in closed culture vessels attains a considerable height above the substratum, and also throws up a large number of conidiophores under these conditions (*see* Fig. 168). No perithecia or sclerotia have yet been discovered. This quick-growing species, which can be easily cultivated on the usual mycological substrata, flourishes particularly well in the incubator (above 30° C.). Nothing is known about the enzyme, which plays an active part in the decomposition of the Soja bean. Like the preceding species, this fungus does not belong to the European flora, though both will grow well here.

Aspergillus glaucus, Link (*Eurotium Aspergillus glaucus*, A. de Bary), is the ordinary green mould, which grows everywhere, especially on dried plants, old black bread (pumpernickel), skins, jam, old leather articles, herring pickle and other materials. This species has long been known, and is met with in the literature under various names: *Eurotium herbariorum*, Link; *Aspergillus herbariorum*, *Eurotium Aspergillus glaucus*, de Bary; *Eurotium glaucum* (*E. repens* also seems to be the same fungus). A. de Bary (IX.) in 1859 identified the ascospores (VIII.) of the so-called *Eurotium herbariorum* with the conidiophore form of *Aspergillus glaucus*, Link, and showed the two to be one and the same fungus. The young conidial herbage is pale green to verdigris-coloured, but darkens quickly to a dirty greyish green or greyish brown, the mycelium also changing colour by the deposition of pigment granules, and becoming pale yellow, which turns a dirty rust-brown. Consequently, old vegetations are often entirely discoloured and ugly, new sowings being requisite for the identification of the fungus. Hence culture experiments are necessary, the characteristics of old growths being unreliable. Many of the different earlier *Aspergillus* species, established on the basis of such material, are probably nothing more than old vegetations of *Aspergillus glaucus*, and should be struck off the list. Some herbages exhibit conidia exclusively, whilst others produce only numerous golden yellow perithecia (for example, on cranberry jam). The conidiophore shown in Fig. 169 (1–3 mm. high) is readily distinguishable from other species; the globule, sterigmata and conidia present characteristic features. The globule, which is not sharply demarcated from the stalk, is spherical to knob-like, measuring about 60 μ across, and thickly covered all over with very short, simple sterigmata (up to 14 μ by 7 μ), dividing into unusually large, prickly, globular or slightly elongated conidia (7–30 μ and more in diameter). These latter are remarkably large in proportion to the sterigmata (about half as broad as these are long), which are plump, their length being only about double the width, and not slender and pointed as in many other species. *A. glaucus* has larger conidia than any other well-known species, and none of the others produces perithecia with such

readiness and abundance. These are small (about 100–200 μ in diameter), pale brown-yellow-coloured at first, afterwards ugly brown capsules with a simple, delicate envelope, and enclosing numerous rounded oval asci. Each ascus contains 5–8 colourless, smooth, ellipsoidal spores, exhibiting a longitudinal furrow and

FIG. 169.—Aspergillus glaucus.

Conidiophores (1, 2), sterigmata (3) and conidia (4). A portion of mycelium, with overlying perithecia and a conidiophore (magnified) is shown at 5, whilst 6 gives the natural size. Sections of perithecia are given at 7, with young asci (*as*) and first stages of development (*Eurotium coil*); isolated asci (8) and detached spores (9), germinating at *c*. Approximate magn. of 1–2, 50; of 7, 170; of 8, 260; of 9c, 700. (1, 7, 8, 9 (in part) *after de Bary*, the rest *after Wehmer*.)

measuring 7–10 μ long by 5–8 μ broad. The spore wall bursts open and ejects the exospore, which germinates to a new mycelium. The gradual development of the perithecia from spirally coiled hyphæ need only be briefly mentioned here, having been already described in the majority of botanical works. This proceeds both in the presence and absence of light, and therefore—as in the formation of conidia in *A. niger*, &c.—is not hindered by the action of light, as was assumed by ELFVING (I.). The same author's assertion respecting the formation of yeast cells is also dubitable and lacking proof. According to LINDNER (II.) the conidiophores

can be made to branch abundantly by restricting the food-supply or adding antiseptics.

In point of substratum this industrial and domestic fungus is selective, since it does not thrive on (*e.g.*) liquid saccharine media with mineral salts and inorganic nitrogenous food, whereas black bread or—as a good bacteriological substratum—wort gelatin, is favourable. The fungus also prefers moderate temperatures (it will grow at 8°–10° C.) and ceases to develop at blood temperature, consequently its alleged occasional appearance in the human ear—recently mentioned again by Hatch and Row (I.)—is probably a mistake arising from the species having been confused with *A. fumigatus* or *A. flavus*. Nomura (I.) states that it is associated with *A. flavus* in the cocoon fungus ("Uchibaki") which does so much damage to the silk industry, and was first attributed to *Aspergillus* species by Raux (I.). It is certainly a chief source of mould in black bread, and is stated by J. Behrens (III.) to be a frequent, injurious dweller in "shed-ripe" tobacco and cigars (*see* vol. i. p. 167), as well as in hops. According to Spieckermann and Bremer (I.) it is the cause of mould in cotton-seed meal; and perhaps it is among the still undescribed species of *Aspergillus* that damage leather. Aderhold (IV.) found it in acid gherkin pickle; and it thrives on smoked meats (ham), preferring very dry substrata. Whether, in certain cases, it is actually pathological toward plants and takes part in the blackening and spoiling of chestnuts, as was stated by Roze (I.), still remains to be fully investigated Occasionally it is found on the kernels of walnuts and hazel-nuts still in the shell, an abundance of perithecia being produced. The limits of temperature for this species are 7°–37° C., the optimum being 27°–29° C. according to Klebs (I.), though others give the optimum at 20°–25° and the maximum as 30° C. (*Elfving*, *Siebenmann*). More complete morphological data are furnished in the works of A. de Bary (IX.), Wilhelm (I.), Siebenmann (I.), R. Meissner (I.), and Wehmer (XVII.).

The fungi termed *Eurotium repens*, de Bary, and *E. Aspergillus medius*, Meissner (I.), are presumably the same as *A. glaucus*, since there are no tangible differences between them exceeding the usual limits of variation. It is, nevertheless, highly desirable that this doubtful point should be finally settled by careful investigation. Whether the *Eurotium rubrum*, Spieckermann and Bremer (I.), found in mouldy cotton-seed meal is a different species also seems questionable, and requires elucidation.

Aspergillus flavus, Link, greatly resembles *A. oryzæ* in the yellowish green superficial colour of the herbage, and also in the shape of the conidiophores; but is readily distinguishable by the smaller dimensions of the latter (less than 1 mm. high). This species, which has been identified as pathogenic in animals, has a preference for warmth, the optimum temperature being about

37° C. It is frequently observed in cases of mycosis of the human ear (where it is sometimes confounded with *A. glaucus*), and also occurs on bread, portions of plants, and dried excrement. Even at blood temperature it thrives luxuriantly on all kinds of mycological substrata, and rapidly produces extensive yellow-green growths of mould. The superficial colour is rarely pure yellow, and the older growths (several weeks or months) are very liable to turn colour, becoming finally an ugly dark brown. The conidiophores (Fig. 170), which generally measure less than 1 mm. (0.5–0.7 mm.), carry a spherical or club-shaped globule, which rarely springs in a sharply defined manner from the pale, warty stem; and the simple, slender sterigmata, which are generally disposed radially, though sometimes confined to the summit, develop large conidia (average 5–6 μ in diameter), which are generally of an irregularly globular shape, and smooth (sometimes finely granular), separate, by constriction, into chaplets, which readily become dissociated. The coloured heads measure up to about 90 μ, the globules 30–40 μ in diameter, the sterigmata usually about 20–60 μ. The conidia vary between 4 and 8 μ in diameter, but in any event the species (with *A. glaucus* and *A. oryzæ*) belongs to the large-spored class. No perithecia have been observed, but Wilhelm (I.) in 1877 described small, black, nodular sclerotia (about 0.7 mm. in diameter), with a thick skin and pale core, which remained sterile in germination tests. These appear to be formed by simple intertwining and fusion of morphologically uniform filaments. Further morphological details are furnished by Wilhelm (I.), Siebenmann (I.), and Wehmer (XVII.).

Fig. 170.—Aspergillus flavus.

Conidiophores with spherical to club-shaped globules and simple sterigmata (1–4), the outer wall of the (frequently septate) stem being roughened by colourless granules (5). 6. Conidial herbage (about 2/1). 7. Conidia. Magn. of 1–4, 140; of 5, 400; of 7, 500. (*After Wehmer.*)

According to Nomura (I.), this fungus is the chief source of

injury in the cocoon disease of silkworms, but does not play any other industrial *rôle*, though sometimes found as a subordinate fungus on mouldy cotton-seed meal. In the literature it is often, but erroneously, classed as *A. flavescens*, Wred., a species which should be struck out as merely synonymous; and, in view of the lack of perithecia, de Bary's name, *Eurotium A. flavus*, is also inappropriate. The *A. subfuscus*, Johan-Olsen (III.), still encountered in the literature, is probably only *A. flavus*.

Aspergillus fumigatus, Fresenius, a cosmopolitan green to greyish green (not yellow-green) species, characterised by a high optimum temperature (about 40° C.) and rapid growth, is chiefly of medical interest (pathogenic), but occasionally acts injuriously in industrial processes carried on at high temperatures, such as certain fermentations (*e.g.*, lactic fermentation). According to Behrens, it occurs on the ribs of fermenting tobacco leaves (*see* vol. i. p. 167); and on one occasion Wehmer (I.) also found it, in the form of large patches, on woollen fabrics. It likewise attacks vegetables (decaying potatoes, bread, malt, beer wort, &c.) in the incubator; and, according to F. Cohn (XIV.), it also produces a thermogenic effect (*see* vol. i. p. 151). Of greater importance is its frequent occurrence in the body cavities of men and animals (*e.g.*, the human ear and the lungs of various birds), where it produces otomycosis and pneumomycosis, which latter malady, according to Rénon (I.), is almost invariably found among workers in certain trades, such as pigeon-fatteners and hair-combers (in Paris). Conidia introduced into the arterial circula-

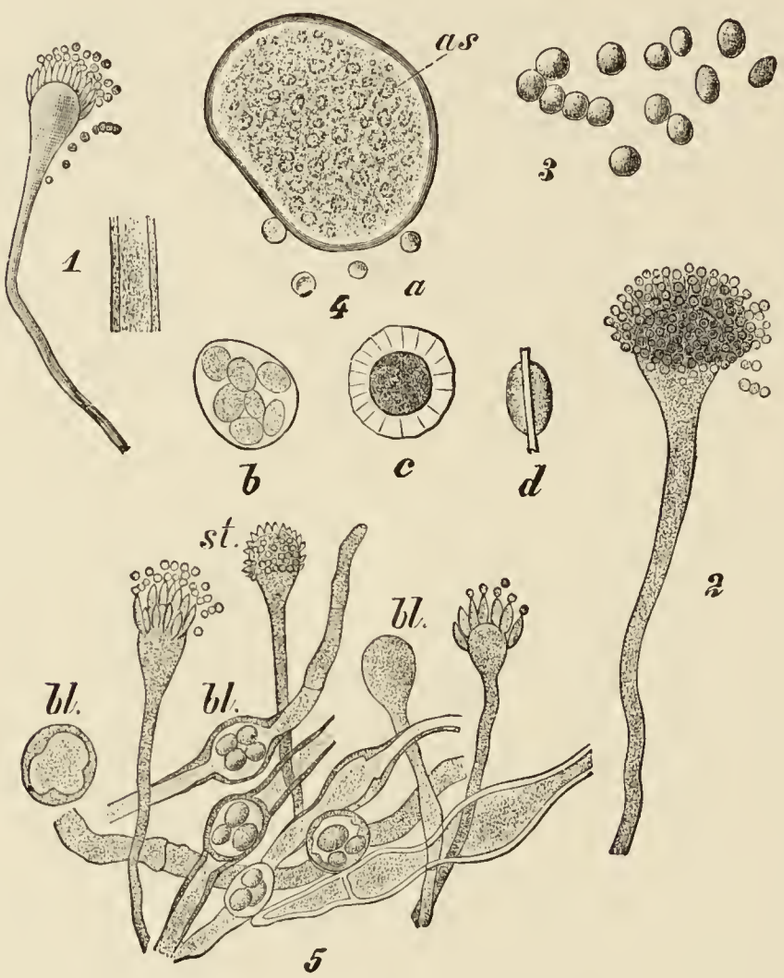


Fig. 171.—Aspergillus fumigatus.

1–2. Club-shaped conidiophores (in optical section at 1). 3. Conidia. 4. Ascus and ascospores. 5. Hyphæ (*bl*) with peculiar globular swellings, and conidiophores, from herbage. Approx. magn. of 1, 2, 5, 140; of 3, 1000; of 4*a*, 70; of 4*b*, 719; of 4*c* and 4*d*, 2250. (4*a–d after Grijns*, the rest *after Wehmer*.)

tion of animals germinate in the body and produce serious illness, which has mostly a fatal termination. The species was first discovered by Fresenius (I.), in 1841, in the bronchi and air cavities of a bustard. The conidial herbage, however, is not—as the name would imply—smoky grey, but penicillium-green, though quickly turning to grey and even to dirty brown. It is readily identified by the dwarf conidiophores (Fig. 171) 0.1–0.3 mm. long, with club-shaped globule (10–20 μ), thick, simple, slender, upright sterigmata (6–15 μ long), grouped on the crown, and with long chains of very small (2–3 μ) conidia, mostly globular. Hence, racial variations apart, the species cannot easily be mistaken for any other. Though J. Behrens (XIV.) mentioned the occurrence of perithecia, and Siebenmann (I.) sclerotia, we are indebted to Grijns (I.) for a description of the true perithecia. According to this worker, they are small, globular, nut-brown bodies, measuring 250–350 μ in diameter, with a special integument, from which the true, dark red perithecium (which has a very fragile, stratified coloured wall) can be extracted without difficulty. The interior consists of a colourless network of filaments, surrounding a number of colourless, oval, thin-skinned asci (14–9 μ), each of which contains eight red lenticular, tough-skinned spores (4–4.5 μ), surrounded by a pale, radially striped equatorial ledge. Hence a considerable difference exists between the frontal and lateral appearance of these spores, which, moreover, do not become coloured until shortly before maturity. These asci, which recall those of *A. nidulans* (Eidam) in the appearance of the shell, were found in large numbers by Grijns on the surface of the herbage and a number of cultures derived therefrom. Owing to the resemblance of the ascospores to those of *A. nidulans*, Vuillemin (I.) considered—which is hardly probable—that Grijns was really dealing with the last-named species. Further morphological details are furnished by Fresenius (I.), Siebenmann (I), Behrens (III.), Wehmer (XVII.), and Grijns (I.). *A. nigrescens*, Rob., and *A. bronchialis*, Blumentritt (I.), appear to be synonymous with *A. fumigatus*, though Blumentritt (II.) recently confirmed the existence of small variations from the cultures of *A. bronchialis*. Costantin and Lucet (II.) wish to subdivide *A. fumigatus* into a number of forms differing in part by their pathological behaviour; and they also describe new allied species: *A. Lignieres* and *A. virido-griseus*. With regard to races of *A. fumigatus* and pathogenic species, compare Savoff (I.), Savouré (I.), Bodin (I.), Guéguen (III.), and Macé (II.).

Aspergillus luchuensis, Inui. According to Inui (III.), this recently described mould fungus plays a similar part in the preparation of "Awamori"—a beverage resembling whisky—in the Loochoo islands to that filled by the rice *Aspergillus* in making Saké, is similar, morphologically, to *A. Wentii*, though more like *A. niger* in colour. The conidiophores (Fig. 172),

which are 1–2 mm. in height, develop blackish brown heads (40–80 μ thick), whose spherical (rarely knob-like) globules (20–30 μ in diameter) are thickly covered with radially arranged, conical sterigmata, bearing spherical conidia (4–5 μ thick), covered with tiny wart-like protuberances. No perithecia have been observed up to the present. This species saccharifies starch—

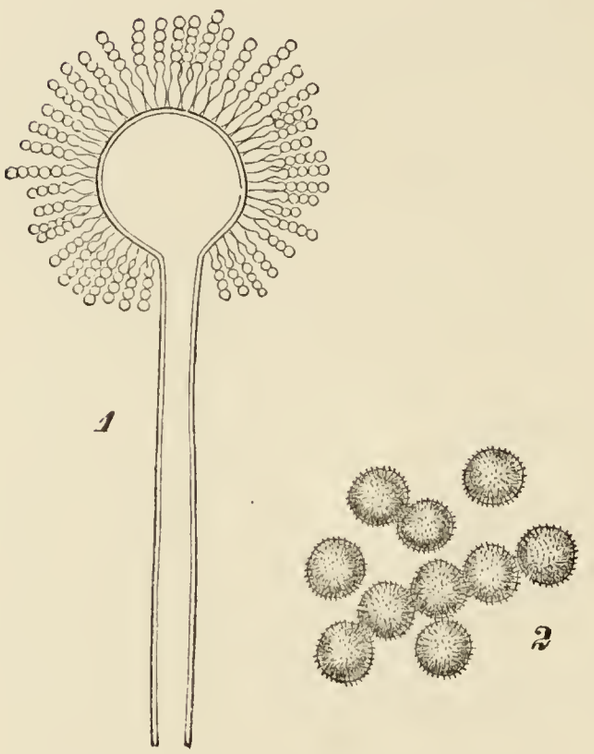


FIG. 172.—Aspergillus luchuensis. Conidiophores and conidia. Approx. magn. of 1, 300; of 2, 1000. (*After Inui.*)

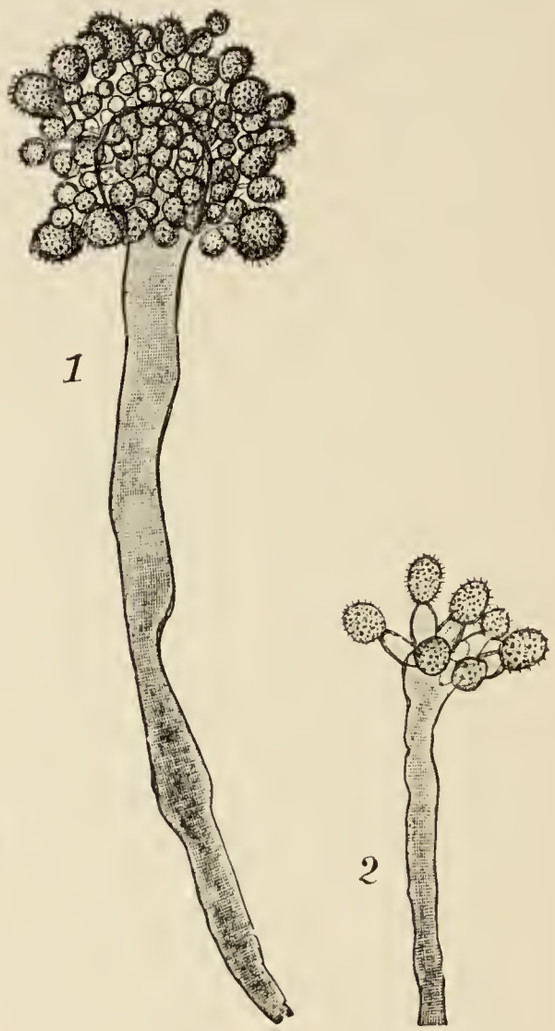


FIG. 173.—Aspergillus Tokelau. Conidiophores of different sizes from diseased human skin. Approx. magn. 300. (*After Wehmer.*)

in which respect it deserves further investigation—and is very similar to *A. Wentii*, apart from its colour and somewhat smaller dimensions. The optimum temperature of growth is between 30° and 35° C.

Associated with this species in Awamori koji, INUI (III.) found another, which is morphologically analogous, but of a greyish brown colour (*A. perniciosus*), which, however, is merely regarded as an impurity, and is subordinate, or entirely absent, in good koji.

Aspergillus Tokelau, Wehmer. This species was discovered by TRIBONDEAU (I.) in the infectious "Tokelau" or Samoa disease, attacking the natives of certain of the Pacific islands. It was

named "Lepidophyton" at first, and is chiefly of medical interest, though worthy of note as parasitic on the human skin. Whether the species plays a more comprehensive part in that disease (formerly known as "Trichophytis") remains to be ascertained; but at any rate the fungus is a true *Aspergillus*, and indeed, according to WEHMER (XIX.), a well-defined new species, characterised by large hairy conidia (up to 12 μ in diameter), resembling those of *A. glaucus* (*see* Fig. 173), growing on conidiophores

FIG. 174.—Aspergillus clavatus.

Conidiophores in various stages of development, with elongated globule and simple sterigmata, shown in optical section at *c*, with incipient conidia at *e*. A slightly magnified herbage is seen at *f*. Conidia (*g*), sterigma (*h*), section of stem (*i*). Approx. magn. of *a* and *b*, 30; of *c* and *d* 60; of *e*, 120; of *g* and *h*, 1000. (*After Wehmer.*)

which at times are like those of *A. glaucus*, and at others those of *A. fumigatus*.

Aspergillus clavatus, Desmazières, a species often found on vegetables, and, according to P. LINDNER (XXXIII.), more especially on green malt, on which it forms a pure green (not yellow-green!) herbage, is otherwise devoid of practical importance. It is distinguished by the peculiar elongated globule

(similar to a gun sponging-rod, and measuring 150 μ by 35 μ), such as are observed in *A. pseudoclavatus* (with branched sterigmata) and *A. giganteus;* and was described by Desmazières (I.), in 1834. The short, simple sterigmata (about 8 μ by 3 μ)—*see* Fig. 174—divide by constriction into small, oval (not globular!), smooth conidia (4.2 by 2.8 μ) in long chains, enveloping the elongated heads with a greyish green dust. The length of the conidiophore stems, which are 15–25 μ thick, reaches about 2 cm., but seldom exceeds that figure.

Other species mentioned in the literature as occurring on plants are the large, greenish yellow *A. penicillopsis* (Hennings), Raciborski (II.), as well as the dwarf, olive-coloured *A. Delacroixii* (Delacroix), Saccardo and Sydow, observed by Delacroix on cocoa beans. Both these species deserve further investigation. Among other well-known species we will only mention the green *A. varians*, Wehmer, *A. minimis*, Wehmer (observed on leaves and on sugar solutions), and *A. ostianus*, Wehmer (found on leaves and boiled rice), which latter species is characterised by a pale ochreous pigment. There are also a number of more or less completely described species, chiefly observed on vegetables, and for the most part not yet cultivated, including the new *A. calyptratus* and *A. Koningi*, Oudemans (II.) and *A. citrisporus*, F. von Höhnel (I.).

As a giant among its kind, mention may also be made of *Aspergillus giganteus*, Wehmer (XVII.). The conidiophores of this fungus (which grows on sour wort) resemble those of *A. clavatus* in the shape of the globules and sterigmata, and attain an average length of 1–2 cm., and therefore about ten times that of most of the other species. Before the heads begin to colour, the herbage has a mucor-like appearance, and it is only later that it is clearly distinguishable from the greyish yellow or dark mucor vegetation by the greyish green tinge of the conidial heads (1000 by 120–125 μ) on their slender, pale, saffron-yellow stems. The short sterigmata (9–12 by 4–5 μ), which are invariably simple and thickly cover the surface of the globule (500–800 μ by 80–100 μ), produce comparatively small, smooth, oval conidia, measuring on the average 4 μ by 2.6 μ. The species thrives on the usual substrata at room temperature and is easily cultivated. A noteworthy feature is the readily detectable perforation of the wall of the globule below the sterigmata, in the form of a narrow channel (when viewed in section, and a tiny circle when viewed in plan) appearing inside the larger one corresponding to the diameter of the sterigma, and causing the globule to seem as though covered with small concentric circles (*see* Fig. 163, 11). No perithecia have yet been observed.

§ 285. Aspergillus Species with Branched Sterigmata[1] (Sectio Sterigmatocystis).

Aspergillus niger, van Tieghem = *Sterigmatocystis antacustica*, Cramer = *Sterigmatocystis niger*. This well-known and widely spread species, which has been frequently studied from a chemico-physical standpoint and has a literature of its own, can be identified by the brownish black conidial herbage, with imposing, stiff, slender conidiophores, several millimetres in height. In any event, every *Aspergillus* of this colour described in the literature under some other name needs careful identification. Specific names, such as *A. nigricans*, Wreden (1869); *A. nigrescens*, Robin (1851); and *A. nigricans*, Cooke, should be abolished entirely; and at least half a dozen others are in the very doubtful class. The proper specific name *pro tem.* is *Sterigmatocystis antacustica*, which was given by Cramer (II.) to the fungus he discovered in the passage of the human ear in 1859. As was shown by Wilhelm (I.), the fungus afterwards (1867) termed *A. niger* by van Tieghem coincides with the above species by also possessing branched sterigmata. The morphological examination of the structure of the conidiophores (Fig. 175), which necessitates the removal or bleaching of the dark masses of conidia, reveals a pale, rigid stem, about 15 μ thick, carrying a sharply defined spherical globule (diameter about 80 μ), with slender radial primary sterigmata (26 μ by 4·5 μ) each with 3–4 ornamental secondaries (8 by 3 μ), and long chains of small globular, smooth or warty conidia (about 3–4 μ) as carriers of the dark colour. Moreover, the reports of various authorities do not altogether agree, the dimensions of the conidia being oftentimes given as 3·4–4·5 μ, and the length of the sterigmata as 20–100 μ. This must be specially emphasised, in view of the diagnosis of the black species, to be described later. Of course the heads vary in size, and the result obtained depends on which of them have been measured, unless the average be taken. In unfavourable circumstances, for instance, unsuitable media, the conidiophores languish (few sterigmata and simple, conidia pale, &c.), as was observed by Duclaux (XX.), and more recently by Molliard and Coupin (I.). as well as by Lutz (II.). C. Engelke (I.) states that, under certain conditions, a conidial form, similar to that of *Botrytis*, *Sceptromyces Opizii*, Corda, is produced; but this somewhat improbable report requires confirmation on the basis of indubitably pure cultures.

Sclerotia have been frequently observed in this fungus, the first to discover them being K. Wilhelm (I.) in 1877; but unaccompanied by any development of asci. According to

[1] The division is by no means sharp, some species exhibiting both simple and branched sterigmata.

Brefeld (IV.), they are formed by the simple intertwining and fusion of morphologically equal hyphæ, and take the appearance of yellowish, hard, tough-skinned and nearly globular nodules, 1–3 mm. in diameter. They lie scattered over the surface of the herbage—inside as well, according to Wilhelm (I.)—but are rare, and only found occasionally. This easily cultivated fungus, which, though a lover of warmth, will continue to grow slowly a few degrees above freezing-point (optimum temperature about 40° C., minimum about 7° C.), and exhibits a preference for certain acid substrata (gall-nut extract, solutions of tannic acid, and also solutions of fruit acids containing sugar and other nutrient substances)—in consequence of which it is said by Wehmer (XXIII.) to be easily captured—is of little practical importance. On the other hand, it is all the more important as an experimental fungus in the study of fungo-physiological questions. It plays a practical part in the preparation of gallic acid from tannin, and also in opium manufacture (compare § 292). The physician is acquainted with it as a not infrequent inhabitant of the human ear, in otomycosis, though its appearance in this case seems to be secondary. Its share in the retting of flax is doubtful. According to Bordas (II.), it is the cause of the cork disease (known in France as *piqûre* or *tache jaune*) affecting many cork oaks on the side exposed to the weather. The bottle corks made from this diseased material are liable to impart a corked taste to wine, and contain the *Aspergillus*, either alone or associated

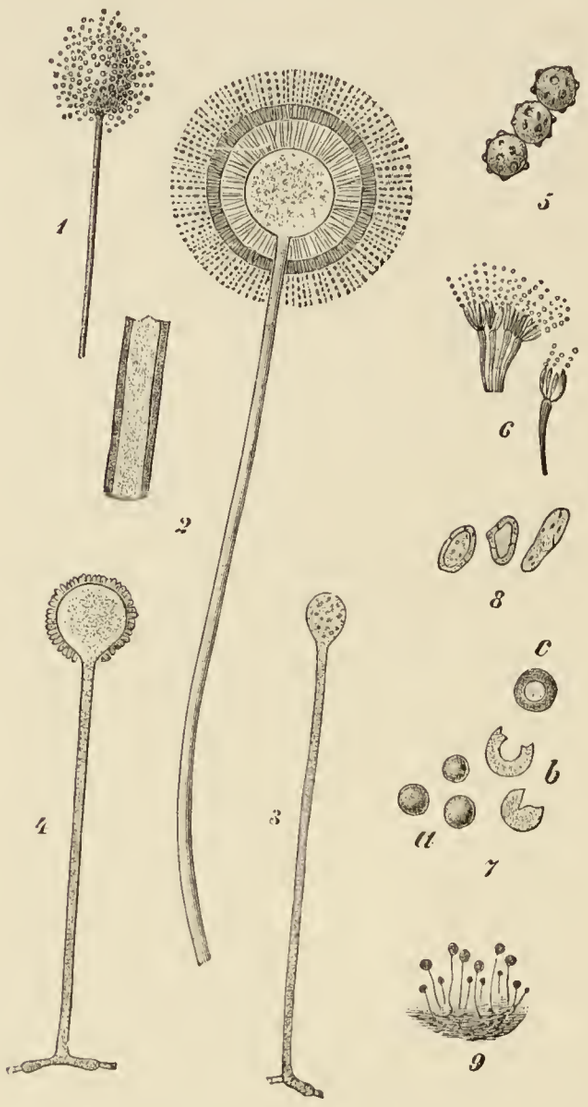


Fig. 175.—Aspergillus niger.

1 and 2, Conidiophores, in optical section at 2, after decoloration and clearing, showing the spherical globule and double radial wreath of branched sterigmata, as well as the conidial zone (semi-diagrammatical); 3 and 4, young conidiophores before and during the production of sterigmata (optical section); 5, globular, warty conidia; 6, separately prepared sterigmata; 7, sclerotia, after unsuccessful germination experiment (fallen apart at *b*); 8, tough skinned spotted cells from the interior of the sclerotia; 9, conidial herbage. Approx. magn. of 1–4, 40; of 5, 1000; of 6, about 154; of 7, natural size; of 9, about 2. (*After Wehmer.*)

with other mould fungi. Behrens (XVI.) states that it not infrequently exerts an injurious action in germination tests on seeds, inoculation tests having resulted in stunting the embryos of numerous species, so that a pathological character is assumed in these circumstances. The numerous chemical influences of this fungus, including the very decided capacity for producing oxalic acid, are described more fully in chapter lvii. Linossier (II.) states that the, presumably ferruginous, black pigment (the so-called aspergillin) is of some physiological importance in the life of the plant; but this is doubtful in view of its nature as an excretion product of the conidia, although both Molisch (I.) and Kanter (I.) assert that iron is indispensable for the fungus. Greater interest seems to attach to the yellow pigment in the hyphæ, examined by Milburn (I.). Here we can only refer briefly to a whole series of recent investigations by Czapek (III.), R. Chodat and Bach (I.), Raciborski (II.). Lode (I.), Ono (I.), Hattori (I.), Kny (II.), Bourquelot and Hérissey (II.), Saida (I.), Iwanoff (I.), Kosinski (I.), Richter (I.), Emmerling (V.), Lutz (II.), Friedel (I.), Maximow (I.), Kostytschew (I.), Koernicke (I.), Kanter (I.), Heinze (II.), Jousset (I.), Orlowski (I.), Molliard and Coupin (I.), Kurzwelly (I.), Kosjatschenskow (I.), Lesage (IV.), Pantanelli (I.), Altenburg (I.), Charpentier (I.), Krasnosselsky (I.), E. Meissner (I.), Porodko (I.), R. Meissner (IV.), Todur (I.), Garnier (I.), and Coupin (I.), dealing with the chemical composition, nutrition, respiration, the production of enzymes, influence of stimulants, radiation, resistance of the conidia to injurious influences, &c. The literature on this fungus previous to 1901 has been collected by Wehmer (XVII.), who gives no fewer than 79 references. The numerous enzymes produced by this fungus are dealt with in the next chapter.

Fungi allied to *A. niger* occasionally inhabit the interior of certain fruits. Thus, Corda found in dates a species which he named *Ustilago phœnicis*—the *Aspergillus phœnicis* of Patouillard and Delacroix (I.), who identified it as a species of *Sterigmatocystis*, and Hennings (II.) also recognised as a *Sterigmatocystis* (*St. ficuum*), the *Ustilago ficuum* discovered by Reichardt (I.) in dried figs. According to G. von Lagerheim (I.), the two have since been found to be identical. The question now arises whether this date and fig fungus, which has not yet been compared, in pure cultures, with *A. niger*, is really different from the latter. This point is by no means clear; and a short description of the fungus may be given here, on account of its injurious effect on the fruits in question. According to Hennings (II.), the conidiophores fill the interior of the figs with a compact black mass of conidia, and their heads measure 76–100 μ in diameter, the globule being 45–60 μ across, and closely set with club-shaped primary sterigmata (15–28 by 6–9 μ). The

dark, slender sterigmata (5–8 by 2–3 μ), mostly present, produce long chains of globular, blackish violet conidia, usually 4 μ thick, which are said by Hennings to be smooth, but which G. von Lagerheim (I.) asserts to be provided with granular ledges. According to the latter authority, sclerotia are also formed. The fungus produces oxalic acid, saccharifies starch and inverts saccharose, all of which properties are found in *A. niger*, and the dark pigment of the conidia behaves in the same manner. The date disease ("Mchattel") caused by this organism is of frequent occurrence in the valley of the Nile. Hennings states that gastric troubles ensue when figs affected by this fungus are eaten. The species is indigenous to Egypt and Tunis (in dates and figs).

A similar species, *A. strychni*, has recently (1904) been described by Lindau (I.), as filling with black conidial powder the mummy-hard masses of the dried fruit of *Strychnos leiosepala* in Angola. The stiff conidiophores, 2–4 mm. in length, carry a black head, 250–330 μ thick, the dark, spherical globule measuring 58–86 μ in diameter. The primary (septate) sterigmata measured up to 100 μ in length (mean 85 μ), the diameter being 7–20 μ, whilst the secondaries measure 10–11 μ by about 3.5 μ. Here also the diameter of the dark, spherical, hairy conidia is about 4 μ. The dimensions of the heads and sterigmata are considerably larger, it is true, but the fungus ought to be cultivated for comparison with *A. niger*.

In 1896 MacAlpine (I.) described a black *Aspergillus* (*Sterigmatocystis pulverulenta*) found in all parts of *Phaseolus vulgaris*, L., which furnishes dark, spherical warty conidia, 4 μ in diameter, greatly resembling Lindau's fungus in dimensions. Culture experiments with all these species, for the purpose of observing their mutual relations, are highly desirable, it being essential to know how the form and dimensions of such fungi turn out under controllable conditions.

The fungus growing on the aged fruit of *Welwitschia mirabilis* and known as *Aspergillus Welwitschiæ* (Bresadola), P. Hennings (formerly termed *Ustilago W.* by Bresadola), is also an ordinary *A. niger*, as has already been admitted by Hennings in a private communication on the subject. The same remark may also apply to the *A. ustilago* discovered by Beck in the fruit buds of *Phyllanthus Emblica* (East Indies), as well as to many others. Of course it is not impossible that other, very similar, brownish black *Sterigmatocystes* may exist—see, for example, P. Lindner (XXXIII.), who briefly mentions two unnamed forms of this type. The *A. atropurpureus* discovered by Zimmermann (I.) on rotting coffee berries at Buitenzorg is similar in all respects, except that the conidia are larger, being 6–8 μ in diameter. Up to the present, the conidia of *A. niger* have not been observed to share the fluctuation dimensions of the conidiophores.

Forms that are otherwise identical with *A. niger*, but differ in the slower and less abundant formation of conidia, should hardly be classed as separate species ("small species"), as was done by Costantin and Lucet (I.) in the case of *Sterigmatocystis pseudonigra*, this method leading to confusion in many respects. Gasperini (I.) found on gall-nuts, apple kernels, and solutions of tannic and citric acids, a species, *A. violaceofuscus*, which produced conidia measuring 3–3·5 by 5–6.5 μ, though recalling *A. niger* in habitat and other features.

Aspergillus candidus I., Wehmer (XVII.), occurs preferably on old, decayed vegetables of various kinds (mouldy *pumpernickel*, rotten cucumbers, rotten grapes on the vine, spoiled cabbage-broth, mouldy cotton-seed meal, and mouldy grain), as well as on putrescent urine, old cheese, &c. The ordinarily sluggish growth of the cultures on the usual substrata also indicates that its food requirements are rather peculiar; and it seems to prefer an alkaline reaction of the medium. Probably several of the white species described in the literature will have to be amalgamated with this one; but at present it is impossible to say if it is identical with Link's old species. The surface, which is perfectly snow-white, turning creamy in old cultures, and even brown in those on wort gelatin, exhibits two forms of conidiophore: one with spherical globule and branched sterigmata, corresponding exactly with those of *A. niger* (*see* 9 in Fig. 163), whilst the other is much simpler and smaller, the sterigmata being unbranched. The conidia are mostly ellipsoidal, smooth or covered with fine dots, and 2.5–4 μ in diameter. The *A. albus*, described by Wilhelm (I.) in 1877, with its spherical globule and branched sterigmata—differing from the specimens found nearly always on spoilt barley by P. Lindner (XXXIII.)—probably corresponds to the larger form. A critical investigation of the white species, on the basis of culture experiments, is highly desirable, this group being at present in a chaotic state, unless one is content with imperfect descriptions and artificial specific names (*see* the forms arranged by Wehmer (XVII.) and Lindau (I.)).

Aspergillus nidulans (= *Sterigmatocystis nidulans*, Eidam). This species, which is pathogenic when injected into the blood (optimum temperature about 40° C.!), and is also sometimes found in the human ear, was first discovered by Eidam, in 1883, in a humble-bees' nest. It is a scarce, handsome green species, and is rendered interesting by its sclerotia, which, however, has only been observed and studied in a single instance, Eidam having failed to discover it again. In 1904 Saito (I.) had a specimen, which he did not examine further, but states that the fungus occurs in the air in Japan, associated with *A. glaucus*. The tough-skinned conidiophores (Fig. 176) on the green surface (which afterwards becomes discoloured) measure up to 0.6–0.8 mm., but are frequently only one-third to one-half that size. The branched

sterigmata of the insignificant, club-shaped globule (15–20 μ thick), which recalls *A. fumigatus*, are usually confined to the upper half, and generally produce globular, smooth (or finely dotted), very small conidia (3 μ in diameter) in long chains adhering in the form of tough masses. Septation and branching of the stems (sometimes very irregularly) seem by no means infrequent. According to Eidam's observations on the development of the

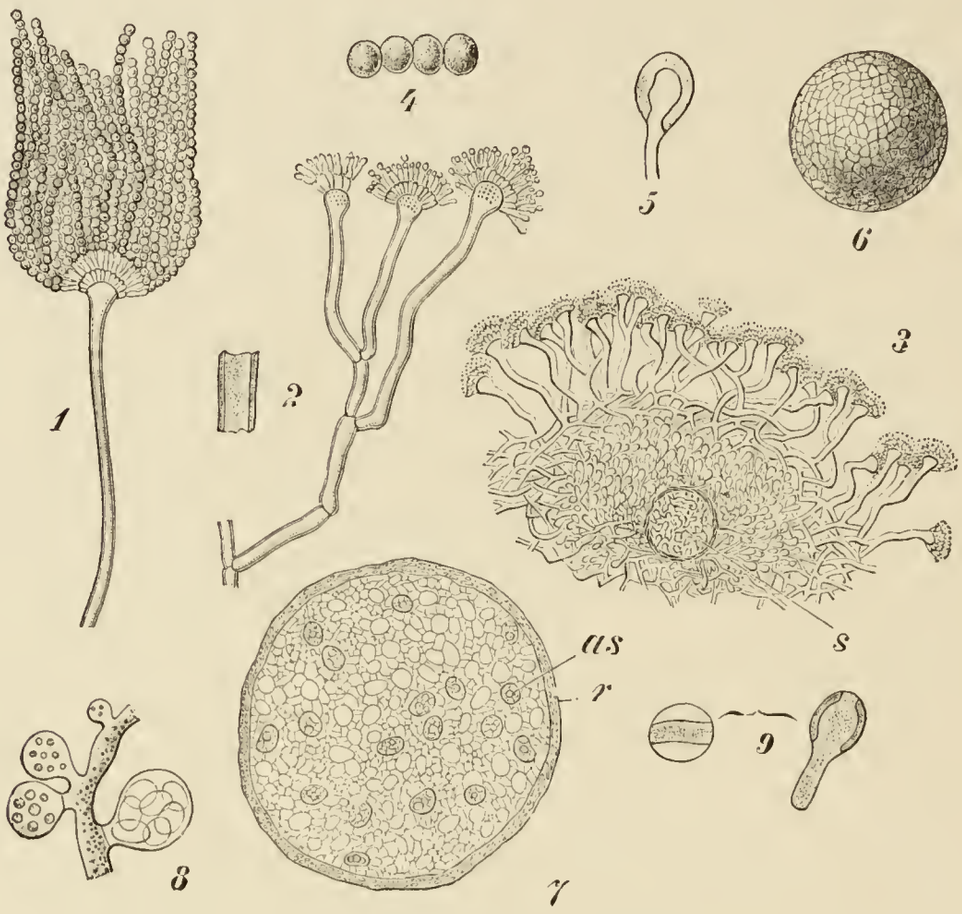


FIG. 176.—Aspergillus nidulans.

Conidiophores with branched sterigmata (1 and 2), conidia (4), ascospore with globular capsule (5), prepared separately at 6, and shown in section at 7, with asci (8), spores (9), one of them with germinating tube. Magn. of 1 and 2, 330; of 3 120; of 4, 1000; of 6, 85; of 7, 170; of 8, 400. (*After Eidam.*)

ascospore, this latter is formed from two hyphæ (instead of one, as in the case of *A. glaucus*), the one developing into the tough, stratified, pseudoparenchymatous integument, whilst the other furnishes the internal tissue forming the asci. Several weeks pass before the development is complete, and the ascospore, which is then provided with tough, dark blackish red walls, is ripe. The gradually evolved, ovoid ascus, 10–11 μ in length, encloses eight smooth, lenticular spores (about 5 μ by 4 μ), provided with a longitudinal furrow and a tough purple epispore, which bursts in two during germination. Analogous to those of *A. fumigatus* and *A. Rehmii*, the sclerotia (0.2–0.3 μ in diameter) are surrounded by a shell of peculiar, yellowish hyphæ, which are distended like bubbles—this feature is absent in *A. glaucus* and *A. pseudoclavatus*.

We need do no more than mention the ascosporogenic *A. pseudonidulans* (Vuill.), recognised by Vuillemin (I.) as a *Sterigmatocystis*.

A. Rehmii, Zukal, and *A. pseudoclavatus*, Puriewitsch, are also species with branched sterigmata. Both of them are rare and of little practical importance, but noteworthy as being among the few reported as producing ascospores, which, however, are quite different from those of *A. nidulans*.

Aspergillus Rehmii (*see* 3 in Fig. 165) was discovered on gall-nuts and decayed oak bark by Zukal (I.) in 1893. The sulphur-yellow to ochreous coat develops dwarf conidiophores (0.4–0.5 mm. high), bearing elongated ovoid globules (20 μ by 30 μ), slender sterigmata, and small globular to ellipsoidal conidia (2.5–4 μ across). The black, fragile perithecia (0.1–0.2 mm.), the skin of which is formed of a single layer of regularly disposed rows of cells, are surrounded by a compact shell formed of yellow hyphæ, which in many cases are swollen into globules. The asci, which are formed immediately, are ovoid, on short stems and rapidly become mucinous, develop elliptical, tough-skinued, smoke-grey spores, measuring 5 μ by 3.5 μ and numbering 8 in each case. The ascospores are formed by the intertwining and fusion of morphologically uniform hyphæ. This species may be regarded as doubtful.

Aspergillus pseudoclavatus, Puriewitsch, agrees, in the structure of the conidia (up to the branched sterigmata) entirely with that of *A. clavatus*. The globule measures 260–300 μ by 60–70 μ; and the greyish green, ellipsoidal conidia, measuring 3.5–4 μ by 2.5–3 μ, are identical in size and shape with those of that species. The small naked globular perithecia, which measure 60–70 μ in diameter, and are provided with a wall formed of a single layer of cells, enclose only 6–7 asci, each with 8 colourless spores. The perithecium is apparently developed from two hyphæ. The optimum temperature of this species, which was discovered on old yeast cultures by Puriewitsch (IV.) in 1899, is about 25° C.

Of the other best known *Sterigmatocystes* we need mention only the following: the brownish yellow *A. sulfureus*, Fresenius (on bark); *A. ochraceus*, Wilhelm (on bread and damp portions of plants), which develops sclerotia abundantly, but no asci; the green *A. elegans*, Gasperini (on decaying lemons); *A. variabilis*, Gasperini (on decaying fruit), with both simple and branched sterigmata. Allied to these are a number of more or less doubtful or imperfectly described species, found chiefly on vegetables, and included in Saccardo's list (IV.), and also critically sifted by Wehmer (XVII.). The *A. ochraceus*, described and closely examined by Wilhelm (I.), produces a large number of brown, nodular sclerotia, formed by the intertwining and fusion of ordinary hyphæ (as in the case of *A. niger*), but not developing asci. This seems to be identical with the *A. auricomus* of Guéguen (I.).

Recently, Vuillemin and Mirsky (I.) described *A. versicolor* (*Sterigmatocystis v.*), and Guéguen (III.) an *A. syncephalis*. The former is of interest, owing to the variable colour of its cultures, and has latterly been repeatedly investigated by Mirsky (I.), Vuillemin (II.), Friedel (II.) and by Coupin and Friedel (I.). The conidiophores are similar to those of *A. niger*, but the optimum temperature of growth is much lower, and no development takes place at all at 37°–39° C. The mycelium is a rusty brown, and no perithecia or sclerotia are formed. The red pigment, which is soluble in alcohol, is developed in the green cultures exclusively. The fungus also appears in a reddish form (with pink conidia), which, however, reverts to green sooner or later. No morphological details seem to have been published in connection with this species.

The following are probably synonyms, or at all events unrecognisable, owing to imperfect description, though they have found a place in the more recent literature: *A. luteus* (v. Tiegh.); *A. flavescens*, Wred. (same as *A. flavus*, Link), *A. nigricans*, Wred. (also Cooke); *A. nigrescens*, Rob. (both probably *A. niger*); *A. terricola*, March. (probably *A. flavus?*); *A. griseus*, Link, (*A. fumigatus?*); *Eurotium malignum*, Lindt (probably *A. fumigatus*, Fres. ?); *A. quininæ*, Heim; and *A. subfuscus*, Johan-Olsen (*A. flavus?*). In any case, the only way to justify these names is by describing the fungi in such a manner as to admit of their identification; otherwise the reader is left in doubt. Even the scientific literature does not, unfortunately, always give the correct names; Green (I.), for example, referring to the well-known *Aspergillus oryzæ* as "*Eurotium oryzæ*."

§ 286. The Genus Penicillium.

The *Penicillium* group, which, though less important, both scientifically and practically, than Aspergillus, possesses considerable interest on account of its characteristic conidiophores, comprises a number of species which are more or less analogous, and are chiefly met with in practice as producing mould on vegetables, inhabiting cheese, or acting as putrefactive fungi.

The microscopically small and delicate conidiophore, which is morphologically on a far lower stage of development than that of *Aspergillus*, differs from an ordinary vegetative hypha solely in the method of branching and the fairly upright growth, being inappreciably thicker, and just as thin-skinned and septate as the latter. The slender sterigmata, which are developed successively in whorls or tufts, occupy the undistended ends of main and lateral branchings, which grow to an almost uniform height and are mostly upright. The lateral branches are usually two to four in number, sometimes alternate and sometimes in whorls, a considerable amount of variation being, however, observed in the

structure of the conidiophores belonging to the same species. This method of branching produces the characteristic brush shape of the conidiophores. The sterigmata usually diverge in a very appreciable manner, and vary in number from two to ten, their relative length (referred to the head) and pointed shape varying

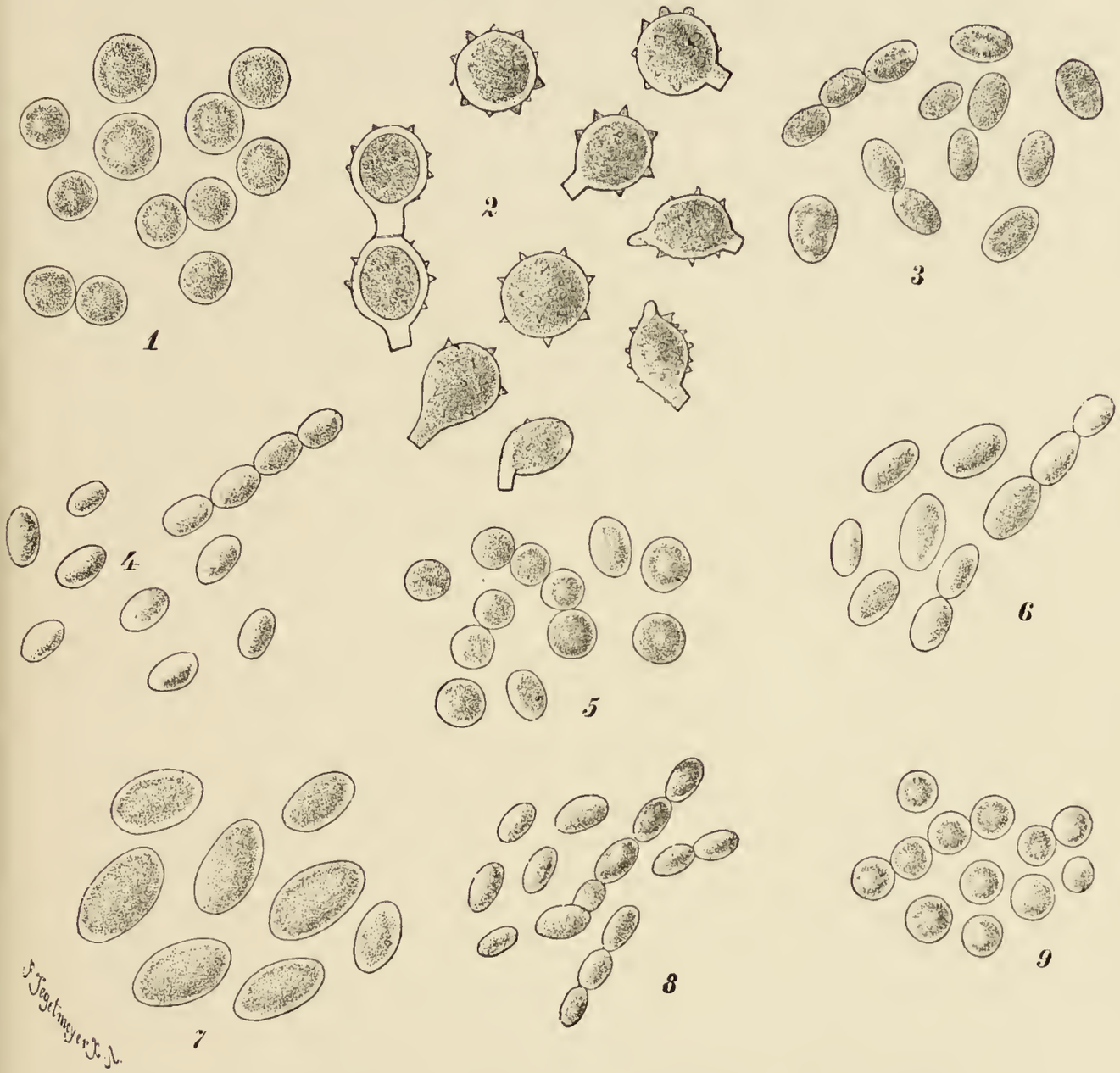


FIG. 177.—Conidia of various species of Penicillium, all drawn to the same scale. (Magn. about 1200).

1. P. Camembert (conidia 3.1–4.5 μ in diameter); 2. P. brevicaule (7–10 μ by 5.7–6.8 μ); 3. P. purpurogenum (2.8–3.3 μ by 2 μ); 4. P. claviforme (3 μ by 2 μ); 5. P. rubrum (2.8–3.5 μ in diameter); 6. P. italicum (4–5 μ by 2–3 μ); 7. P. olivaceum (6–10 μ by 4–6 μ); 8. P. luteum (2.3–3 μ by 1.4–2 μ); 9. P. glaucum (2.5–3 μ in diameter.

Measured on growths from pure cultures on wort gelatin. (*Original.*)

with the species, but being generally constant in one and the same species. The conidia of the commoner species ("*P. glaucum*," *P. luteum*, *P. italicum*) are globular to ellipsoidal, mostly glabrous, thin-walled, and almost colourless when taken singly, but

producing the characteristic colour of the growth when observed in the mass. They are mostly small (about 2.5–5 μ in longest diameter), but in isolated instances (*P. olivaceum*) may grow to a length of 10 μ. The younger members of the long chains are often appreciably smaller, of different shape, and firmly attached together throughout, only becoming loosened after they have grown considerably.

The germination of *Penicillium* conidia, observed by E. Loew (I.), presents no special features.

Several species are distinguished by a tendency to the formation of a coremium (*see* vol. ii., p. 22), which in some cases occurs spasmodically, being apparently dependent on circumstances (*P. luteum*, *P. glaucum*), whilst in others it is a regular feature (*P. granulatum*, *P. claviforme*) under nearly all conditions. The arboriform coremia of *P. luteum* are noticeable on account of their size (up to 1 cm. in height) and ornamental appearance. Those of *P. claviforme*—described by Bainier (I.)—differ from the others in that handsome isaria-like clubs are formed, which initially appear white, but afterwards turn green on the top from the presence of conidiophores. This fungus, the surface of which remains sterile, the conidia being produced solely on the clavate stroma, about 1 cm. in height, should more properly be grouped with *Isaria*.

Ascospores, in the form of small, coloured globular nodules of highly diversified character, have been found in four to five species. In the case of *P. luteum*, *P. aureum*, *P. insigne* (?) they are soft-skinned, with continuous development. In the last two the skin is pseudoparenchymatous, whereas in the first one they consist of hyphæ, somewhat loosely connected at first, but afterwards coherent. Tough sclerotia, forming asci after a lengthy period of rest (intermittent development), are formed by *P. glaucum*, Brefeld, and similar though sterile forms are found in *P. italicum*. The perithecia of *P. aureum* (which seems closely allied to *P. luteum*) are said by van Tieghem (IV.) to possess a yellow mycelial integument as well. Morini (I.) reported the occurrence of perithecia in *P. candidum*, Link, but gave no further particulars regarding the form of the conidia; and, since pictorial representations are lacking, the question must be left undecided. The same also applies to *P. Wortmanni*, Klöcker (*see* p. 346, vol. ii.). More detailed particulars on the progress of development are scarce and also contradictory in many respects. The ascospores are ellipsoidal, with the epispore tough, glabrous (*P. aureum*), warty (*P. insigne*, *P. Wortmanni*), or thickened in ridges (*P. glaucum*, Bref., *P. luteum*, Zuk.), the epispore being with (*P. glaucum*, Bref.) or without (*P. luteum*, Zuk.) a longitudinal furrow.

Ascospores not having been detected in a large number of species, the criteria of differentiation of the various species include the colour of the vegetation (mostly green in all shades from bluish

to brownish, though yellowish, white, and brown species are known); the branching of the conidiophores and the size and shape of the conidia, together with other, slighter characteristics, especially those of a physiological nature, such as pigmentation, energy of growth, gelatin liquefaction, acid production, food-stuff requirements in respect of the various sources of carbon, nitrogen, &c. Except in the case of the collective species "*P. glaucum*," little was known until recently of their requirements as to temperature. No mention has yet been made of species that thrive at blood-heat, and the maximum seems to be lower than 37° C. Stoll (I.) quite recently published certain observations in this connection with regard to six species more closely examined, morphologically and in cultures, by him, from which it appears that only *P. purpurogenum* and *P. rubrum* thrive best at higher temperatures (30° C. in the one case and 30°–35° C. in the other), the optimum temperature for the remainder being below 30° C., viz., *P. italicum*, 25° C.; *P. olivaceum*, 23°–25° C.; "*P. glaucum*" and *P. brevicaule*, 20°–23° C. The colour of old herbages, especially when grown under uncontrollable conditions, is, of course, useless as a means of differentiation, and probably a number of grey, brown, and dark-coloured species mentioned in the older literature owe their existence to these fictitious differences. As in the case of *Aspergillus*, the colour is frequently dependent on the character of the substratum, an alkaline reaction of the latter appearing to cause the green to turn greyish brown.

In the present unsatisfactory state of knowledge on the *Penicillium* group, the number of species taking part in the ripening of cheese, and the production of mould and decay in fruit, is about six or seven, though the list will probably be increased to some extent in time. A noteworthy fact, in comparison with *Aspergillus*, is the absence of any species pathogenic to animals, or of technical value outside Europe; at least, the few that are said to be pathogenic, inhabiting mucous membrane and animal substrata, have a very doubtful existence as distinct species (*P. quadrifidum*, Salisbury; *P. pruriosum*, Salisb., &c.), and there is a wide field open for subsequent research. A so-called *P. minimum*, found by Siebenmann (I.), in the ear of a patient, needs further explanation. F. Dierkx (I.) in a recent preliminary communication, gave no less than twenty-two new species (almost completely ignoring those already known), but gave no illustrations or sufficient description of them. Moreover, the habitat of the newly found "species" being unstated, it is difficult to accept them as genuine, and the matter is not advanced at all by this communication, despite the accuracy of the principles laid down by that author, who lays stress, *inter alia*, on the necessity for culture experiments for describing a species. A publication by Stoll (I.), shortly before the completion of the present manuscript, adds to our knowledge of the *Penicillium* group, by detailing a series of observations on comparative cultures of several species. Further

elucidation of this difficult subject may be anticipated from the investigations of Thom (I.), which up to the present have only been outlined.

It hardly needs emphasising that the form and size of the conidia are constant for one and the same species, and that reports on the transformation of growths with ellipsoidal conidia into such as produce globular conidia—as described by Guéguen (II.)—must be regarded very critically.

Nothing certain can be stated with regard to the number of species in existence. Undoubtedly a large proportion of the fifty odd alleged species included in Saccardo's list (IV.) will have to be deleted, especially since the older descriptions are insufficient for identification. Scarcely one-half of the above number have been clearly characterised, and only a portion of this moiety can be regarded as authentically established. Nearly half the thirty-two species counted by Lindau (II.) are unrecognisable or doubtful, the old descriptions given by Preuss, Corda, and Bonorden being insufficient as a starting-point, it having been customary at that time to simply describe, without troubling about the previous work of others, in a way that is quite incommensurate with modern requirements. The greatest confusion exists at present with reference to the fungus termed "*Penicillium glaucum*," of which there appear to be several closely allied species included under this collective name in the literature. In fact we are only on the threshold of real knowledge in connection with the *Penicillium* group. A summary of the *Penicillium* species is given below, the technically important members, that have been more accurately described and are dealt with fully later on, being marked with a *. Fuller particulars are set forth by Saccardo (IV.) and by Lindau (II.).

Summary of Penicillium Species.

1. Conidial herbage, green :
   P. glaucum * (Link ?) Bref., Sclerotia with subsequent formation of asci ; *P. italicum*.* Wehmer, sterile sclerotia ; *P. olivaceum*,* Wehmer ; *P. luteum*, Zukal, soft-skinnned ascospores ; *P. rubrum*, Stoll ; *P. purpurogenum*, Stoll ; *P. aureum*, Corda, soft-skinned ascospores (perithecia) ; *P. radiatum*, P. Lindner (sclerotia ?) ; *P. Wortmanni*, Klöcker, soft-skinned ascospores (like *P. aureum* and *P. luteum*) ; *P. Duclauxii*, Delacroix (*P. luteum ?*), *P. Camembert*,* ad int. (*see also* under 4) ; *P. Roquefort*, ad int. ; *P. claviforme*, Bain. ; *P. granulatum*, Bain.
2. Conidial herbage yellowish to brownish or brown :
   P. brevicaule,* Sacc.
3. Conidial herbage reddish to red :
   P. roseum, Lk. (?).
4. Conidial herbage white to light grey :
   P. candidum, Lk., sclerotium with formation of asci ; *P. Camembert*,* a.i. (herbage temporarily a faint green) ; [*P. insigne*. (Winter) Schröter, with formation of perithecia (= *Gliocladium penicilloides*)].

As already mentioned, it hardly seems advisable to subdivide this morphological genus at present, even in cases where the course of development of the separate species seems to assign them to different places in the system. Thus, the great difference between the ascospores of *P. glaucum*, Bref. and *P. luteum*, Zuk. involves their allocation to two different genera, whilst any species exhibiting true perithecia would have to be placed in a third genus, leaving the numerous unallotted species to rank as "fungi imperfecti" in a fourth group. For this, however, it is preferable to wait until the species are better known, retaining in the meantime the genus *Penicillium* as a group of species classed together by their conidiophores.

§ 287. The Species of the Genus Penicillium.

The species most frequently encountered, generally of technical or pathological importance, will be dealt with first, chief among them being *Penicillium glaucum* (Link ?), Brefeld ; (*Pen c. crustaceum*, Fries ?). The *P. glaucum*, Link, of the literature is evidently a collective name for a series of closely allied green species, a thorough examination of which is highly desirable. The colour of the growths, the branching of the conidiophores and the size and shape of the conidia are very similar in all. If it be desired to preserve this specific name from extinction—it is impossible now to say what Linné, Link, Fries and others had before them—it would be most appropriately bestowed on the species more closely studied by Brefeld (II.), which produced very small, spherical conidia (2.5 μ in diameter), and sclerotia, all differing from this form being named afresh. That a large number of these do exist is sufficiently demonstrated by the recent investigations of Thom (II.). In these circumstances it is difficult to assign to any particular form the numerous reports in the literature relative to the occurrence and action of the collective species "*P. glaucum*." For instance, the "*P. glaucum*" concerned in the ripening of cheese can apparently be subdivided into several distinct species, readily distinguishable macroscopically, in pure cultures, from each other and from "*P. glaucum*." Two of them, in fact, are described a little later on, under the names *P. Roquefort* and *P. Camembert*. Differing from these again are the species furnishing round spores, and appearing as the cause of decay in ripe fruits, but not yet closely examined. The classification of the so-called *P. glaucum*, Link, species of green mould observed on hops, shed-ripe tobacco, in the leather manufacturing process, and in vinous fermentation (the cause of mouldy flavour in wines) will have to be postponed until they have been more closely compared by the customary mycological methods. This group is comparatively easy to differentiate from the species producing elongated spores (*P. luteum*, *P. italicum*, *P. olivaceum*),

and also from the macroscopically similar members of other genera (*Citromyces* species, *Aspergillus fumigatus*), distinguishable at once by the structure of their conidiophores, though confusion has probably occurred in the literature from the grouping of all green moulds as *P. glaucum*, Link. Nothing, in fact, is so deceptive as the exactly similar green shade common to the

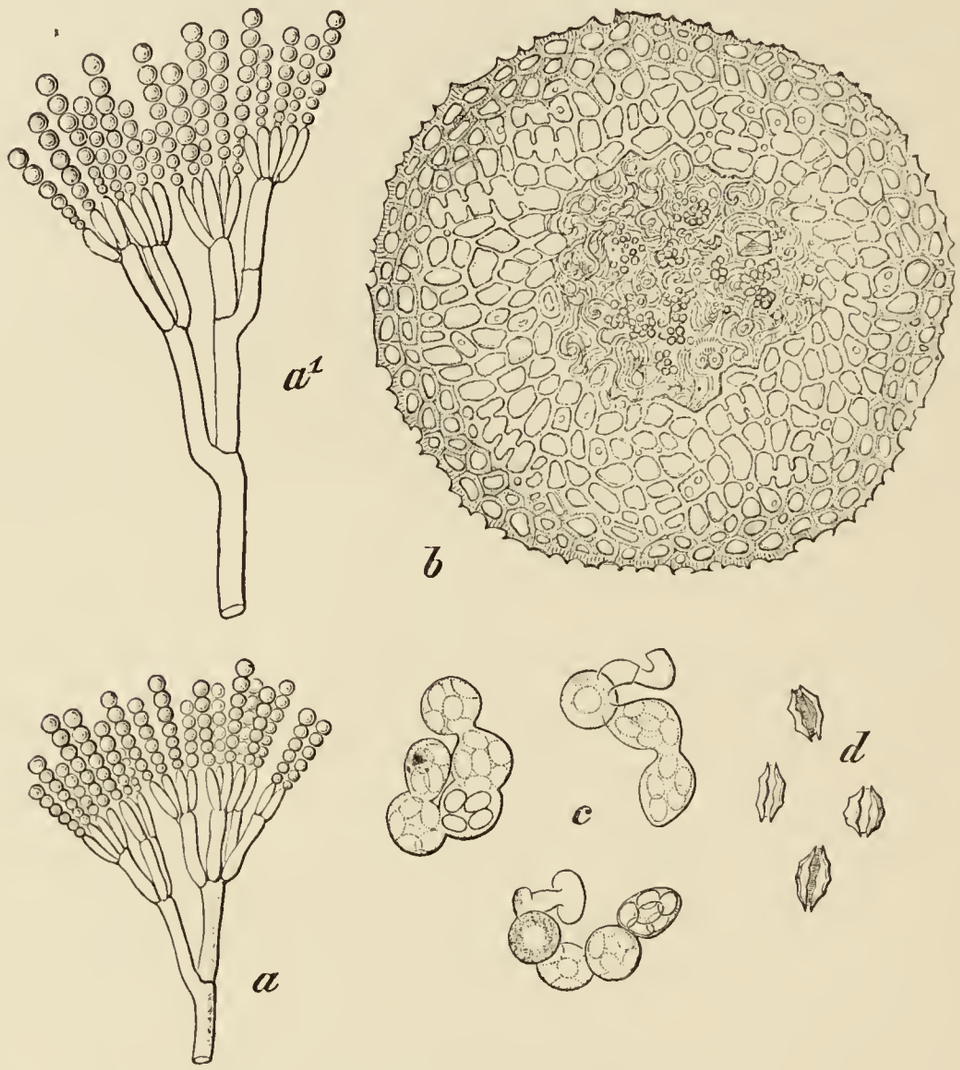


FIG. 178.—Penicillium glaucum.

Conidiophores exhibiting diversified branchings (*a*, *a'*); ascospore with ripening asci (*b*); isolated ascus in course of sporogenation (*c*); spores viewed laterally (*d*). Magn. of *a*, 315; of *b*, 150; of *c*, 630; of *d*, 800. (*After Brefeld.*)

growths of a large number of species, most of the *Penicillium* group being of this colour.

The *Penicillium glaucum* of BREFELD (IV.) exhibits the following characteristics (*see* Fig. 178). The conidia are globular, smooth, 2.5 μ thick, and occur as long coherent chains on pointed cylindrical sterigmata, measuring about 5–13 μ long and 3–4 μ thick. The branching of the conidiophores varies considerably (*see* Fig.), and these organs measure 200–400 μ in length, each twig being crowned with a tuft of (up to 12) sterigmata, which are usually shorter than their bearing cells. The colour of the herbage and vegetation is pale, or dark green, becoming discoloured with age;

structure compact, not woolly, conidia abundant. Under certain conditions, though not very regularly, the species forms small, hard, spherical to nodular sclerotia, resembling grains of sand in size (0.1–0.8 mm. in diameter), and gradually forming asci, after a period of repose, by resorption of the tough central tissue. The closely crowded, globular to ellipsoidal asci (12–15 by 8–10 μ) fall apart eventually, so that when ripe (after about 7–8 months) the interior of the organ, which is surrounded by a stratified skin (2–3 layers), is full of free, pale yellow, ellipsoidal spores (5–6 by 4–4.5 μ). In germination, the individual spores, which have a longitudinal groove and 3–4 transverse ribs, throw off the two halves of the epispore. According to Brefeld's earlier reports, the sclerotium is developed from two special hyphæ (the ascogonium and pollinodium) by a kind of fructification process, the asci being then formed as lateral shoots from the ascogonium. Zukal (II.), on the other hand, describes the formation of the sclerotium as resulting from the fusion of two equivalent, simple, vegetative hyphæ, and has observed the asci developing from the filaments growing out from the wall of the hollow sclerotium into the interior cavity of same.

It seems evident, from the varying dimensions of the conidia, and especially from the reports on the limits of temperature, that the *P. glaucum* described by various authors was not always one and the same fungus. Only the dimensions given for the conidia by Schröter (I.), namely, 2–3 μ, and by Wehmer (XXIII.), namely, 3 μ, agree closely with the figures given by Brefeld (II.), Saccardo (III.) reporting them as measuring 4 μ, Lindau 3–4 μ (globular or ellipsoidal conidia), and Stoll (I.) 3.8–4.3 μ. The latter worker in particular seems to have had before him a very different form, with round spores, since it thrives as well at 37° C. as at 8° C., the maximum temperature being even above 40° C., whereas, as a matter of fact, most of the forms of this class die off completely at 37° C. Others have found the minimum and maximum temperatures for *P. glaucum* as 1.5°–2° C. and 33°–35° C. respectively. If Grawitz formerly habituated the fungus to temperatures of 38°–40° C., and then made successful inoculations on animals, he could hardly have been working with a form of *P. glaucum*. The form grown by Stoll (I.) gave a pure white instead of green vegetation on agar-agar after several re-inoculations, thus producing a white form, analogous to *P. candidum*, Link, which, however, reverted to the green form and produced the normal conidial pigment, when transferred to ordinary media. Actual proof is lacking in support of Guéguen's assumption (II.) that the species known as *P. glaucum* varies considerably in the form of its conidia, and that a form with round spores can pass over into a form with elongated spores. This is, moreover, very unlikely, and the probable explanation is that similar but really different species were present in the mixture,

since the shape and size of the ripe conidia have hitherto been found very constant, and there is no really accurate experience of any variability in the morphological characteristics of one of these forms, if we except the irregular branching of the conidiophores. Reports on sclerotia are given by Winter (IV.) and Guéguen (II.), and on the formation of coremia by Brefeld (II.) and Hennings (II.).

The two following species can be differentiated from Brefeld's fungus on the ground of Thom's investigations (II.), and also differ from each other in form and culture. Thom named them "Roquefort mould" and "Camembert mould" respectively; but, in the absence of any specific names, they may be provisionally termed *P. Roquefort* and *P. Camembert*.

Penicillium Roquefort, Thom's (II.) Roquefort mould, hitherto generally called *P. glaucum*, Link, differs clearly from Brefeld's *P. glaucum* by the size of its conidia, which are about twice as large as those of the latter. The position of this species, which is of regular occurrence, in the conidiophore-bearing stage, in the green veins of ripening Roquefort cheese, is left an open question by Thom. The conidiophore is 200–300 μ high and 4 μ thick, the average height of the conidia heads is 90–120 μ; and the branchings are arranged in irregular whorls, carrying sterigmata 9–11 μ long and 2.5 μ across. The conidia are bluish green, mostly spherical, smooth and large, being 4–5 μ in diameter. The colour of the vegetation is dark green, afterwards turning to a dirty brown, the underside being yellowish white. No ascospores have been detected. Only a slight liquefactive action was exerted on sugar gelatin; and red litmus was rapidly turned blue. The germination of the conidia and development proceed rapidly, an abundant mycelium, with conidia, being frequently produced within thirty-six hours. This rapid growth distinguishes the species from *P. Camembert*, and the conidia are less sensitive to drought, sometimes retaining their germinating power for months. According to Conn, Thom, Bosworth, Stocking and Issajeff (I.), a bitter taste is imparted to the cheese. Thom states that the species is characteristic for Roquefort cheese, though it occurs on many other substrata and appears to be distributed everywhere.

Penicillium Camembert, the Camembert mould of Thom (II.), is a distinct species which plays a constant part in the ripening of Camembert cheese. Conn, Thom, Bosworth, Stocking and Issajeff (I.) call it simply Camembert fungus, leaving the species undefined owing to lack of sufficient description. Thom (II.) has also recently described it more closely under the name Camembert mould (*P. album*, Epstein ?), and it is probably identical with Roger's (I.) *P. candidum* from Brie cheese (1898), and Epstein's (I.) *P. album* from Camembert cheese (1902), which have not been morphologically described.

In re-naming the species, *P. Rogeri* would perhaps be more applicable than *P. Epsteini*, suggested by Lindau (II.). Thom states that the vegetation is white at first and decidedly woolly (not smooth !), the colour gradually changing to pale greyish green, and afterwards greyish white. The conidiophores are 300–800 μ long and 3–4 μ thick, the conidia heads are up to 175 μ in length and slightly branched. Sterigmata are not numerous (8–11 μ by 2.4–3 μ), the ripe conidia are globular (cylindrical to ellipsoidal while young), bluish green, large, 4.5–5.5 μ thick and smooth. The mycelial threads are about 5 μ in diameter. Conidia are formed on the free surface only, not in cavities in the substratum. Sugar gelatin is liquefied under the colonies only, litmus being turned red at first, but quickly blue again. Cheese inoculated with this species is covered over in a week with a woolly white mycelium. The fungus does not seem to occur in the open ; and Thom regards it as a typical dairy species which will not grow under other conditions. Even as an infection, it rarely occurs on other kinds of cheese. The conidia lose their power of germination if kept perfectly dry for a few weeks. It is said to peptonise milk without any previous coagulation, and to assume a faint yellow colour, without emitting the pungent ammoniacal smell produced by "*P. glaucum*" (Roquefort-P.). The slight acidity set up in the substratum at first, soon disappears. In pure cultures, the species can be distinguished from the two preceding ones at a glance.

Penicillium luteum, Zukal, forms green vegetations, distinguishable from the other species by their faintly brownish (olive) tone. The sterile mycelia are often characterised by a bright lemon yellow coloration, which is afterwards masked by the incipient conidia, and is then only visible at the edges, if at all. It differs from the ordinary species by its small ellipsoidal conidia and very long sterigmata. It frequently occurs on substrata that are prone to mould (skins, fruits, paste, &c.), especially preferring those of an acid character (lemons), and according to Behrens (IX.), it causes fruit to rot, by producing poisonous substances. On account of its tenacity and rapid growth, it is a source of trouble in places where it has once found a lodgment, and when infecting other fungi it frequently kills them off rapidly. According to Wehmer (XXXIV.), this is especially the case with *Citromyces*, on the vegetations of which it produces brown, slippery, dead patches which rapidly spread outwards. The tenacity of life on the part of the conidia is, however, very slight; and as a rule they all die off in one or two years. The delicate conidiophores (Fig. 179), more closely examined by Wehmer (XX.), branch like the two preceding species, but are characterised by a tendency to form whorls, so that the main filaments usually exhibit only a single whorl of 2–4 branches of the first order bearing tufts of sterigmata, though a variety of

deviations occur. The sterigmata (17 by 2.1 μ) are more pointed, and longer (in comparison with the head) than in most other species, the conidia decidedly elongated (ellipsoidal), very small

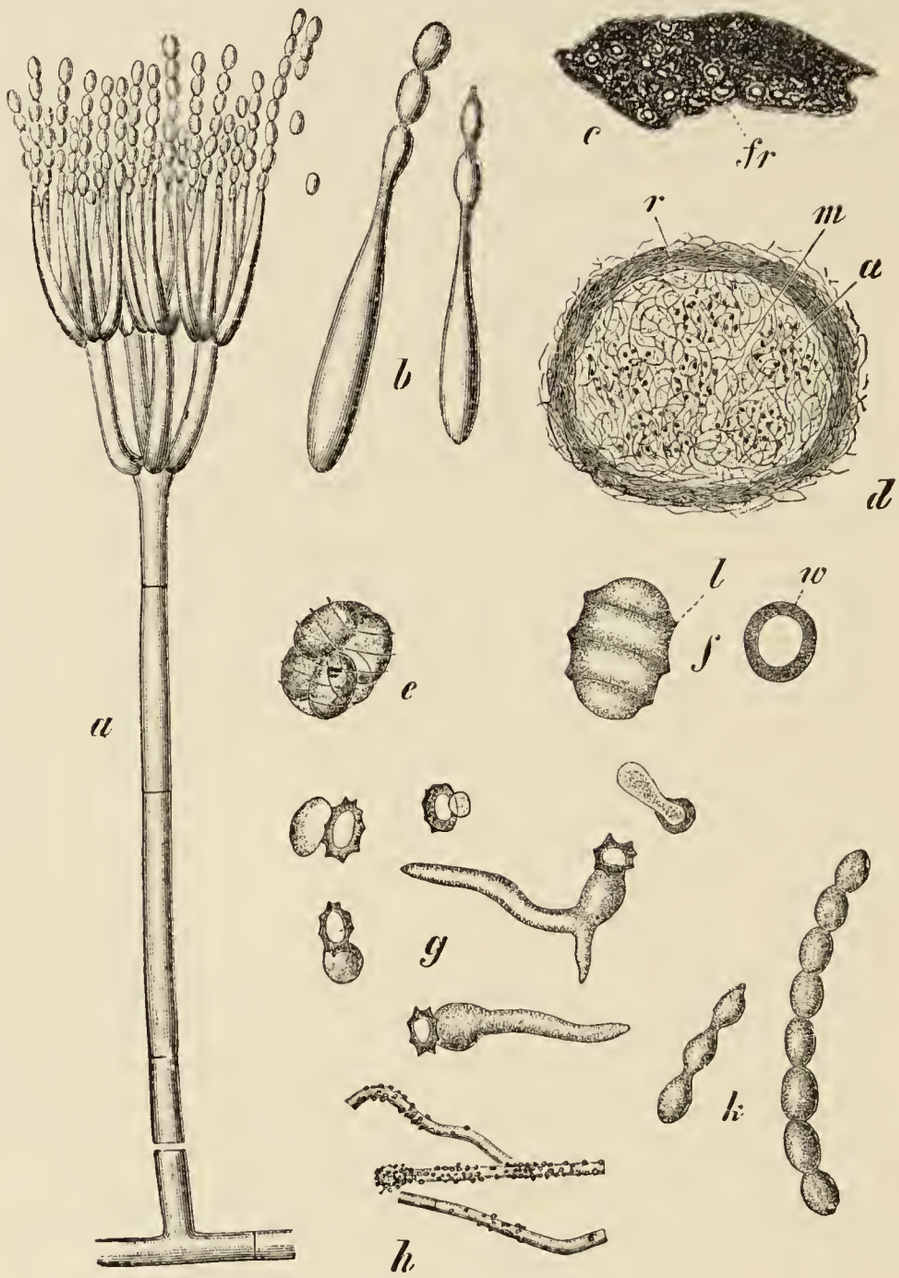


FIG. 179.—Penicillium luteum.

Typical conidiophore (*a*), sterigmata (*b*), and conidia (*k*); *c*, ascospores on the vegetation (nat. size); *d*, section of ascospore with medulla (*m*), skin (*r*) and groups of asci (*a*); *e*, free ascus; *f*, spores viewed from the side and in section, showing barrel-hoop fillets; *g*, germination of ascospore; *h*, hyphæ with yellow granules. Magn. of *a*, 1000; of *b*, 2000; of *d*, 15; of *e*, 1200; of *f*, 2400; of *g*, 900; of *h*, 500; of *k*, 2000. (*After Wehmer.*)

(2.3–3 by 1.4–2 μ)—*see* also 8, Fig. 177—smooth, delicate, hanging together firmly in long chains, dull grey in colour, but greenish grey when heaped up. According to WEHMER (XXI.) coremia are frequently developed, occasionally extensive, very handsome, and up to 1 cm. in height. The ascospores, which were first observed by ZUKAL (III.), and afterwards more par-

ticularly described by Wehmer (XX.), are usually abundant, occurring on the surface of the vegetation as lemon to golden yellow, thin-skinned bodies, more or less spherical, 1–2 mm. in diameter, turning dark orange with age, and ultimately becoming discoloured. The integument, which is about 100 μ thick, formed of loosely woven hyphæ and of a golden yellow (afterwards brownish red) colour, encloses a colourless network of filaments with embedded nests of ellipsoidal asci (measuring 9–11 by 6–8 μ), each of which contains 4–8 (average 5) barrel-shaped, tough-walled spores (4–5 by 2–8 μ). In contrast with those of *P. glaucum*, Brefeld, these spores have no longitudinal furrow, but are provided with 3–4 delicate transverse fillets, which do not eject the epispore in two halves during germination, but allow the contents to escape through fine cracks and form a voluminous secondary spore, which then develops. In a few weeks the integument of the fruit becomes very brittle, and encloses a pale yellow dusty mass of liberated spores. These ascospores, which at first consist of loosely intertwined bundles of hyphæ with separated groups of asci, would undoubtedly justify the classification of the species outside the *Aspergillaceæ*, the fructification being similar to that of *Gymnoascus* and differing completely from *P. glaucum*, Brefeld. The bright yellow colour of the young mycelium and fruit case of *P. luteum* is due to yellow granules (a pigment soluble in alcohol and classed by Zukal as a fungus acid), abundantly secreted by the hyphæ and forming a dense coating upon them. These are lacking in *P. glaucum*, and are not invariably met with in *P. luteum*. The fungus readily acidifies saccharine nutrient media by the formation of free citric acid.

Penicillium italicum, Wehmer, is a mould which, according to Wehmer (XXIX.) is found only on certain substrata (pine-apples, lemons, oranges, and similar southern fruits), and differs from those already described by the bluish grey shade of the green surface. The structure of the conidiophores (*see* Fig. 180) corresponds with that of *P. glaucum*, Brefeld, but the conidia are ellipsoidal instead of spherical. This fungus, which is very commonly imported with the fruits in question, is the cause of extensive putrefaction, for instance, in the case of ripe pine-apples, the entire contents of the closed cases being sometimes destroyed in this way during transport. The rapid spread of the mould on the surface is accompanied by an equally rapid penetration of the flesh of the fruit, which is spoiled in consequence. The delicate, colourless conidiophores, which are only of the thickness of hyphæ and about 250 μ long, carry 2–3 upright branches, arranged at unequal heighths and provided with a tuft of (2–6) sterigmata, like the main stem, but not always at the same height. The delicate ellipsoidal conidia, extending in long chains from the slender, tapering sterigmata (measuring about 10 by 3 μ), hang

together at first like the cells of a closely septated hypha, but afterwards become more rounded, increasing considerably in volume and becoming looser, their fairly uniform dimensions then being about 4–5 by 3 μ, though sometimes as much as 6.1 by 4 μ. Individually almost colourless, they give rise to the characteristic shade of the vegetation when closely packed together. The fungus develops abundant sclerotia, differing but slightly in size, form, and tough structure from those of *P. glaucum*,

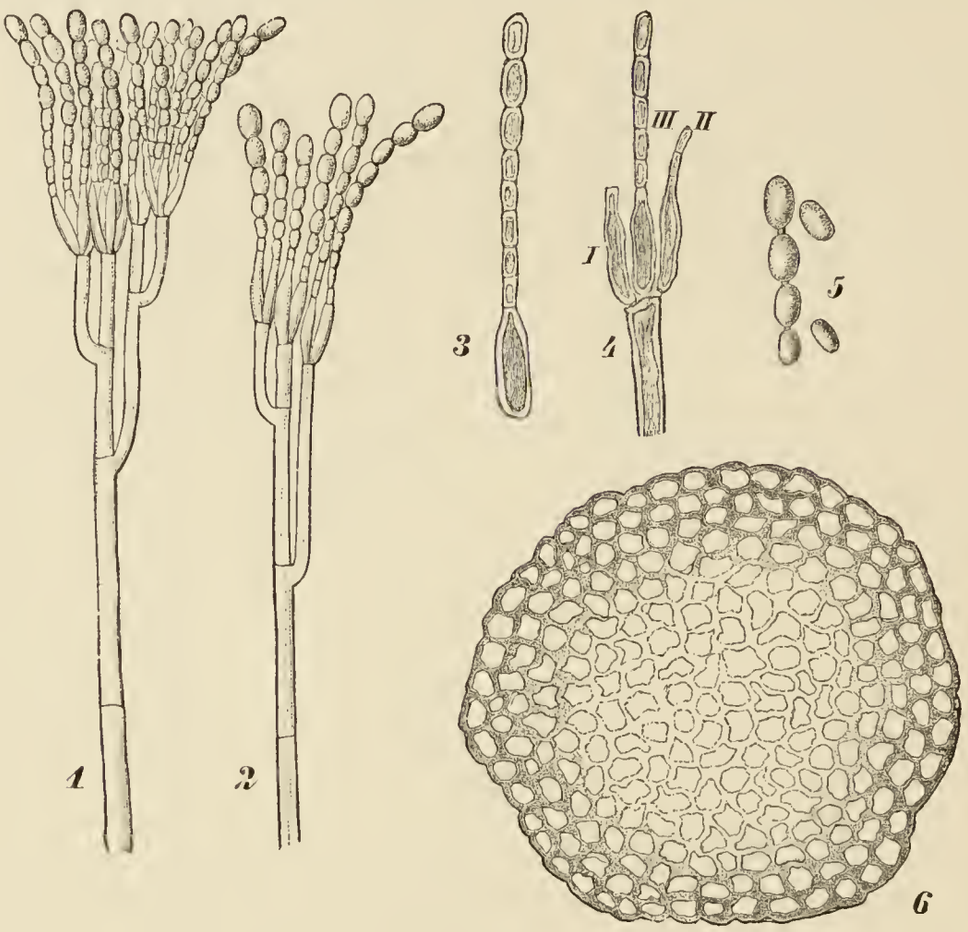


FIG. 180.—Penicillium italicum.

Conidiophores (1, 2), sterigmata (3, 4), and conidia (5). Section through an old sclerotium (6), the coloured strata of the rind shaded more darkly. Approx. magn. of 1–2, 400; of 3–4, 600; of 5, 700; of 6, 90. (*After Wehmer.*)

Brefeld. They form small, smooth, brown, fairly uniform, hard, brittle globules, about 300 μ in diameter, either enveloped in mycelium or bare, and are easily separated at any time by rubbing the vegetative coating between the fingers. Since, up to now, all experiments with a view to the development of ascospores have failed, the sclerotia must, for the time being, be regarded as sterile, in which respect they are on a par with those of *Aspergillus flavus*, *A. ochraceus*, and *A. niger*. Nothing definite is yet known as to their life history. The fungus is readily cultivated on the usual mycological substrata, and when grown on sugar solutions containing inorganic salts, forms tough, closely matted coatings, colourless below and pale to greyish green above, a large number

of conidia being produced. According to Stoll (I.), the optimum temperature is 25° C., and the minimum 10° C. The liquefaction of the gelatin is effected very slowly, or may be entirely absent (according to the composition).

Penicillium olivaceum, Wehmer, is said by Wehmer (XXIII.) to occur, like the foregoing species, almost exclusively as a putrefactive organism on southern fruits, the two being sometimes found together. It is also occasionally met with on European fruit, Zschokke (I.) having found it as the cause of gradual putrefaction on pears. His description, however, might apply to *P. luteum*, which has also been found on fruit by Behrens (IX.). The colour of the vegetation is an olive-green, like that of *P. luteum*, but brighter, and lacking the yellow granules excreted by the sterile hyphæ of the latter.

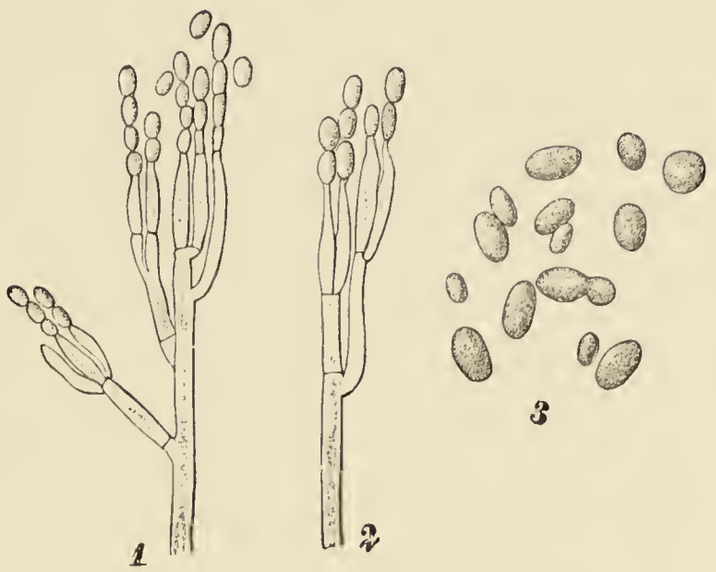


Fig. 181.—Penicillium olivaceum.

Conidiophores and conidia. Approx. magn. of 1–2, 400; of 3, 500. (*After Wehmer.*)

The conidiophores are handsome, but scarcely visible to the unassisted eye, so that the herbage does not appear filamentous. The conidiophores (Fig. 181), which measure up to about 200 μ in length, are less regular in structure than those of the preceding three species, there being no well-defined average system of branching. The branches are 1–3 in number, each carrying a few (2–3) sterigmata, about 14 by 3 μ. The conidia are ellipsoidal, like those of *P. italicum*, but much larger, averaging 6–7 by 4 μ, though sometimes attaining 10 by 6 μ, and joined together in chains which readily fall to pieces, only the younger and much smaller ones ($\frac{1}{3}$–$\frac{1}{2}$ μ) being firmly connected. The conidia are therefore twice as large as those of the preceding species. No fructification has yet been observed. On artificial substrata, the species forms a yellow-green coating of mould. The optimum temperature of growth is 23°–25° C., the minimum being about 10° C. The liquefactive action on gelatin is very slight. Further particulars and observations on the cultivation of the species are given by Stoll (I.).

Penicillium brevicaule, Saccardo, observed by Saccardo (III.) with other moulds on decayed paper, has been recommended by Gosio (II. and III.) as a reagent for the detection of arsenic, since, when grown in media containing traces of that

substance, it forms the pungent compound, diethylarsine (*see* p. 407). The morphological and biological conditions have recently been more closely described by Stoll (I.), though not exhaustively so. The growths are brownish yellow to brown in colour, according to the age and substratum. The conidiophores (Fig. 182) are delicate and small, irregularly branched,

FIG. 182.—Penicillium brevicaule.

Formation of conidia on special conidiophores (1 and 2), as also directly on the mycelium (3), the former on wort gelatin, the latter on an agar-agar culture. The conidiophore 1 carries only young, elongated conidia in course of development, those on 2 being ripe and partly shed, together with three younger ones not yet septated (*see* also fig. 177, 2). Approx. magn. of 1 and 2, 500 and 800 respectively; of 3, 400. (*Original.*)

and usually with but few twigs and sterigmata, the latter being rather long (about 16 by 3.5 μ) but not very characteristic. According to Stoll, there are two forms of the smooth, yellowish conidia, one being spherical (about 6.5 μ in diameter), the other pear-shaped (10 by 6 μ). Saccardo mentions spherical

conidia, sometimes warty (5–7 μ). These statements, however, require correction, the conidia being extremely variable in cultures on different substrata, both elongated, pear-shaped, ellipsoidal, spherical, smooth, prickly and warty forms being met with. The typical development on a good substratum (wort gelatin), however, furnishes ripe conidia that are decidedly warty globules with broad stems, the globules themselves soon falling asunder. At an earlier stage they are elongated, sometimes pointed, and also provided with a decided stem (*see* also Fig. 177). Mature specimens measure 6.8–9.2 by 5.7–6.8 μ. Consequently these conidia differ in a marked degree from those of all other species of *Penicillium*. No sclerotia ascospores have yet been observed. The species grows at only a moderate rate on the usual bacteriological substrata, and liquefies gelatin. The optimum temperature of growth is about 20°–23° C., and development is sluggish below 15° C. According to Stoll, a decided liberation of ammonia is produced on alkaline gelatin, but not on sugar or acid gelatin. Occasionally the spores are produced directly on the mycelium, without sterigmata or supports (*see* drawing). This observation needs reinvestigation, as indeed does the whole morphology of the species, the existing communications on the subject being scanty.

The following species are less known, apparently scarcer and of no practical importance:

Penicillium purpurogenum, Stoll, was described by Stoll (I.), who obtained the species from Král, according to whom the original culture was isolated by Fleroff from impure Japanese koji. In respect of the conidiophores, colour of the vegetation, and the formation of pigment, it resembles *P. luteum*. The vegetation is dark green to dark greyish green; the conidiophores are delicate and branched in whorls, each twig being usually provided with four elongated, pointed sterigmata (7 by 2 μ). The conidia are ellipsoidal, very small (2.8 by 1.7 μ), and uniform in size and shape. The optimum temperature is about 30° C., growth ceasing below 15° C., though the fungus continues to develop at incubation temperature. It produces a yellowish red to purple-red pigment, but only on substrata containing carbohydrates. The sterile mycelia are bright yellowish red.

Penicillium rubrum, Stoll, is a species of unknown origin, isolated by Grassberger, and described by Stoll (I.) in 1904. The hyphæ are coloured yellow to yellowish red by excreted granules; the conidial herbage dark green, and the conidiophores are delicate and often branched in whorls, the ends of the twigs carrying 4–5 long, pointed sterigmata (9.6 by 2 μ). The conidia are globular, very small (2.3 μ in diameter), and joined in readily detachable short chains. The optimum temperature is 30°–35° C. The species continues to grow at incubation temperature, but does not develop below 15° C. It produces a yellowish red to rusty brown pigment, but only on substrata containing carbohydrates,

and the pigment differs from that of the preceding species. No fructification bodies have been discovered. Particulars of its behaviour in cultures, as compared with the two preceding species, are given by Stoll (I.).

Penicillium bicolor, Fries, is a species described by Oudemans (IV.) as having been isolated from soil; and, unless found to be identical with some other species, should probably be re-named. It forms greyish green coatings or cushions, with a sulphur-coloured rim (similar to *P. luteum*), but the conidia are spherical (2.3 μ in diameter). The conidiophores are divided up into two or three groups of fours, with cylindrical, pointed sterigmata and long conidial chains. It is probably difficult now to say what species was actually examined by Fries, though the yellow periphery of the cushions distinguishes it from "*P. glaucum*."

Penicillium claviforme, Bainier, was observed by that worker (I.) in 1905, on powdered oak bark at a drug store. The species is rendered so remarkable from the formation of club-shaped growths (1–2 cm. in height), which are white at first, but afterwards green, resembling those of *Isaria*, and also occurring extensively in pure cultures, that is not easily confounded with any other kind. The conidia measure 4.2 by 3.1 μ, and are of a pure colour. The species should probably be classed with *Isaria*.

Penicillium granulatum, Bainier, was observed on oak chips in the woods. It produces a yellow pigment. The conidia are globular to ellipsoidal, measuring 2.6 by 2.1 μ. This species, like the preceding one, was cultivated by Bainier (I.).

The following species need more accurate description, and are probably to some extent synonymous or doubtful.

Penicillium roseum, Link, forms reddish growths on vegetables. According to Oudemans (I.) the conidia measure 5–6 by 2–2.3 μ. The species is apparently rare, and also requires further investigation.

Penicillium radiatum, P. Lindner, differs from the other species by its tough-walled, dark-coloured conidiophores. The conidia are green and spherical. This species was found on cranberries by P. Lindner (XXXIII.), on which habitat it forms black, spherical sclerotia. Further investigation is needed for more complete particulars.

Oudemans (II.) has recently found (in 1902) several species in forest humus. These, however, can only be briefly mentioned, more complete investigation being necessary; from the description and drawings given, it is doubtful whether they really constitute new species.

Penicillium geophilum, Oudemans, produces conidiophores about 360 μ high and 6 μ thick, which are septated and provided with a whorl of bottle-shaped twigs (sterigmata), up to 30 μ in length, from which the conidia separate direct by abstriction. The conidia are spherical, green in colour, and 3–4 μ in diameter.

The species, with its unbranched conidiophores, moreover, belongs to the genus *Citromyces*.

Penicillium humicola, Oudemans, is yellow-green. The conidiophores measure about 110–120 by 1–1.5 μ (probably a typographical error ?), and are branched in whorls. The conidia are 2 μ in diameter. Oudemans' diagrammatic drawing exhibits no special features.

Penicillium desciscens, Oudemans, is similar to the preceding species. The conidiophores show repeated branchings. The description gives the diameter of the conidia as 2–3 μ, but, according to the drawing, they are ellipsoidal (*P. luteum ?*).

Penicillium silvaticum, Oudemans, is brown. The conidiophores measure 210 by 2–3.5 μ, and are septated, with a whorl of bottle-shaped sterigmata, from which separate the pale brown, spherical conidia, 2–3 μ in diameter. The unbranched conidiophore excludes this species from the *Penicillium* group. It is apparently identical with *P. geophilum*, and should be classed with *Citromyces*.

Penicillium candidum, Link, forms white herbages, the conidiophores and conidia apparently coinciding with those of "*P. glaucum*." The spherical conidia measure 2–3 μ in diameter. The species grows on all kinds of vegetables. Accurate reports are lacking, but Morini (I.) speaks of sclerotia and asci, the latter being ovoid, 24–30 μ long, and containing eight smooth ovoid spores measuring 6.5–9 by 3.5–5 μ.

According to Roger (I.) a white *Penicillium*—termed *P. candidum*, but not fully described—plays a part in the ripening of Brie cheese, which it covers with a pale herbage. Epstein (I.), who also discovered this fungus, named it *P. album*, but its identity with the older *P. album*, Preuss (1851), has not been established; and it is also doubtful whether it coincides with the above-mentioned *P. candidum*, Link. More probably it is the same as *P. Camembert*, which is slightly green at first, afterwards turning greyish white.

Penicillium Duclauxii, Delacroix, forms herbages, which are white or sulphur-yellow at first, afterwards turning olive-green. The sterigmata are spindle-shaped, the conidia rounded-ellipsoidal, and measuring 3–4 μ in diameter. The species has been found on grapes that have lain in water. According to the description by Delacroix, the species is probably *P. luteum*.

Penicillium insigne (Winter), Schröter, forms white conidial herbages and elongated ellipsoidal conidia. The structure of the conidiophores closely resembles that of *P. luteum*. It was described by Winter (IV.) as *Eurotium insigne*, and, according to Schröter (I.), is identical with *Gliocladium penicilloides*, Corda. The pale yellowish brown, globular perithecia (0.25–1 mm. in diameter) have a smooth, thin, pseudo-parenchymatic skin. The asci are elongated ellipsoids (35–50–28–35 μ), and contain eight

pale brownish yellow, spherical, prickly, tough-skinned spores, 15–20 μ in diameter. The species is found on the excrement of dogs and geese.

Penicillium aureum, Corda, forms yellow conidial herbages, turning to olive green, with very small, oval to spindle-shaped conidia (3–1.5 μ). The perithecia are thin-skinned, similar to those of the preceding species, but enveloped in a yellow covering of matted hyphæ. The spores (5 by 3 μ) are smooth, yellow, and ellipsoidal. Corda observed the species on decayed wood, and VAN TIEGHEM (III.) found it on the husks of *Bertholletia*. Further investigation is necessary, though in many respects it so closely resembles *P. luteum* that the two might be considered identical.

Penicillium Wortmanni, Klöcker, forms ascospores, which are stated by KLÖCKER (VI.) to be similar to those of *P. luteum* and *P. aureum*, though they are not smooth or provided with transverse ledges, but with stumpy warts, as in the case of *P. insigne*. How far the resemblance to the latter extends cannot be decided until a more complete description of the conidiophores is available.

The *Penicillium aromaticum*, observed by JOHAN-OLSEN (III.) during the ripening of Norwegian "gammelost," but not described, is probably nothing more than the *Penicillium* of Roquefort cheese. Particulars are also lacking of the *Penicillium* forms—which certainly included the Camembert *Penicillium*—observed by COSTANTIN and RAY (I.) in Brie cheese. According to the results of investigations by de Seynes, the *P. cupricum* of Trabut (1895), is merely a form of the ordinary "*P. glaucum*," modified by the substratum (copper sulphate solution).

§ 288. The Genera Citromyces and Allescheria.

The genus *Citromyces*, Wehmer, comprises only a few forms, some of which are remarkable, physiologically, for their energetic power of acidification. It differs from *Penicillium* by the absence of branchings and by the swelling of the conidiophores (often into a globular form), and from *Aspergillus* by the slenderness of these organs and by the successive development of the sterigmata. The globule is spherical, club-shaped or insignificant. The conidiophores resemble hyphæ, are mostly aseptate, and, especially in aged specimens, provided with a colourless, thin-skinned, terminal, club-shaped to spherical globule. They are usually simple, slender, and project in large numbers from the mycelial filaments, the stalks being delicate and barely distinguishable from the vegetative hyphæ. The slender, tapering sterigmata are disposed in 5–10 whorls or tufts, pointing upward and inward, so that the head, deprived of conidia, resembles a calyx. The conidia are mostly spherical, very small (under 3 μ),

green in the mass, and arranged in long chains. Ascospores are unknown.

Citromyces Pfefferianus, Wehmer, is undistinguishable from "*Penicillium glaucum*," even in colour, by the unassisted eye. It occurs as a tough, pure green mould, turning to greyish green, grey or brownish with age, on sour fruit, sugar solutions, sugar

Fig. 183.—Citromyces Pfefferianus.

Conidiophores, at *b* and *d* after removal of the conidia, showing the variable globule with simple sterigmata; *c* is a malformation, a sterigma growing out into a new conidiophore. Conidiophores, slightly magnified at *f*. Hyphæ at *g*, from a growth in a calcareous nutrient solution, showing spherical, granular, or compact enveloping deposits of calcium citrate. Ripe and germinating conidia at *h*. Magn. of *a*–*e*, 400; of *f*, 240; of *g*, 400, of *h*, 600. (*After Wehmer.*)

preserves, and lemon-juice; and in the open air as a fine green coating, occasionally on old mushrooms, &c. (*e.g.*, *Pholiota squarrosa*). The delicate, colourless conidiophores, measuring 3 μ in diameter (*see* Fig. 183) are scarcely 70 μ high, forming a dense herbage and carrying a globule, 4–8 μ across. On this the sterigmata, 9–14 μ long and about 3 μ thick, are arranged in a whorl or are irregularly distributed over the surface, a considerable part of which is usually left exposed. All the parts are

thin-skinned and colourless, the only exception in this latter respect being the conidia, which are globular and 2.3–2.8 μ in diameter. Ascospores are unknown. According to Wehmer (XXXIV.), this fungus converts the sugar of the nutrient solution into free citric acid—in which connection *see* chap. lvii., § 291.

Citromyces glaber, Wehmer, agrees in its principal characteristics with the preceding species. The vegetations are closely interwoven and produce an abundance of conidia, which are of a rather darker green and almost smooth on the surface, and not bristly like the first species, the under side being dark, and often fissured. The conidiophores have globules up to 15 μ thick; the sterigmata and conidia are indistinguishable from those of the other species. The fungus stains boiled rice by means of a yellow pigment; and, according to Wehmer (XXXIV.), it also incites citric acid fermentation.

Probably several other allied species exist that are undistinguishable except from their appearance and behaviour in cultures; and the forms with unbranched conidiophores, referred to in the literature as *Penicillium* species, should also be included in this class. The same applies to the old, but indefinite *P. radians*, observed by Bonorden on rotting leaves, and also to two of Oudemans' species mentioned above (*P. geophilum* and *P. silvaticum*), as well, perhaps, as to the *P. radiatum* of P. Lindner (XXXIII.).

Mazé and Perrier (I.) recently established four species (*Citromyces citricus*, *C. tartaricus*, *C. oxalicus* and *C. lacticus*), without, however, specifying their morphological characteristics; so they cannot be regarded as species in the sense of the naturalist. These two workers, instead of adopting the morphological basis of classification, apply the term *Citromyces* to all fungi producing citric acid. The practice of establishing genera according to physiological characteristics is specially indefensible in the case of forms that are morphologically well defined, and would also completely break up our system of natural history.

The genus *Allescheria*, Saccardo and Sydow, differs from *Penicillium* by the sympodially branched conidiophores. Moreover, it is represented by only a single species. This was formerly described by Costantin (IV.) as *Eurotiopsis Gayoni*, Cost., on the basis of a generic name already applied by Karsten to a genus of *Nectroidaceæ*, but should be named *Allescheria Gayoni* (Cost.) Sacc. and Syd. It also requires to be carefully compared with *Monascus purpureus*, Went. Lindau (III.) proposed to call it *Eurotiella*; and Ed. Fischer (II.) has described it as *Allescheria Gayoni*, Saccardo and Sydow.

Allescheria Gayoni, Sacc. and Syd. (*Eurotiopsis Gayoni*, Cost.) is a species that has been more closely investigated by Laborde (VI.) on account of its property of saccharifying starch. It produces a red pigment, and incites alcoholic fermentation, but, though of chemico-physiological interest, has no practical import-

ance, and will therefore be only briefly mentioned. It forms white or reddish herbages, with sympodially branched conidiophores, developing long chains of ovoid conidia by abstriction. The conidia are relatively large, measuring 12 by 10 μ. The ascospores (resembling those of *A. glaucus* = "*Eurotium*") are spherical and small (50–80 μ in diameter), with rounded 8-spored asci, the spores measuring 6 by 4 μ. It forms purple-red patches on starch paste and other vegetable substrata. In any event the conidium-producing form bears little resemblance to the previously mentioned *Aspergillaceæ*.

CHAPTER LVII.

CHEMICAL ACTIVITY OF THE ASPERGILLACEÆ.

By Prof. Dr. C. WEHMER.

§ 289. General Review.

The present chapter deals specially with certain chemical effects of the *Aspergillaceæ*, many of the representatives of this family being worthy of note, having formed the subject of numerous investigations in this connection. This has already been discussed in various other parts of the present work, so that all we have to do now is to arrange the facts briefly, for the characterisation of the family in this respect as well.

The presence of enzymes, as the means of producing effects of decomposition, has been confirmed in all the species examined for this purpose. The number of enzymes identified seems to be still increasing, so that nearly all the enzymes known are found associated in *Aspergillaceæ*. Instances of the decomposition of carbohydrates (disaccharides and polysaccharides), glucosides, fats and proteids, by invertase, maltase, lactase, amylase, (diastase), inulase, cellulase (cytase), pectinase, melecitase, raffinase, emulsin, lipase, protease, &c., have been noticed; and mention has also been made of lab enzyme, amidase and tannase, as well as of oxidising and reducing enzymes. *Aspergillus niger* and the collective species "*Penicillium glaucum*," have particularly served as the subjects of experiment in these researches; but similar observations have also been recorded with regard to *A. oryzæ*, *A. Wentii*, *A. glaucus*, *Penic. luteum*, and isolated instances of other species of *Aspergillus* and *Penicillium*, as well as in the case of *Allescheria Gayoni* (*Eurotiopsis Gayoni*). Unfortunately the value of the results has been considerably impaired by the uncertainty regarding the identity of the so-called "*Penicillium glaucum*" examined by the different authors. Only in a very few instances have the enzymes in question been actually isolated, their presence having been, as a rule, deduced from the reaction with the culture liquid or with extracts from the triturated growths of mould.

In addition to enzyme action, true fermentative action in the stricter sense is found in only a few species; and in only one, namely *Allescheria Gayoni*, has any decided alcoholic fermentation been observed up to the present. On the other hand, oxidising fer-

mentations have been noted in several species, namely: oxalic acid fermentation by *Asp. niger*, citric acid fermentation by *Citromyces Pfefferianus*, *Citr. glaber* and *Penicillium luteum*. Whether these phenomena are separable from the living fungus and can be produced by the lifeless substance has not yet been investigated.

The dissociation of racemic compounds into their optically active components by micro-organisms has been already dealt with in vol. i. chap. xxii. The circumstance is only referred to now because nearly all the determinations were made by the help of *Aspergillaceæ*, more particularly *Asp. niger* and "*Penicillium glaucum*," though in many cases the purity and identity of the species may be doubted. Several experimenters have also worked with *Asp. flavescens* (probably *A. flavus*) and *A. griseus*, the latter name possibly masking the identity of some better-known species (*e.g.*, *A. fumigatus*, Fres.). Unfortunately, descriptions of the fungi are lacking, so that the results are practically worthless. Attention has already been drawn to the circumstance that the *Penicillium glaucum* of the earlier workers was an imperfectly identified species, and can only be regarded as a collective term applied to green moulds of indefinite nature.

Our knowledge of the pigments produced by several of the species, and the conditions under which these pigments are developed, is still in an imperfect state. The same also applies to the poisons formed by the pathogenic species, though the less important decomposing action of several species on readily oxidisable substances (alcohols and organic acids) has been repeatedly examined. The chemical activity of our fungi is almost invariably connected with the presence of atmospheric oxygen, submerged vegetations being unable to bear the complete exclusion of oxygen for more than a short time, even when sugar is administered. Further particulars of the processes are given by Pfeffer (III.) and Duclaux (XXI.), in the lectures on plant physiology by Jost (I.), and in Czapek's (IV.) recently published work on the biochemistry of plants.

§ 290. Saccharification of Starch.

The diastatic property of the *Aspergillaceæ* is rightly placed in the foreground as the one of greatest practical importance. It has been utilised technically from the oldest times; and special historical interest attaches to the diastase of the Japanese *Aspergillus oryzæ*, this being the first enzyme from thread fungi to become better known, and forming the pioneer of the long series of fungus enzymes discovered during the last two decades of the nineteenth century. In 1860, Berthelot isolated yeast invertase, and the property of inverting saccharose, possessed by the extract from "mould fungi," was mentioned in 1864 by Béchamp. After Gayon's discovery of the inverting action of *Asp. niger* in 1878,

the subsequent investigations of that fungus were not commenced until the eighties.

In 1876, Korschelt (II.), who was the first to publish a complete description of the method of saccharifying rice with *Asp. oryzæ*, practised in Japan, not only mentions that a diastase, capable of converting starch into dextrins and maltose, is secreted in the hyphæ of that fungus, but also tried to ascertain the optimum temperature (40°–50° C.) for the action of this enzyme, which he named eurotin (from *Eurotium oryzæ*, the earlier name of the fungus), and which greatly resembles malt diastase. The statements in the literature —*e.g.*, by Oppenheimer (III.)—ascribing the discovery of this diastase to later workers, consequently need correction. From the begining of the eighties it received attention at the hands of the majority of investigators, viz. : Atkinson in 1881, F. Cohn in 1883, Büsgen in 1885, and Kellner, Mori and Nagaoka in 1889. Then came Takamine's endeavours to utilise the properties of this *Aspergillus* beyond the confines of his native land, and the researches (extending up to the present time) into *Aspergillus* diastase (Taka-diastase) and its capacity, especially in comparison with enzymes of other origin. Reference to this matter has already been made in § 242, dealing with the technical application of the enzymes. In addition it may be stated that the extract from *Asp. oryzæ* or from koji contains not merely an amylase, but a mixture of various enzymes, whose divergent effects (decomposition of saccharose, maltose, &c.) cannot be ascribed to a single enzyme, since Atkinson demonstrated dextrose to be a saccharification product.

The saccharifying influence on starch, that Effront (X.) claims to be stimulated by a suitable mixture of different substances (phosphates, aluminium salts, asparagin, &c.), is adversely affected even by small quantities of alcohol or common salt, though additions of 20–30 per cent. are required to suppress it entirely. According to Kellner, Mori and Nagaoka (I.), 2 per cent. of common salt will lower the effect of the mixed enzymes to 50.2–58.3 per cent. of its original value, 20 per cent. reducing it to less than 10 per cent ; whilst 2 per cent. of alcohol will bring it down to 82 per cent., 10 per cent. of alcohol—according to Kozai (II.)—to 50 per cent., and 28 per cent. of this reagent to 1 per cent. Less than 1 per cent. of free acids (lactic acid, hydrochloric acid) also produced complete retardation. These factors play an important part in the technical utilisation of the fungus in the preparation of rice-wine, Soya and Miso.

Asp. oryzæ is by no means the only amylolytic species of this family, the same power being apparently shared by most of them, though in a less degree. So far as the species have been examined, starch paste (with the usual additional nutrient substances) forms a suitable substratum for all, and therefore the presence of the enzymes capable of acting on that medium is indicated. It is

hardly necessary to enumerate the whole of these species. Experiments of this kind were commenced by Duclaux (XXI.) in 1883 with *Asp. niger*, and afterwards with *A. glaucus*, "*Penicillium glaucum*," and *Allescheria Gayoni* (*Eurotium Gayoni*), by Fernbach (III.), Bourquelot, Hebebrand (I.), Laborde (VI.), and Wehmer (V.). Observations on the saccharifying properties of extracts from the mould vegetations have recently been communicated by Schäffer (IV.), with regard to a number of species (*Aspergillus niger*, *A. Wentii*, *A. fumigatus*, *A. glaucus*, *A. oryzæ*, *Penicillium glaucum*, *P. luteum*, *P. italicum*, and *P. rubrum*.

Fernbach (IV.) and Bourquelot isolated from the cultures or growths of *Asp. niger* the amylase (diastase) previously mentioned by Duclaux (XXII.). The first-named worker also found that the preparation obtained by precipitation with alcohol has its activity seriously impaired by even small quantities of free organic or inorganic acids. This may also explain the circumstance, observed by Wehmer (V.) that the liquefaction of starch by this fungus (which produces free oxalic acid), is sometimes incomplete. Duclaux (XXII.) states that the fungus will also corrode and dissolve raw starch by means of a maltase differing from the ordinary kind, dextrose being formed. According to Laborde (VI.), the enzyme in question (amylomaltase) from *Asp. niger*, *Penicillium glaucum*, and *Allescheria Gayoni*, is able to transform starch directly into dextrin and dextrose, and not, as in the case of malt diastase, into maltose—which substance it is also able to hydrolyse. In this author's opinion (which, however, has not been left unchallenged), the amylomaltase secreted by these three fungi is not only different from the maltase of barley malt, but is also a different substance in each case, a conclusion formed on the basis of comparative behaviour under external influences, such as the action of acids and the optimum and maximum effect produced. This point, however, needs further investigation. Petit (IV.) states that both *Penicillium* and *Aspergillus* also convert into dextrose the dextrin $(C_6H_{10}O_5)_3$ formed during the saccharification of malt. Hebebrand (I.) has written on the diastase of *Penicillium;* and Gosio (VII.) on *Penicillium brevicaule*, which also saccharifies starch.

With regard to the conditions under which diastase is formed by *Asp. niger* and *Pen. glaucum*, reference may be made to p. 62, vol. ii. The continuous production of amylase in cultures of *Asp. niger* on sugar solution was assumed in 1889 by Duclaux, but no proof was advanced; so that the work of this experimenter, fruitful as it was, affords no experimental proof of the various new statements.

§ 291. Acid Fermentations.

In contrast with the various enzyme actions of the *Aspergillaceæ*, the production of free organic acids—the sole process to which we

apply the term "acid fermentation"—is a rare occurrence; and up to the present this is the only family of *Eumycetes* in which this process is carried on in the same way as by the bacteria. Just as in the latter case the chief products of this fermentation are acetic acid, butyric acid, and lactic acid, so with the *Eumycetes* the products are oxalic acid and citric acid. In the case of phanerogams, similar processes furnish preferably citric acid, tartaric acid or malic acid. The accumulation of such acids in any appreciable quantity, whether in the vacuoles of higher plants or in the nutrient solution of micro-organisms, is invariably a physiological peculiarity confined to certain species or families (*Aurantiaceæ*, *Crassulaceæ*, *Vitaceæ*, &c.), and one that is difficult to analyse closely. In the majority of cases the free organic acid is merely an intermediate product, which is afterwards decomposed by complete oxidation; and the question whether the formative stage has been traversed too rapidly or the decomposition stage too sluggishly must remain open for the present. Of course, the presence of salts of organic acids, which are commonly met with, does not necessarily imply acid fermentation, since the occurrence of these salts does not argue the pre-existence of free acids, but is more frequently the result of the availability of bases during metabolism.

At present we are probably only on the threshold of knowledge with regard to acid fermentation, and continued systematic investigation may reveal both additional fungi and acids concerned in the process. Even now a few reports—still, however, incomplete—are available on the point. Thus, it is known that *Asp. oryzæ* will acidify saccharine nutrient media, though the nature of the acid has not been determined. Graf (I.) found the acidity of a 28-days-old culture on wort to be equivalent to 40 c.c. of decinormal baryta per 20 c.c. of culture liquid, as compared with an acidity of only 2.45 c.c. in the case of "*Penicillium glaucum*" and of 56.6 c.c. with *Asp. niger*, for the same volume of liquid. The fact discovered by Lind (I.), that *Asp. niger* and "*Penic. glaucum*" will corrode thin plates of lime (*see* p. 61, vol. ii.), is apparently—as in the case of algæ, rich in calcium oxalate—at least partly due to such acidity.

The statement by Sanguinetti (I.) that formic acid and acetic acid are present in cultures of *Asp. oryzæ*, lacks probability, and should be confirmed by means of pure cultures; and the same applies to Heinz's (II.) assumption that acetic acid is produced by *Asp. niger*. Moreover, the statement that a so-called *Lactomyces* fungus will ferment sugar solutions to lactic acid must be regarded as lacking both proof and probability, although the German authorities granted a patent (No. 118, 063, of Feb. 26, 1901) for it, especially as no thread fungi have yet been found to produce that acid. This opinion is endorsed by Czapek (IV.). The souring of culture liquids by certain *Mucorineæ* has been dealt with

already in the present volume (pp. 73, 74). The *Aspergillaceæ* recognised as producers of free acids include primarily *Asp. niger*, *Penicillium luteum*, and two species of *Citromyces*, the first of these furnishing oxalic acid and the last three citric acid.

Decided oxalic-acid fermentation has, so far, been observed solely in the case of *Aspergillus niger*, merely indications being found with *Asp. glaucus*, *Penicillium glaucum*, and also with certain non-*Aspergillaceæ* (*Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Rhizopus nigricans*), the slight traces of an excess of free acid being only prevented from further decomposition by immediate neutralisation. In other groups of the vegetable kingdom, however, free oxalic acid is formed during metabolism, and this acid may remain free (*Rheum* and also *Oxalis* species), though it is usually thrown down at once by the calcium carbonate supplied by the soil-water (Cacti, the buds and bark of various shrubs, algæ). The application of the term "fermentation" to the process in the case of fungi does not affect its similarity of character in all these cases, though, of course, it must not be classed indiscriminately with the formation of oxalates.

The mere occurrence of calcium oxalate crystals in fungi has long been known, and frequently observed in cultures of *Asp. niger*, this being also reported by Schröter (II.). Their origin in gelatin cultures of *Penicillium glaucum* was mentioned by A. Hansen (I.) in 1889, and in sclerotia of the same fungus by Brefeld in 1874. A. de Bary (II.) referred to the formation of soluble oxalates by *Sclerotinia sclerotiorum*; and Duclaux (XXII.), in 1889 mentioned casually (and without any experimental proof) the formation of oxalic acid or oxalates in cultures of *Asp. niger* on different substrata. Zopf (XIV.) in 1889 found oxalate crystals in cultures of a species of yeast, and also in cultures of various acetic bacteria in peptonised sugar solutions with added gelatin; and thoroughgoing observations on this point were published by Banning (I.). The proof that oxalic acid in the free state is produced by fungi, especially *Asp. niger*, solely in presence of carbohydrates or chemically allied substances, was afforded by Wehmer (V.) in 1892; and at the same time an attempt was made to bring the previously known facts to a focus and refute the budding hypotheses on the relation between the occurrence of oxalic acid and the formation of protein. These discoveries led to a series of definite determinations on the oxalic fermenta tion of this fungus, the result being to rank the process with other fermentations. According to Wehmer (XXVI., XXVI.*a*, and V.) the process goes on in the following manner:

As soon as the vegetation has developed from the sown spores, the nutrient solution of the pure culture of *Aspergillus* at room temperature begins to turn red Congo paper blue, and to liberate gas in presence of calcium carbonate—both certain reactions for

the presence of free acid. The acidity gradually increases to a maximum, declining once more during the next few weeks, to gradually fall to zero when the experiment is prolonged, the reaction being even alkaline finally. The capacity of this fungus for destroying free acid can be demonstrated by placing the mature vegetable growths on dilute solutions of oxalic acid (containing 0.5 per cent. of crystalline acid). The limit of the accumulation of acid averages about 0 2 per cent. of the volume of the liquid. The amount of sugar is immaterial, but the general conditions of the environment are important.

The acidification is primarily dependent on the organic nutriment presented, sugars, or chemically allied substances being essential, whereas no free acid is produced when salts of organic acids, amides or peptone are used, though an abundance of oxalates is formed. A decisive influence is also exerted by the inorganic bodies present, especially the source of nitrogen for the growing fungus, the liberation of acid being absent when ammonium chloride or sulphate is substituted for potassium, calcium, or ammonium nitrate (even in presence of sugar); and, in fact, these additions will prevent the formation of acid in cultures that would otherwise acidify at once. Temperature also plays an important *rôle* from the outset, and has a determining effect on success, lower temperatures favouring the accumulation of acid, whilst high temperatures have an adverse effect, so that at the optimum temperature for the growth of the fungus (about 37° C.), acidification ceases to occur, the highest production (up to about 1 per cent.) being attained at a few degrees above the minimum growth temperature (about 7° C.). The fungus being actually capable of far more readily decomposing free oxalic acid at higher temperatures (and even when 0.4 per cent. is present), the accumulation at lower temperatures is therefore solely the result of retarded oxidation, that is to say, enfeebled oxidising action. The whole shows clearly that, contrary to preconceived ideas, a relative scarcity of oxygen is not the cause of the production of oxalic acid, since all the growths in these experiments had an equal supply of oxygen, and the acid must therefore be regarded as the product of incomplete oxidation only in the sense that this oxidation has been prevented by some adverse influence or other.

A remarkable influence is exercised on the process by the addition of salts able to combine with the acid. In this case the accumulation of the combined acid is progressive, and finally attains extraordinary dimensions. The resulting calcium oxalate may amount to more than 100 per cent. of the sugar originally present, so that 15 grms. of sugar furnish about 10 grms. of (anhydrous) oxalic acid (corresponding to about 7 grms. of sugar), without the crop of the fungus being affected. In this way the fungus produced the following quantities of calcium oxalate

from 1.5 grms. of grape sugar in presence of added chalk, at 15°–20° C.:

After 11 days	0.282 grm.	After 72 days	1.340 grms.
,, 16 ,,	0.570 ,,	,, 100 ,,	1.642 ,,
,, 27 ,,	0.650 ,,	,, 120 ,,	1.615 ,,
,, 46 ,,	1.122 ,,	,, 247 ,,	1.730 ,,

In the absence of added chalk, the amounts precipitated from the same nutrient solution were only:

After 9 days	0.005 grm.	After 66 days	0.298 grm.
,, 16 ,,	0.070 ,,	,, 78 ,,	0.130
,, 23 ,,	0.170 ,,	,, 97 ,,	0.103
,, 46 ,,	0.255 ,,	,, 120 ,,	0 018
,, 54 ,,	0.248 ,,	,, 175 ,,	0.014

At higher temperatures (34°–35° C.), under otherwise equal conditions, only traces of oxalate were formed, viz.;

Af er 4 days	0.000 grm.	After 32 days	trace
,, 8 ,,	0.008 ,,	,, 42 ,,	,,
,, 15 ,,	0.028 ,,	,, 68 ,,	0.068 grm.
,, 18 ,,	0.000 ,,		

On the other hand, at 7°–9° C., without chalk, 0.624–0.820 grm. was found after about seven months; and with chalk, even at a temperature of 34°–35° C., there was obtained from the same amount of sugar (1.5 grms.), 1.133 grms. of calcium oxalate at the end of forty-six days, and 1.340 grms. after seventy-two days.

In order to withdraw the acid from the further action of this physiologically interesting fungus by fixation, it is not even necessary to convert it into an insoluble salt, the same effect being produced by the aid of soluble salts, such as alkali phosphates of alkaline reaction, and even neutral alkali phosphates, the latter being transformed into acid oxalates. Apparently the appearance of alkali acid phosphates in phanerogams (*Oxalis*, &c.) is based on this circumstance. Conversely, it is interesting to find that *Penicillium glaucum* decomposes both free acids and alkali oxalates much more readily than *Aspergillus* does, and, therefore, if for no other reason, cannot be a generator of acidity to any extent.

Although, in general, experiments of this kind with *Asp. niger* proceed with the certainty of a chemical test, variations are not unknown in individual cases. The isolated instances in which Wehmer (XVII.) and Emmerling (VI.) found no acidification must probably be allocated to this category. Other factors may, perhaps, have contributed, since, according to a previous discovery by Wehmer (XXVIII.), the addition of even a trace of iron salts to cultures grown in the light can favour the redecomposition of the acid. References to the formation of oxalic acid by *Asp. niger* are also found in certain recent investigations, such as in

those of Emmerling (VI.) and Heinze (II.). We have naturally excluded here the cases in which the production of oxalic acid is regulated by the liberation of bases during metabolism (*e.g.*, the salts of other organic acids, amides, peptones, &c., are consumed). In such cases oxalates are produced instead of any surplus of free acid, the process here—which is independent of temperature—being of a different character, and in the absence of the liberated bases no accumulation of acid would take place. To this class of process relate several earlier and later investigations mentioned in the literature, where the workers, *e.g.*, Zopf (XIV.), Banning (I.,) &c., employed additions of amides, peptones, meat extract or gelatin, instead of merely sugar solutions and mineral salts. In presence of actively oxidising organisms, the chemical nature of these substances necessarily entails the formation of oxalates or carbonates. Up to the present, no competitor exhibiting the same decided peculiarity as *Asp. niger* has been found among the thread fungi, yeasts or bacteria.

A work on the production of oxalic acid by *Asp. niger*, published in 1905 by Charpentier (I.), who was unfortunately not acquainted with the existing literature of the subject, merely repeats what was already well known; and his remarkable conclusion that the production of acid is a result of the exhaustion of the nutrient medium shows such an inaccurate conception of the true state of affairs as to require no serious refutation. According to Heinze (II.), acetic acid is formed along with the oxalic acid—a statement requiring further confirmation, at least so far as pure cultures are concerned. In view of the ease with which acetic acid is decomposed by the fungus in question, as reported by Pfeffer (VI.) and Duclaux (XXII.), this formation is not very probable, nor has it been properly demonstrated. Moreover, the circumstance that Heinze's three experiments with a two-fold and three-fold quantity of nutrient solution, the amount of oxalic acid was correspondingly greater than with 200 c.c., is—as emphasised by Wehmer—the natural result of the regulation of the production of acid, and not due to the lower content of nitrogen. That Heinze observed the formation of potassium nitrate by this fungus from peptone, gelatin, &c., is incredible from the sum of his reports. The brief statements of Kostytschew (I.) regarding the production of acid by the intramolecular respiration of *Aspergillus* are of too general a character to allow definite conclusions to be drawn from them.

Citric acid fermentation ranks along with that in which oxalic acid is produced, and relates solely to the formation of free citric acid, but not to the production of citrates so generally observed among the phanerogams and fungi. A parallel to the exciters of this fermentation is found among the phanerogams, in the *Citrus* species, just as *Aspergillus niger* is physiologically allied to the *Rumex* and *Rheum* species. We shall now deal concisely with the chemistry conditions and course of the process, as

observed by Wehmer (XXVIII.), more particularly in the case of *Citromyces Pfefferianus* and *Citr. glaber*. According to the same worker (XXIX.), both *Penicillium luteum* and *Mucor piriformis* are feeble acid-formers.

Being an oxidation fermentation, the process is dependent on the presence of an abundance of oxygen, in the same degree as the acetic and oxalic acid fermentations. When air is excluded, neither *Aspergillus* nor *Citromyces* spores will develop at all, and even the mature growths only survive a short time under these conditions. Although citric acid must also be regarded as the product of an incomplete oxidation, its formation is not the result of an insufficient supply of oxygen, but the consequence of a decomposition arrested through other causes. True, the temperature does not seem to be such a decisive factor as in oxalic acid fermentation, and further determinations are necessary in order to elucidate the dependence of the process on external conditions. In this case also the chemical character of the organic nutriment is an essential factor, carbohydrates or allied substances alone, and not peptones, amides, salts of organic acids, &c., enabling the production of free acid to take place.

In its incipient stage, the acidification, which takes place without any visible liberation of gas, can be detected by the blue reaction with Congo paper; and an addition of chalk produces brisk effervescence. The acidity gradually increases, the limit being reached at about 8 per cent., without any apparent influence on the development of the fungus. The latter then begins to redecompose the accumulated acid, and the acidity decreases, no trace of free acid being perceptible at the end of a few weeks longer; hence it is undoubtedly merely an intermediate product that has momentarily escaped further decomposition. In this case, also, the anticipated effect of fixing the acid in the form of salts is realised, neutralisation preserving the acid from redecomposition; at the same time the formation of acid is accelerated and the total amount is considerably increased. Consequently, whilst the acidity increases but slowly in the absence of chalk, the addition of this substance to the acidifying culture results in a continuous liberation of gas, much more apparent than in the case of oxalic acid fermentation, and followed in a short time by an extensive deposition of calcium citrate.

The citrate is separated from the unaltered calcium carbonate by dissolving it in hydrochloric acid, neutralisation with ammonia, and boiling—which precipitates the citrate—the mass being dried at 110° C. and weighed, so that the quantitative yield can be approximately determined. The average weight is from one-third to one-half the quantity of sugar, in the form of the crystallised acid (with 1 molecule of water), this being recovered in the usual manner by freeing it from the lime salt with sulphuric acid, filtering from the resulting gypsum, and concentrating to the point of crystallisation. Hence, in well-conducted

experiments, nearly half the sugar is converted into citric acid, without any appreciable hindrance to the development of the fungus. As we have seen, *Asp. niger* is also able to transform about one-half the sugar (dextrose) into oxalic acid, the resulting weight of acid being more than three-quarters that of the original sugar.

$$C_6H_{12}O_6 + O_3 = C_6H_8O_7 + 2H_2O$$
180 — 192 (+ 1 molecule H_2O = 210)
Dextrose. — Citric acid.

$$C_6H_{12}O_6 + O_9 = 3C_2H_2O_4 + 3H_2O$$
180 — 270 (+ 2 molecules H_2O = 306)
Dextrose. — Oxalic acid.

In both cases about one-half the material is consumed in satisfying the needs of the fungus; but possibly an alteration in the conditions of experiment might increase the proportion of the product. The resulting calcium citrate remains at first dissolved in the culture liquid, and it is only as the concentration increases that it separates out largely in the form of a bulky crust, consisting of coherent acicular or granular concretions, at the bottom of the vessel. When precipitated in the above manner from hot solution, it has the composition $(C_6H_5O_7)_2Ca_3 + 4H_2O$, containing therefore about three-quarters of its weight of crystallised acid ($C_6H_8O_7 + H_2O$), which is recovered in a pure state, free of impurities.

With reference to the technical importance of a process of this kind for manufacturing citric acid, it may be mentioned that the market price of the acid is about six guineas per cwt., whilst that of the raw material is only about one-tenth that figure. Nevertheless, there are certain difficulties, not easily overcome in practice, with respect to the nature of the apparatus required for manufacturing large quantities of acid, as well as in connection with the risk of infection and the fluctuating character of the fermentative power. The "Fabriques de Produits Chimiques" at Thann and Mülhausen, under the management of Scheurer-Kestner, have been occupied with this question for a long time.

The chemistry of the process also merits a brief description. The conversion of sugar (dextrose) into citric acid is a matter not merely of oxidation, but of the simultaneous splitting up of the normal carbon chain of the sugar molecule. In accordance with the formula of the acid, the one carbon atom is transferred to a side chain:

COOH	COH
CH_2	CH.OH
COH.COOH	CH.OH
CH_2	CH.OH
COOH	CH.OH
Citric acid.	CH_2OH
	Dextrose.

Hence the process appears more complicated than other fermentations; and, contrary to statements in the literature, no one has yet succeeded in obtaining the acid by the simple oxidation of sugar.

There is little to be gained by going into the question of the biological importance of such acid fermentations, since it affords no explanation. Although, in the case of oxalic acid fermentation it might be that the accumulating acid is injurious to competitors for the available food, this can hardly apply to citric acid fermentation. In both cases the acid is lacking at the time when it would be most effective, namely, at the commencement of vegetation; and where it is afterwards present in abundance, it no longer possesses any value in this respect, the fungus having already fully occupied the substratum. Moreover, the accumulation injures the fungus, and finally, liquids containing citric acid also permit the development of certain other fungi. A far more important problem is whether the organism is not itself injured by the waste of substance entailed by the accumulation of acid, as is certainly the case, for instance, in alcoholic fermentation. This does not appear to be so with oxalic acid fermentation, and, according to Wehmer (XXVIII.), it is not appreciable in the case of citric fermentation, despite the higher physiological value of this acid, the only way in which it can be estimated being by careful quantitative determinations. In general, however,—from the standpoint of practicability—it may be said that in the interests of the organism fermentations of this kind are better dispensed with, since they imply a more or less uneconomical utilisation of the substratum.

Mazé and Perrier (I.) have latterly occupied themselves with the formation of citric acid. They observed this to occur from alcohol and glycerin, and ascribe its origin to an incipient scarcity of nitrogen in the culture, though it is independent of the presence or absence of oxygen. The acid is said to result from a process of disassimilation when the substratum has been exhausted of assimilable nitrogen; and its formation is preceded by a decomposition of the sugar into alcohol and carbon dioxide. It would probably be easy to refute these statements by experiment. Mazé and Perrier seem to be acquainted with only a preliminary communication by Wehmer (XXX.), and not with his more exhaustive work (XXVIII.).

§ 292. The Fission of Disaccharides and Trisaccharides, Glucosides and Polysaccharides (Starch Excepted).

The inverting enzyme of the *Aspergillaceæ* was discovered a few years anterior to their diastasic enzyme; and subsequently various other enzymes were recognised. Up to the present, these have chiefly been detected in *Asp. niger* and *Pen. glaucum*, the latter

being invariably a collective term for a number of very similar species not more closely identified.

Asp. niger splits up all the sugars here in question (except milk sugar, which still remains doubtful) before consuming them, and therefore secretes invertase (sucrase), maltase, trehalase, melecitase (?) and raffinase. This has been demonstrated by a series of observations, dating from the year 1878, by Gayon (V.), Duclaux (XXII.), Fernbach (V.), Bourquelot, Hérissey and Gillot (III.). Bourquelot especially—both alone and in collaboration with Hérissey and Graziani—has repeatedly investigated these enzymes, and enriched our knowledge of them. The first report of the inversion of saccharose by the fungus dates from 1878, namely, by Gayon (V.); and Duclaux has repeatedly treated the question since 1883. According to Fernbach, who detected the enzyme in 1890, and also examined the deterrent influence of light on its action, the formation of invertase is *not* dependent on the presence of saccharose in the culture liquid. In 1893 it was studied by Bourquelot; and in 1896 this worker, in association with Hérissey (I.), demonstrated the enzymatic splitting up of the trisaccharide melecitose into dextrose and turanose—the latter a disaccharide analogous to maltose, and one that cannot be further modified by the fungus, though it can be by the action of acids. The same worker obtained, in 1893 (also by alcoholic precipitation), an enzyme (trahalase) capable of hydrolysing trehalose into two molecules of dextrose, and differing from invertase and maltase though it may be identical with the amylase of E. Fischer (III.) which acts in the same way. Bourquelot finally demonstrated the enzymatic fission of maltose and raffinose (VII.)—the latter by means of a separate enzyme—and also (VI.) that of the trisaccharide gentianose into dextrose (2 molecules) and lævulose, subsequent to the intermediate formation of gentiobiose (capable of subdivision into 2 molecules of dextrose) and lævulose (invertase and emulsin), in part of which researches he collaborated with Hérissey (III.). With regard to raffinase, opinions are still divided, some considering its action limited to the splitting up of melibiose (Bau's "melibiase") into galactose and dextrose, and that the conversion of melitriose into melibiose and dextrose is accomplished by invertase. According to E. Fischer, it is very similar to maltase; with which melecitase is also probably identical. Furthermore, according to Bourquelot (V.), gentiobiose and turanose are also capable of being split up by specific enzymes. The action of maltase was also investigated by Hérissey (I.), and that of raffinase by Gillot (III.), both working with the same fungus. Consequently, a considerable literature exists on the sugar-decomposing enzymes of *Asp. niger* alone.

"*Penicilllum glaucum*" behaves in a very similar way, this fungus having already been found to contain invertase (Duclaux,

1883), maltase and trehalase (by Bourquelot (VII.) and others since 1880), and raffinase (by Gillot (III.) in 1900). The optimum temperature (45° C.) of *Penicillium* maltase is about 30° lower than that of *Aspergillus* (Bourquelot). The observation made in 1864 by Béchamp (XII.), on the inversion of saccharose by the filtrate from crushed mould fungi, probably relates to "*P. glaucum.*"

Bourquelot and Graziani (I.) failed to discover any invertase in the culture liquid in the case of the saccharose-inverting *Penicillium Duclauxii* (which is probably identical with *P. luteum*), the enzyme being, perhaps, retained by the mycelium.

A. oryzæ has long been known for its inverting power (Atkinson, 1881, Kellner, Mori and Nagaoka, 1889), by virtue of which it effects the enzymatic fission of maltose, though not lactose. According to Kozai (I.) it can also degrade raffinose (melitriose). Our present ideas on the subject no longer allow us to ascribe these effects to a single enzyme (the eurotin and invertase of older workers); and indeed Kellner doubted the uniform nature of "invertase." Lactic acid in small quantities (0.05 per cent.) acts as a stimulant in respect of the fission of saccharose, but even as little as 0.1 per cent. retards the action, and 0.6–0.7 per cent. restricts it entirely; indeed the action sinks to about one-fifth with 0.5 per cent.—compare Kellner, Mori and Nagaoka (I.). The action of alcohol and common salt is probably about the same as in the case of amylase.

A special position is occupied by *Allescheria Gayoni* (= *Eurotiopsis G.*), inasmuch as it contains lactase, but not invertase. In addition, maltase and trehalase were detected in this fungus by Laborde (VI.) in 1897. In the fermentation of solutions of invert sugar, the lævulose was attacked at an appreciably more rapid rate than dextrose. Up to the present, this is the only member of this family of fungi that has been observed to split up lactose enzymatically, previous to consuming it. The question whether the same is done by *A. niger* and *P. glaucum* was discussed by Duclaux (XXII.), as long ago as 1889, but was left unsettled. More recent experiments by Schäffer (IV.) also led to no definite result, although this worker thought he observed a slight action on lactose in the case of *A. niger*, *A. oryzæ* and *P. glaucum.* He also found that saccharose solution is inverted by the extracts furnished by vegetations of all the *Aspergillaceæ* examined (*A. Wentii*, *A. fumigatus*, *A. glaucus*, *A. oryzæ*, *A. niger*) and *Penicillium* (*P. luteum*, *P. rubrum*, *P. italicum*, *P. glaucum*), whilst lactose exhibits an incomparably greater resistance. He likewise states that maltose is converted into glucose by extracts of the fungi in question.

A good deal of information is also available on the fission of the glucosides, the formation of emulsin being mentioned in the case of *Asp. niger*, *A. oryzæ*, *A. fumigatus*, *A. Wentii*, *Penic.*

luteum, *P. rubrum*, *P. italicum*, *P. glaucum*, and *Allescheria Gayoni*. This enzyme was also isolated from *Asp. niger* in 1893 by Bourquelot (III.), who—partly in collaboration with Hérissey (II.)—studied its behaviour toward several glucosides: amygdalin, salicin, coniferin, helicin, populin, arbutin and æsculin; all of which were split up by the extract from the vegetative fungus, though negative results were obtained in the case of digitalin, solanin, hesperidin, convallamarin, jalapin, &c. The precipitate thrown down by alcohol from the solution concentrated *in vacuo* has the same effect. Hérissey (II.), made a more exact comparison with almond emulsin, from which it differs in several respects, populin and phloridzin, for instance, being split up (into benzoyl, saligenin and phloretin respectively) by the *Aspergillus* emulsin alone. It, however, could not be separated from the other enzymes of this fungus. According to Gérard (VI.), *Penic. glaucum* also secretes an enzyme that can be isolated by lixiviating the fungus, acts like emulsin and splits up amygdalin and salicin. *Asp. glaucus* agrees with the two fungi just named in respect of its behaviour to solutions of glucosides. All three of the fungi were tested, in the form of living vegetations, by Puriewitsch (V. and VI.). On a solution of helicin the fungus died off under the influence of the resulting salicylic aldehyde, salicin was decomposed with formation of saligenin, the dextrose being consumed by the fungus at once; and similar results were obtained with arbutin, coniferin, æsculin, hesperidin and phloridzin. The fission of amygdalin into dextrose, benzaldehyde and hydrocyanic acid was observed only in the case of extracts, or after etherising the fungus, the living vegetations producing neither benzaldehyde nor hydrocyanic acid, so that in this case the fission seems to proceed in a different manner. The author believed that decomposition into sugar and amygdalic acid was effected by an enzyme allied to invertase; but this is certainly incorrect (*see* below). The secretion of enzyme was suppressed by the addition of larger quantities of sugar, which therefore prevents the fission of the glucosides in a manner analogous to the action of diastase on starch. J. Behrens (IX.) found emulsin in *Penic. luteum*; and this fungus also splits up quercitrin. According to Laborde (VI.), the living vegetations of *Allescheria Gayoni* (*Eurotiopsis G.*) will split up the glucosides (amygdalin, salicin and coniferin), with formation of sugar. Results differing to some extent from those of Puriewitsch were obtained by Brunstein (I.) in 1901, who tested *Asp. niger*, *A. oryzæ*, *A. Wentii*, *A. glaucus* and *Penic. glaucum* in presence of helicin, salicin, arbutin, amygdalin, coniferin, myrosin, saponin and glycyrrhicin, all of which were split up by the living vegetations, except myrosin, which gave doubtful results. *Asp. glaucus* and *Asp. Wentii* split up helicin, without formation of salicylic aldehyde, salicylic acid being produced; *Asp. niger*, *A. oryzæ*, and

P. glaucum furnished salicylic aldehyde, this being oxidised to salicylic acid by *Asp. oryzæ*, &c. The latter product was in turn consumed by several of the fungi, especially by *Asp. Wentii*. On the other hand, the hydroquinone formed from arbutin had a poisonous effect. Amygdalin was split up by all the species into sugar and cyanhydrin, which underwent secondary oxidation to mandelic acid, with liberation of ammonia. The fission of amygdalin and helicin by the living fungus, especially by extracts from the vegetations, was also demonstrated in the same year (1901) by Schäffer (IV.), in respect of a larger number of species, *Asp. fumigatus*, *Penic. luteum*, *P. rubrum* and *P. italicum* acting in the same way, in addition to *Asp. niger*, *A. Wentii*, *A. glaucus*, *A. oryzæ*, and *P. glaucum*. In this case, also, potassium myronate was not attacked. The actual organism with which Hérissey (III.) obtained similar effects, namely, the so-called *Asp. fuscus*, Bonorden, is uncertain, Bonorden's description being insufficient for its identification.

The fission of the polysaccharides has also been investigated. The occurrence of an enzyme splitting up inulin was shown, in the case of *Asp. niger* and *Penic. glaucum*, by Bourquelot (III.) in 1893; but, according to Kellner, Mori and Nagaoka (I.), it is lacking in *Asp. oryzæ*, and, according to Laborde (VI.), in *Allescheria*, though the latter forms reducing sugars from gum Arabic. A closer investigation of this enzyme has recently been undertaken by Dean (I.), in the case of *Penic. glaucum* and *Asp. niger*. He finds that it does not issue from the hyphæ spontaneously, so that it belongs to the endo-enzymes. It is injuriously affected by acids and alkalis, even in small quantities; and its optimum temperature of action is given as 55° C. Schäffer (IV.) states that inulase is also secreted by *Asp. oryzæ*, *A. Wentii*, *A. fumigatus*, *A. glaucus*, *A. niger*, *Penic. luteum*, *P. rubrum*, *P. glaucum* and *P. italicum*. On the other hand, the enzymatic solution of "true" cellulose seems to be a matter of rare occurrence with all the members of this family; for, though it is true that Miyoshi (III.) observed bursting of the cell walls on the hyphæ of *Penicillium* being stimulated chemotactically, the same result occurs when mechanical pressure is applied (*see* p. 62, vol. ii.). J. Behrens (IX.) also confirmed the incapacity of *Penic. glaucum* and *P. luteum* to dissolve cellulose, though both were able to dissolve the substance of the middle lamellæ, and therefore—like *Asp. niger*—secrete pectinase. Two species, *Asp. oryzæ* and *Asp. Wentii* are reported as able to grow through the substance of soft-boiled rice and Soja beans; and according to Prinsen-Geerligs (I.), *Asp. Wentii* penetrates and dissolves the cell walls, setting the contents free. In this case, however, the material is not true cellulose, and consequently the nature of the enzyme has still to be determined. It has also been stated by Newcombe (I.), as well as by Okamura and Takakusu (I.), that

Asp. oryzæ, secretes cytase (cellulase); but as the walls of the barley endospore, which were treated (by Newcombe at least) with the enzyme mixture from *Asp. oryzæ* (the so-called "Taka-diastase") consist merely of a hemicellulose (Reinitzer) attackable even by malt amylase, this result is not decisive. In this case the walls were dissolved even before the starch (in twenty-four hours as compared with about eight to twelve days). Oppenheimer (III.) regards this case as one of cellulose solution, and (as reported by Miyoshi) has obtained the same effect with *Penic. glaucum*; but Miyoshi found precisely the opposite. Van Iterson seems to have observed a very feeble action effected by *Asp. niger* on blotting-paper.

The fermentation of tannin, which was also considered to be a glucoside, was reported by van Tieghem (XIII.), in 1867, to be effected by two mould fungi, *Asp. niger* and *Penic. glaucum*. This worker considered that the fission of tannin into gallic acid and glucose was a "true fermentation phenomenon," *i.e.*, a manifestation of vital activity, and not the effect of a substance secreted by the mycelium of the fungus. Fernbach (III.) and Pottevin (II.) afterwards demonstrated contemporaneously that this view is incorrect, and that *Asp. niger* secretes an enzyme (tannase) that is precipitable by alcohol, and is able of itself, in a sterilised solution, to split up tannin (digallic acid) into gallic acid, a yield of 98.7 per cent. of this acid being obtained from pure tannin. In cultures the sparingly soluble gallic acid separates in fine crystals from the tannin solution. This process has been patented (Ger. Pat. 13,187, of 1901) for the production of gallic acid on a commercial scale. The dextrose (12–15 per cent.) observed by van Tieghem as accompanying gallic acid in the product from commercial tannin is not a fission product from the glucoside, but an impurity. Moreover, tannase is formed only in the case of cultures on substrata containing tannin. Its optimum temperature is about 67° C., and it splits up tannates as well as phenyl- and methyl-salicylate. The same enzyme is probably concerned in the formation of gallic acid in opium fermentation, Calmette (II.) stating that *Asp. niger* plays the chief part in the fission of tannin during that process, and also in the inversion of the sugar into dextrose during this prolonged fermentation, which occupies ten to twelve months. Both the dextrose and dextrin are oxidised into calcium oxalate, without the alkaloids being affected. *Asp. niger* is well known as a fungus preferring acid substrata (solutions of organic acids), on which it thrives; and since it also occurs on gall-nuts and extracts of these, its spontaneous appearance in tannin- and opium-fermentation is easily accounted for. It is certain that spontaneous green, vegetative growths of *Penicillium species*, that need further investigation, play a chief part in the fission of tannin. Moreover, the gallic acid fermentation of gall-nut tannin was ascribed

to "organised ferments," even anterior to van Tieghem. Thus Laroque (I.) in 1850 credited this "ferment" with the power of exciting alcoholic fermentation, without discriminating between the various organisms. On the other hand, Robiquet (I.) in 1852 brought about the same fermentation by means of an enzyme (pectase) in gall-nuts, which enzyme was also said to convert pectose into pectin. In comparison with these opinions, the views afterwards expressed by van Tieghem may be regarded as reactionary. Nevertheless the gradual modification of the ideas held on this point is not without interest.

An enzyme capable of saponifying fat was isolated in small quantity from "*Penic. glaucum*" by Camus (III.) in 1897. The extract from *Asp. niger* gave only a very weak effect in the hands of the same worker (IV.), though this fungus will grow luxuriantly on certain fats (*e.g.*, olive oil in presence of nutrient salts). Gérard (II.) in 1897 demonstrated the occurrence of lipase in *Penicillium*, by means of the method elaborated by Harriot and Camus (I.), but found that the emulsin of this fungus cannot split up fats. Laxa (II.) in 1902 showed that triturating the hyphæ of *Penicillium* liberates an enzyme capable of splitting up butter fat with considerable energy. According to Bremer (I.), a gradual effect of fission is produced on cotton-seed oil by *Aspergillus* species (*A. glaucus* and *A. flavus*). Laborde (VI.) states that *Allescheria* (*Eurotiopsis*) also will split up oil and butter fat energetically, with formation of acid. Lipase was stated by Garnier (II.) to occur in the cultures of *Asp. fumigatus A. flavus*, *A. glaucus*, *A. niger*, *A. nidulans*, and especially in *A. versicolor*. The fission of fats in the sludge of clarifying tanks has been dealt with already (*see* pp. 64, 65, vol. ii.).

§ 293. Formation of Alcohol.

With a single exception none of the *Aspergillaceæ* excites an appreciable alcoholic fermentation. It is true that several species have been credited with forming alcohol; but, even where this has been shown beyond doubt, the quantity produced is insignificant. Sanguineti (I.), states that *Asp. oryzæ* forms alcohol from saccharose, starch and dextrin (up to 4 per cent. by weight in ten days), so that—assuming that, as reported, this organism can form 20 grms. of alcohol from 50 grms. of saccharose in the time mentioned—this fungus should be regarded as an important exciter of fermentation. Sanguineti's isolated experiments, however, need further confirmation. According to Pasteur (XXV.) *Asp. glaucus* forms about 1 per cent. of carbon dioxide and alcohol when submerged in wort—though not when exposed to the air—the mycelia separating into a number of rounded cells; and similar minute quantities of alcohol are said to be produced, in culture liquids, by *Penic. glaucum*. Gosio (VII.) reports in similar fashion with regard

to *Penic. brevicaule*. Doubts, whether justified or not, have been thrown on this alleged capacity in the case of *Asp. glaucus*, *Asp. niger* and *Penic. glaucum;* though the statement of ELFVING (I.), that he found up to 4.2 per cent. by weight of alcohol in cultures of "*Penic. glaucum*," is rather strange. One cannot reject offhand the possibility of this substance being present in fungus cultures to a larger extent than is now believed; and the matter requires closer attention. Perhaps the alcohol has hitherto escaped notice owing to the circumstance that the vegetation of certain species readily decomposes ethyl alcohol (*see also* p. 80, vol. ii.). For instance, according to Laborde, *Eurotiopsis* can decompose up to 10 per cent. and *Asp. niger* (according to Duclaux) up to 6–8 per cent., whilst this substance, in the form of a 3 per cent. solution (when accompanied by mineral food-stuffs), is a suitable nutrient material for both *Asp. niger* and *Penic. glaucum*—compare WEHMER (V.) and COUPIN (I.). Hence, when—as is usually the case—these organisms cannot be grown in a restricted supply of air, a rapid oxidation of the alcohol—sufficient to prevent accumulation—must be reckoned with. MAZÉ (II.) regards alcohol as a normal intermediate product of the decomposition of sugar by micro-organisms, and supported this opinion by experiments with *Allescheria Gayoni* (*Eurotiopsis*) in 1902.

This fungus, in fact, constitutes, according to LABORDE (VI.), the single exception already mentioned. It excites normal fermentation in solutions of dextrose, lævulose, maltose and lactose—subsequent to enzymatic fission in the case of the two last, succinic acid and glycerin being formed in addition to alcohol and carbon dioxide. A restricted supply of oxygen is an essential condition, but none of these fungi will survive the total exclusion of that gas. There is no production of spherical yeast, as in many of the *Mucorineæ*, the submerged mycelium retaining its appearance unchanged. From 100 grms. of sugar Laborde obtained, on the average, 46.4 grms of alcohol, 44.4 grms. of carbon dioxide, 2.3 grms. of succinic acid and 1.8 grm. of glycerin, with an increase of 4–5 grms. in the weight of the fungus (total 94.9 grms.). In the case of the first two sugars, this result corresponds to about 2 grms. less than by fermentation with *Saccharomycetes*, the latter furnishing Pasteur with 48.6 grms. of alcohol, 46.8 grms. of carbon dioxide, 3.2 grms. of glycerin, 0.6 grm. of succinic acid and 1.2 grm. of yeast (total, 100.4 grms.). The appearance of the fermenting fungus closely resembles that of *Mucorineæ* under the same conditions, the submerged mycelium developed from the sowing being quickly interspersed with large bubbles of gas, and also exhibiting a tendency to pass over into surface vegetation. In about six weeks the alcohol produced amounted to upwards of 8 per cent. A 14 per cent. solution of invert sugar was attenuated down to 2 per cent. of sugar in sixteen days, the lævulose disappearing comparatively quickly. Inverted lactose gave a more

sluggish fermentation, 4–5 per cent. of alcohol being formed. Galactose by itself was more difficult to ferment, the process ceasing on 2–3 per cent. of alcohol being formed. Maltose (1–2 per cent. of alcohol) and lactose (2–3 per cent. of alcohol) behaved in a similar way; and their fission anterior to fermentation is difficult to determine. In presence of air, the fungus readily consumes alcohol, even when—as already mentioned—10 per cent. is added to the culture liquid. Nearly the whole of the sugar in a 10 per cent. solution disappears, within twelve days, when in contact with the surface vegetation of the fungus at 25° C., without more than 0.2 per cent. of alcohol being detectable. There is nothing remarkable in this, in view of the aforesaid fact (reported by Wehmer) that sowings of conidia of *Asp. niger* and *Penic. glaucum* on a 3 per cent. solution of alcohol (as the sole organic food-stuff) and inorganic nutrient salts, will develop to complete vegetative coatings; whilst, according to Duclaux, these cultures of *Asp. niger* will also decompose 6–8 per cent. of alcohol.

§ 294. The Degradation of Proteids and their Derivatives.

The property of liquefying gelatin is so general among the filamentous fungi, including the *Aspergillaceæ*, that only the exceptions are really of interest. The rapidity of this liquefaction—and probably sometimes also the time of its inception—depends largely on special conditions (the concentration and reaction of the gelatin, the presence or absence of certain substances, the temperature, &c.). Even the same species does not always behave in the same way, and therefore the appraisement of its diagnostic value is probably on a par with the case of bacteria (*see* vol. i. p. 299), though the feature possesses a certain importance in any event. The liquefactive power of *Penic. glaucum* seems to have been first investigated by A. Hansen (I.) in 1889, and that of *Asp. niger* by Bourquelot (XII.) in 1894. A tentative comparison, with streak cultures in 10 per cent. wort gelatin at 15° C., by Wehmer (XII.) showed that *Asp. glaucus* and *A. fumigatus* liquefy a gelatin very slowly, the results not being appreciable until several weeks have elapsed; whereas about half the gelatin was liquefied in ten days by *A. niger*, *A. oryzæ*, *A. candidus*, *A. minimus*, *A. novus*, *A. ostianus*, *Penic. glaucum*, *P. luteum*, *P. italicum*, and *P. olivaceum*; and, according to Wehmer (XVII.), *Asp. clavatus*, *A. flavus*, *A. Wentii* and *A. giganteus* act with equal promptness. Schäffer (IV.) has also published the results of experiments in the same direction, and with about the same fungi. If well-defined conditions be maintained, the results may be utilised for diagnostic purposes; at any rate, the secretion of the liquefactive enzyme is not retarded by the presence of sugar. Only scanty information is

yet available as to the nature of the proteolytic enzyme or enzymes. That of *Penic. glaucum* was extracted by A. Hansen (I.) in 1889 from the vegetations by means of glycerin. The solution converted neutral gelatin into glutopeptone more rapidly than acid gelatin, whether sugar were present or not. Isolation by precipitation with alcohol was found to be impracticable, the resulting precipitate being inoperative. Possibly the quantity obtained was too minute, since the experiments showed that the substance is actually excreted by the hyphæ into the substratum, and acts at considerable distances, as well as through an artificial collodion film.

Stoll (I.) carried out a series of comparative experiments on the proteolytic power of *Penicillium* species, the influence of the reaction of the medium being also observed, normal, acid (acidified with normal sulphuric acid), and alkaline (with normal caustic soda) gelatin and sugar gelatin (containing 2 per cent. of dextrose) being employed at a uniform temperature. *Penic. brevicaule* liquefied alkaline gelatin more quickly than the acid sample (4–6 days), but did not liquefy sugar gelatin, though this latter was very gradually liquefied by *Penic. glaucum* under the ordinary experimental conditions. Increased additions of alkali or acid seemed to favour the action in this latter case, whereas further additions of sugar had a contrary effect. *Penic. olivaceum* liquefied the same acid and alkaline gelatin only after nearly four weeks, whilst sugar gelatin remained unaltered at the end of a fortnight. *Penic. italicum* also had no effect on sugar gelatin, though it acted on acid or alkaline gelatin after about a fortnight, the same behaviour being observed with *Penic. rubrum* and *Penic. purpurogenum*. Hence, with the exception of "*Penic. glaucum*," the addition of sugar prevented the liquefaction of gelatin (*see also* p. 63, vol. ii.). The previously mentioned experiments, and the observations of Malfitano, show that these results must not be taken, unconditionally, as generally applicable, other circumstances, such as the concentration of the gelatin, the presence of other nutrient substances, &c., having to be considered, since *Penic. brevicaule*, for example, is known to have a decided liquefying influence on 10 per cent. wort gelatin (*i.e.*, gelatin and sugar). Further particulars on the behaviour of four species of *Aspergillus* toward gelatin will be found in a recent work by Tiraboschi (II.).

The fact that an extract from *Asp. niger* soon dissolves fibrin and coagulated egg albumen, and also liquefies gelatin was already reported by Bourquelot. Malfitano (I.), who was the first to investigate this point more fully, found that the method of nutrition was immaterial as regards the formation of the proteolytic enzyme ("protease"), this apparently diosmotising only after the death of the cell. It can be recovered by drying and grinding young and still living vegetative growths, and then extracting them with chloroform water, and using alcohol as a

precipitant. The action of the enzyme is retarded by an acid reaction, neutrality being the most favourable condition and alkalinity highly prejudicial. Casein and uncoagulated albumen are also attacked, though less powerfully, whilst coagulated albumen and egg albumen are left intact. Milk casein, thrown down by the lab enzyme, is gradually dissolved. Though nothing certain is yet known about the final product of the reaction, this protease is apparently different from pepsin, pancreatin and papayin. Butkewitsch (I.) also occupied himself with the enzymatic proteolysis effected by the same fungus. According to Duclaux (VII.), "*Penicillium glaucum*" contains tryptic casease in addition to the lab enzyme (*see* vol. i. p. 243). The further degradation of protein by the fungi under consideration results finally in the formation of amino acids and ammonia. *Aspergillus niger*, however, as was shown by Wehmer (V.) in 1892, forms large quantities of ammonium oxalate in solutions of peptone, 5 grms. of peptone furnishing more than 2 grms. of calcium oxalate; and, according to Kosjatschenko (I.), it also produces from the protein of peas, tyrosin, leucin, histidin, arginin and lysin. On the other hand, according to Butkewitsch (I.), "*Penicillium glaucum*" seems to furnish chiefly amino acids (leucin and tyrosin), so that tryptic enzymes are apparently in question, as was proved by Saito (II.) by the formation of tryptophane in the case of nineteen species of fungi. Some practical importance also attaches to the question of the degradation of protein in the ripening of certain cheeses (Brie, Camembert and Roquefort) by species of *Penicillium*, on which point reference should be made to the labours of Roger, Epstein, Jensen and Thom.

The coagulation of milk is effected in 2–10 days by all the species examined on this point by Schaeffer (IV.), viz., *Asp. niger*, *A. fumigatus*, *A. glaucus*, *A. Wentii*, *A. oryzæ* (2 days in this case), *Penic. glaucum* (in 3 days), *P. luteum*, *P. italicum*, and *P. rubrum*. These species also peptonised milk casein, coagulated egg albumen, fibrin (except *Penic. glaucum* and *P. rubrum*), and vegetable casein. Teichert (I.) also pointed out that "*Penicillium glaucum*" has a decided degrading action on casein, and, according to Conn, Thom, Bosworth, Stocking and Issajeff (I.), both the technical *Penicillium* species of Roquefort and Camembert cheese (*P. Roquefort* and *P. Camembert*) also attack cheese by an excreted proteolytic enzyme. Lab enzyme was also found by Saito (II.) in *Asp. oryzæ*. Similarly, according to Swanoff, *Asp. niger* and "*Penic. glaucum*" contain an enzyme (nuclease) which splits up the nucleo-proteids into xanthin bases and phosphoric acid.

Shibata (I.) states that *Asp. niger* produces an enzyme, or group of enzymes (amidases), furnishing ammonia, like urase. The triturated, dead mycelium acts on urea, biuret and certain acid amides (acetamide, oxamide), with formation of ammonia.

On the other hand, urethane, guanidin, allantoin and uric acid remained intact, and the action on benzamide and asparagin was barely appreciable, whilst hippuric acid was split up into glycocoll and benzoic acid. Stoll (I.) also observed the formation of ammonia by *Penic. brevicaule* from ordinary gelatin. An unrecognisable species, *Asp. terricola*, is said by Wiley (I.) to be a powerful ammonia-former in soil; but the production of ammonia compounds from organic nitrogen compounds is not a specific characteristic.

§ 295. Colouring-matters, Poisons, Oxidations, &c.

On saccharine substrata containing traces of arsenic or arsenious acids and its salts, *Aspergillus glaucus*, "*Penicillium glaucum*," *Penic. brevicaule*, &c., liberate strong-smelling diethylarsine (*see* p. 50, vol. ii.). According to R Schmidt (II.), "*Penic. glaucum*" and *Asp. flavus* liberate sulphuretted hydrogen from sulphates, &c., and arseniuret'ed hydrogen from solutions containing arsenic. Dubois (III.) states that *Penicillium* mycelia will precipitate basic copper carbonate (patina), from solutions containing copper, on to bronze. The frequently reported fixation of free nitrogen on the part of "*Penic. glaucum*" and *Asp. niger* by Berthelot (II.), Puriewitsch (VII.) and Saida (I.) need only be mentioned here (*see* vol. i. p. 353). Little is yet known as to the nature of the yellow, brown and red colouring-matters produced by various species (*Asp. niger*, *A. glaucus*, *A. Ostianus*, *Penic. luteum*, &c.). According to Linossier (II.), that formed by *Asp. niger* ("Aspergillin") is an organic compound of iron; but this remains to be proved. Zukal (III.) states that the colouring-matter produced by *Penic. luteum* is a "fungus acid"; at any rate it is a substance soluble in alcohol and reprecipitable by water. A golden yellow pigment is said by Milburn (I.) to be produced by *Asp. niger* under certain conditions, in the form of a granular excretion from the aerial hyphæ. The alcoholic solution is decolorised by alkali, but not by acid, and the pigment is decomposed by light into a reddish brown substance, so that it is found only in cultures kept in the dark. Possibly the dark pigment of the conidia is formed therefrom by oxidation. R. Meissner (I.) carried out tests with the red-brown pigment of *Asp. medius* (probably synonymous with *Asp. glaucus*). The green conidial pigments produced in the vegetative growths of most *Aspergillaceæ* have not yet received attention. The pigment, soluble in alcohol, of *Asp. versicolor*, Vuill., varies between yellow-brown, orange and red, according to the reaction of the nutrient solution; see Vuillemin (II.), and also Coupin and Friedel (I.) on this point. The dependence of the production of yellow to red colouring-matters on the composition of the substratum, especially as regards *Penicillium* species (*P. olivaceum*, *P. purpurogenum* and *P. rubrum*), has been mentioned by Stoll (I.).

The injuries set up by species that are pathogenic in plants and animals (*Asp. fumigatus*, *A. flavus*, *A. nidulans*, *Penicillium luteum*, *P. glaucum*, *P. italicum*, *P. olivaceum*) are probably attributable to the production of definite poisons; but, in the case of those belonging to the second category—a list of which was compiled by Guéguen (III.)—no further particulars are yet available. J. Behrens (IX.) also failed to ascertain anything definite with regard to the active substance in the fungi which cause the rotting of fruit, but it is apparently not an enzyme and is non-volatile. A similar *rôle* is ascribed to free oxalic acid in the case of *Asp. niger*, which is said by Behrens (XVI.) to be dangerous to plant embryos. Lode (II.) failed to detect any poisonous substance in cultures of species that are pathogenic towards animals.

In several instances investigations have been made into the destructive action, exerted more particularly by vegetative growths, on readily oxidisable substances, such as organic acids and alcohols. This is related to the previously mentioned fact that oxalic acid, citric acid and ethyl alcohol are decomposed again by the fungi that have produced them (*Asp. niger*, *Penicillium*, *Citromyces*, *Allescheria*); but whether oxydases are concerned is still unknown. So far as the substances in question form suitable food-stuffs (tartaric acid, citric acid, lactic acid, &c.), this is nothing remarkable; but it is also exhibited, though to a small extent, in the case of the majority of such substances (acetic acid, butyric acid, propionic acid, &c.) when the degree of concentration is low. Laborde (VI.) reports that growths of *Allescheria* (*Eurotiopsis*) slowly decompose oxalic acid, malic acid (even when 2 per cent. is present), acetic acid (2 per cent.), propionic acid, butyric acid (0.8 per cent.), valeric acid (0.6 per cent.) and formic acid (to 1 per cent.), whereas inactive lactic acid was rapidly decomposed (even with 5 per cent.) without being split up into its active components (*see* vol. i. p. 232), and also methyl, propyl, butyl and amyl alcohol in small quantities. According to Duclaux (I.), growths of *Asp. niger* will decompose even 8–10 per cent. (?) of acetic acid, and also lactic acid and butyric acid (0.1–0.2 per cent., of which 0.5 per cent. is the smallest fatal dose. In presence of butyric acid or tartaric acid, the acetic acid was consumed more rapidly than either. Whether, as stated by Duclaux, *Asp. niger* in cultures free from bacterial infection is really capable of converting calcium butyrate into carbonate, and calcium lactate into carbonate and oxalate, is a point that needs closer examination, the mere statement being scarcely sufficient. Formic acid, in quantities up to 0.08–0.09 per cent, is decomposed by *Asp. niger* and *Penic. glaucum*, though—according to Duclaux—larger doses (0.12 per cent.) have an injurious effect, whilst, according to Wehmer, up to 10 per cent. of citric acid, tartaric acid and malic acid are decomposed by both fungi. In this

connection, reference may be made to Pfeffer's (II.) reports on the selective affinity for nutrient substances (*see* vol. i. p. 46).

With regard to oxalic acid—the decomposition of which is mentioned in certain older reports by Warburg (I.) in the case of "*Penic. glaucum*," by Duclaux (XXI.), Werner and others—more accurate researches by Wehmer (XXVI., XXVII. and V.) have shown that 1 per cent. solutions are not attacked by these two organisms, whereas 0.2–0.5 per cent. solutions are completely, though slowly, decomposed. Soluble oxalates are decomposed with greater difficulty—and in the case of *Aspergillus* only under certain conditions—though with even a small growth of *Penicillium*, 1.5 grms. of potassium oxalate have been completely eliminated in sixty days. In all experiments of this kind, however, the nature of the food-stuff and the temperature require to be taken into consideration. It may be remarked, in conclusion, that *Asp. oryzæ* is credited by Aso (I.) with secreting an oxydase, whilst Pozzi-Escot (I.) states that the same fungus produces a reducing enzyme, which he calls "Jacquemase." Extracts of the vegetative growths of all the species examined by Schäffer (IV.)—*see* p. 365, vol. ii.—failed to give with acidified guaiacol solution the orange precipitate stated by Bourquelot (IX.) to be characteristic for oxidising enzymes. Seven species, however, gave a positive reaction with guaiacol and hydrogen peroxide, among them being "*Penic. glaucum*," which, according to Grüss (I.), has no oxidising action. Saito (IV.) claims that *Asp. oryzæ* secretes catalase; and, according to Altenburg (I.), *Asp. niger* secretes an oxydase which liberates iodine from potassium iodide and was more closely examined by Raciborski (III.).

CHAPTER LVIII.

MYCOSPHÆRELLA TULASNEI AND SPHÆRULINA INTERMIXTA, OTHERWISE CLADOSPORIUM HERBARUM AND DEMATIUM PULLULANS.

By Prof. Dr. G. LINDAU,
Private Tutor at the Berlin University.

§ 296. Cladosporium Herbarum.

In the sub-order of the Sphæriaceæ (*see* p. 100, vol. ii.), species from each of two genera of the family of the *Mycosphærellaceæ* come under consideration here, whilst a third one will merely receive cursory mention. The last-named is the fungus—formerly known as *Læstadia Bidwellii*, and now as *Guignardia Bidwellii*—causing the black-rot disease in the vine, further particulars of which are to be found in Handbooks on Phytopathology, such as those of Viala (I.) and Sorauer (III.). The ascospores of this fungus are unicellular, but occasionally bicellular when ripe.

The very numerous species belonging to the genus *Mycosphærella* (formerly *Sphærella*), on the other hand, produce bicellular ascospores ; and those of the genus *Sphærulina* (*see* p. 379, vol. ii.), even tricellular and polycellular ascospores. Of the first of these two genera only one species is of interest to us here, namely, *Mycosphærella Tulasnei*—on account of its conidial fructification—which, until recently, was still described under the name *Cladosporium herbarum*, given to it by H. F. Link, until its connection with the *Mycosphærellaceæ* was established by E. Janczewski (I.) in 1893. This worker succeeded in tracing the development of this new *Ascomyces* from the ascospores up to the production of ripe perithecia (*see* Fig. 184), and in obtaining, as a secondary fructification, the conidia (Fig. 185) with which alone we shall deal in the present paragraph.

Considered from the systematic standpoint, *Cladosporium herbarum* is probably a collective species, and appears to differ slightly in form under different conditions of cultivation. This explains why G. Fresenius (II.) and P. A. Saccardo (I.) described what seems to be different species, as *Penicillium cladosporioides* and *Hormodendron cladosporioides* respectively, whose proper place as forms of *Mycosphærella Tulasnei* was afterwards allotted them

by Janczewski. This allocation has recently been questioned by W. Schostakowitsch (I.), who did not succeed in transforming the so-called *Hormodendron cladosporioides* into *Cladosporium herbarum*. The assumption by Tulasne (II.) that *Cladosporium herbarum* belongs to the cycle of development of *Pleospora herbarum* was shown to be erroneous by the researches of Gibelli and Griffini (I.), by H. Bauke (I. and II.), and by F. G. Kohl (I.). Hence it may be assumed, in the present state of our knowledge, that, of the forms of *Cladosporium herbarum* hitherto described, one group has been recognised by Janczewski as belonging to the cycle of development of *Mycosphærella Tulasnei*, whilst others that have not yet been proved to so belong must continue in the meantime to figure as independent species in the literature. This remark applies, for instance, to that conidial fructification which was described as *Cladosporium herbarum* by Lopriore (I.). This species produces sclerotia which find a habitat on the husks of germinated and ungerminated wheat grains in the soil.

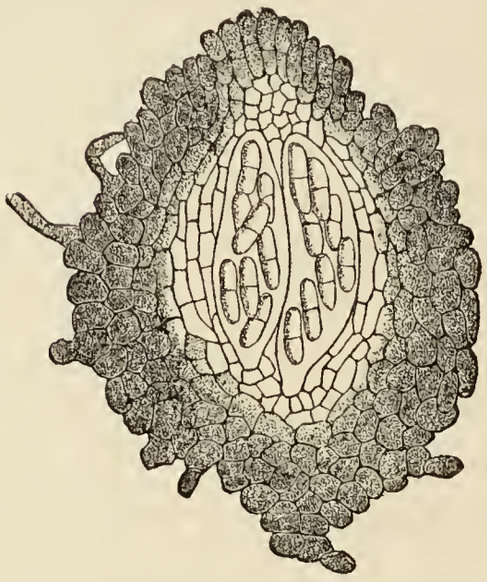


Fig. 184.—Mycosphærella Tulasnei (E. Jancz.).
Longitudinal section through a perithecium. Magn. 325.
(*After Janczewski.*)

The progress of development of the conidial fructification of *Mycosphærella Tulasnei* described as *Cladosporium herbarum*, and first examined under the microscope by E. Loew (II.), is the exact antithesis to that of *Penicillium glaucum*. In the latter organism the outermost member of a conidial chain (the one furthest from the centre of growth) is the oldest and largest, so that the constriction of the several members proceeds from the periphery to the central point (basis) of the fungoid herbage, and is therefore basipetal (*see* p. 20, vol. ii.). The separate conidia are produced in succession immediately below the preceding ones on the conidiophore, which then, in order to counteract the resulting loss of length and to prepare for further constrictions, increases in length correspondingly. With *Cladosporium herbarum*, on the contrary, the faculty of direct constriction on the part of the conidiophore ceases with the production of the first conidium, and all the succeeding ones are formed from this latter—or from the daughter cells produced in the meantime—by budding. In this case, therefore, the lowermost cell is the oldest, the top one being the youngest, so that the production of conidia proceeds from below (from the basis) upward (towards the apex), and is consequently basifugal, or, in other words, acropetalous. The

budding capacity of a conidium is not confined to the formation of a single daughter conidium, a second adjacent bud being oftentimes formed, which is capable of acting in the same way, and thus a richly branched formation ensues, as shown in the course of development in Fig. 186. The basipetal constriction of conidia by *Penicillium glaucum*, on the other hand, is naturally incapable of such a method of development, and is confined to the formation of a simple conidial chain.

According to the observations of Janczewski (II.) a considerable degree of variation prevails in respect of the dimensions of the mycelium and the conidia of *Cladosporium herbarum*, a circumstance explaining the practice of classifying these various forms as different species, before this diversity was recognised. Thus, the length of the ordinary ovoid conidia (Fig. 187) varies from 12 to 25 μ, and the breadth from 5 to 10 μ. The dimensions of the mycelial filaments vary accordingly, so that one worker may be confronted with a giant form, whilst another may have a dwarf specimen. The number of septa within the conidia also varies with the age; two being present in one case, whilst another cell contains only one, and a third exhibits none at all. The external surface of the brown or olive-green membrane of the conidia may be covered with fine needles (crystals ?), though oftentimes it is smooth. On the basis of this characteristic, the systematist has elaborated various species of *Cladosporium;* but in the present state of knowledge we cannot say whether these are specifically distinct forms or only caused by differences of environment.

Fig. 185.—Mycosphærella Tulasnei (E. Jancz.). Mycelial filament with conidia. (*After Janczewski.*) Magn. 250.

The above-described conidiophores are often found in black mildew on dead parts of plants, on damp cellar walls, casks and vats, the surfaces of these being covered with a herbage which is light olive-green when young, but gradually passes through olive-brown into dark brown. Simultaneously

the cells thicken, become filled with drops of fatty oil, and, according to E. Laurent (VI.), also store up glycogen.

In practical fermentation, *Cladosporium herbarum* not infrequently makes its appearance as a source of damage, especially on cereal grains and on malt that is stored in a damp place. The hop plant, too, is occasionally infested and damaged by *Cladosporium herbarum*, particularly in damp weather or when the plants have been rendered susceptible to attack in consequence of other influences. In this case the fungus appears as an olive-green to brown growth on the under side of the

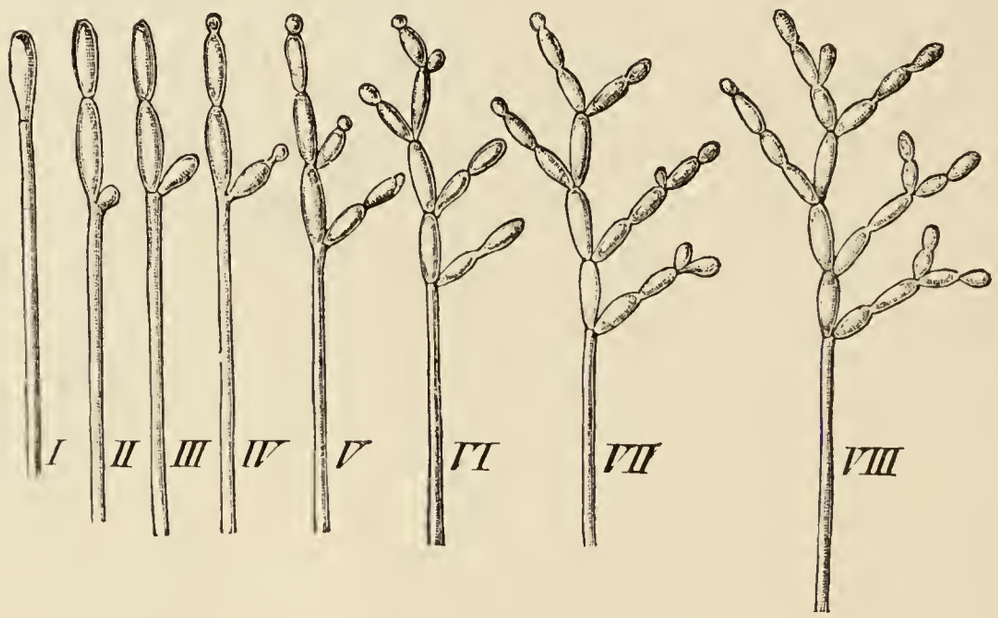


Fig. 186.—Cladosporium herbarum.

Conidiophores showing the successive formation of conidia during continuous observation on grape-juice ; *I*, commencement of constriction ; *II*, after 3 hours ; *III*, after a further $2\frac{1}{2}$ hours ; *IV*, after $10\frac{1}{4}$ hours longer ; *V*, after an additional 6 hours; *VI*, after a further $2\frac{1}{2}$ hours ; *VII*, $3\frac{1}{2}$ hours later ; *VIII*, still later. Magn. 300. (*After E. Loew.*)

leaves. The same fungus also seems to play some part in shed mouldiness in tobacco; and it is frequently noticeable in cellars. For this reason it is by no means surprising to find that the fungus penetrates the corks of wines that are stored in bottle, and contributes to the production of corked flavour (*see* p. 322, vol. ii.) in such wines, as a result of its musty metabolic products. Particulars of this have been collected by J. Wortmann (III.). The fungus is also a frequent cause of damage in cheese dairies, where it plays a part in the blackening of the cheese.

The putrefaction of eggs is not always due to bacteria (*see* p. 218, vol. i.), but frequently to *Eumycetes*, the most active of these being *Cladosporium herbarum*, or, what is practically the same thing, *Hormodendron cladosporioides*. As long ago as 1864 it was shown by Mosler (II.) that uninjured eggs may be infected from the outside by *Penicillium glaucum* and *Mucor mucedo*. Zopf (X.) stated that Montagne cultivated *Dactylium oogenum*

from a rotten egg; and the same is reported of *Malrosporium verruculosum* by O. E. R. Zimmermann. The latter worker also observed *Torula ovicola*, *Penicillium glaucum*, *Stysanus otemonitis*, and its parasites, *Echinobotryum atrum*, and species of *Sporotrichum*. Finally, *Hormodendron cladosporioides* was frequently observed by Zopf (X.) in such eggs. According to the infection experiments carried out by Drutzu, conidia of the said fungus that have been accidentally or designedly placed on the unbroken shell of the egg germinate, penetrate the shell and internal membrane, and develop between the latter and the yolk to an agglomerated, gelatinous, dark brown mycelium, which gradually consumes the albumen; so that in certain cases none of this latter is left, the yolk being enveloped in a thick coat of fungus. On air gaining admission, in consequence of the gradual contraction and drying up of the contents of the egg, conidia are formed. Further particulars of the decomposition of eggs by *Cladosporium herbarum* have been furnished by Cerlese (III.) and Guéguen (IV.). A reliable means of preventing this incursion of aerobic *Eumycetes* is afforded by varnishing or liming the eggs while fresh.

Fig. 187.—Mycosphærella Tulasnei (E. Jancz.).

Conidia from the mycelial filament shown in Fig. 185, two of them being aseptate, two with a single septum and one with two. Magn. 650. (*After Janczewski.*)

According to a report by F. Rathgen (I.), a fungus was discovered in patina (*see* p. 372, vol. ii.) by L. Mond and G. Guboni, and was named by them *Cladosporium æris*. From comparative experiments they were obliged to conclude that this fungus contributes to the destruction of bronze.

§ 297. Dematium Pullulans.

On p. 375, vol. ii., it was stated that the genus *Sphærulina*, belonging to the family *Mycosphærellaceæ* (a sub-order of the *Sphæriaceæ*), is distinguishable from the allied genus *Mycosphærella* by its multicellular ascospores. Of that genus only a single species, *Sphærulina intermixta*, is of interest to the fermentation physiologist. The small perithecia of this fungus are found on withered rose-twigs; and one of the asci from the ascospores of this is shown, with its eight multicellular spores, in Fig. 188. When ripe, the asci are forced out of the perithecium, the ascospores being then liberated in consequence of the swelling up of the wall of the ascus. On finding themselves on a suitable substratum, they swell up immediately, develop longitudinal and transverse septa in their several cells, and also produce daughter cells by

budding, so that a multicellular colony is soon formed. The daughter cells also detach themselves from the mass, and develop in the same way to a daughter colony. Still more commonly the cell-reproduction proceeds chiefly in a single direction, and then furnishes filamentous chains of cells, as shown at 5 in Fig. 188.

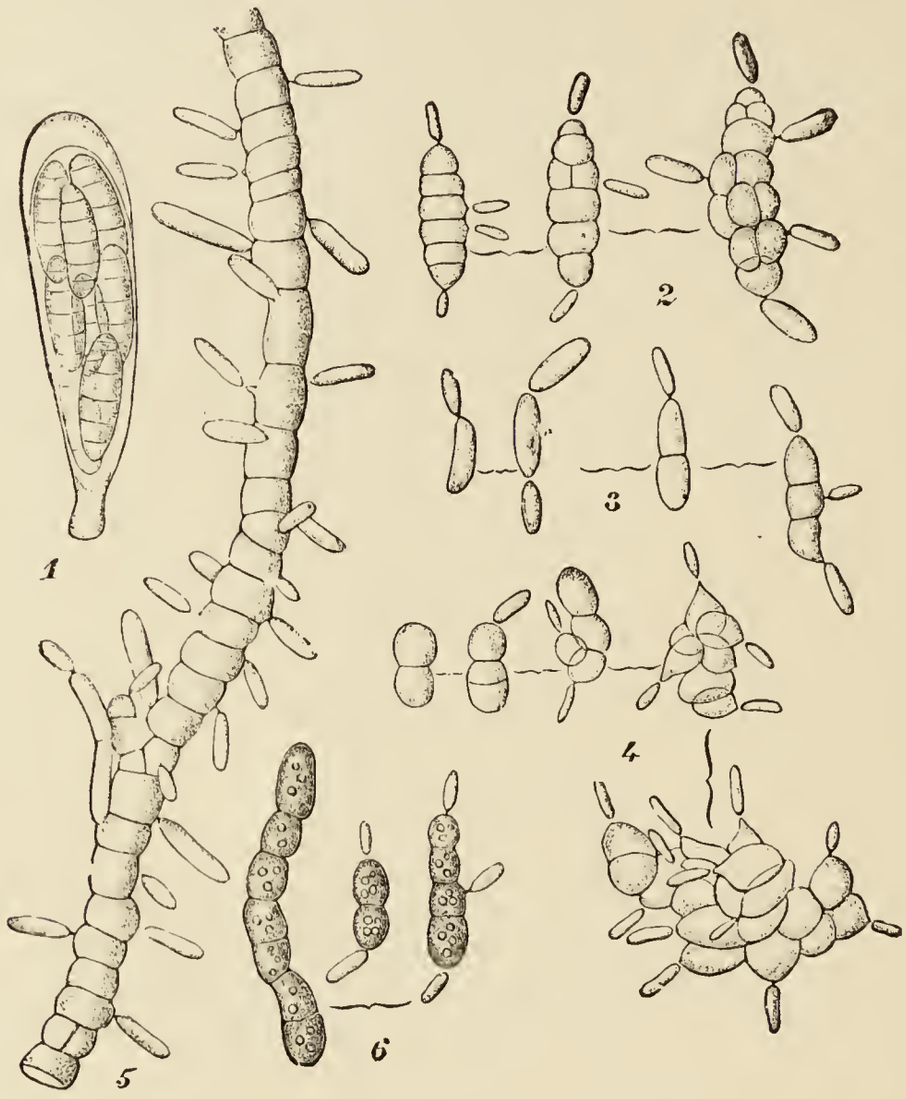


Fig. 188.—Sphærulina intermixta, Brefeld.

1. An ascus with its eight ripe spores.
2. Three ascospores swelling up and germinating.
3. Budding cells separated from the above, in course of reproduction.
4. Colonies from same.
5. Filamentous chain of cells, Dematium form.
6. Gemmæ.

Magn. 350. (*After Brefeld.*)

These chains bear a close resemblance to the *Hyphomyces* described, under the name *Dematium pullulans*, by A. de Bary (III.), and occurring in nature in many kinds of black mildew, on sweet fruits, and on moribund parts of plants. Brefeld (X.) then recognised this *Hyphomyces* as identical with the above conidial fructification, and consequently allocated *Dematium pullulans* to the morphological cycle of *Sphærulina intermixta*. Nevertheless, according to Alb. Klöcker and H. Schiönning (VII.), an unmistakable difference exists between them. The only way in which

this question could be finally settled would be by inducing the *Hyphomyces* to develop perithecia, which no one has yet succeeded in doing. However, for the present, *Dematium pullulans* may be classed as a (still undetermined) species allied to *Sphærulina intermixta*, even if it cannot be regarded as forming part of the morphological cycle of the latter. The identification of species in this group is still in a very defective condition. The morphological similarity is very great, not only between *Dematium pullulans* and the conidial fructification of *Sphærulina intermixta*, but also between both and the conidial fructifications of the *Dothidea ribesia* and *D. puccinioides* described by Brefeld, the *Fumago salicina* examined by Zopf (XI.)—forming the chief constituent of true smut—*Cladosporium herbarum*, and others. Undoubtedly, however, *Dematium pullulans* stands nearest to *Sphærulina intermixta*, and its consideration in this place is therefore justified. On its account alone has mention been made of the said *Ascomycetes*, the ascospores of which, on the other hand, are developed only outside liquids, and possess little interest to the fermentation physiologist beyond the developmental history sketched above. The views of earlier workers, who sought to establish a connection between *Dematium* and the true yeasts (*see* pp. 107, 108, vol. ii.) are entirely erroneous.

Consequently, the form known as *Dematium pullulans* alone constitutes the subject of the following lines. For exhaustive investigations into its structure we are more particularly indebted to E. Loew (V.).

If one of the yeast-like buds (conidia) of *Dematium pullulans* be placed in a suitable environment, it grows to an extensive mycelium, the several members of which throw up numerous ellipsoidal conidia in turn. According to the researches of W. Schostakowitsch (I.) on the influence of external conditions on the formation of the budding cells (*see* pp. 21, 22, vol. ii.), however, these conidia are not produced when the mycelium is made to grow in a strong solution of grape sugar or saccharose. The limit in this respect was determined by O. von Skerst (I.) as about 50 per cent. A temperature of 30°–31° C. has a similar restrictive influence; though by gradual habituation to progressively increasing temperatures it is possible to obtain at last a culture the mycelium of which will produce conidia at 30° C. At 50°–55° C., however, the mycelium is killed, after an exposure varying with its age.

On air being freely admitted to the nutrient solution, the hitherto slender, colourless cells of the mycelium are transformed into short, protuberant forms (gemmæ, *see* p. 24, vol. ii.), the membrane of which thickens and acquires an olive-green to brown tone. The depth of this colour depends, according to O. von Skerst (I.), on the richness of the nutrient solution, and increases therewith. At the same time an abundance of fat collects in the cells in the form of drops, which increase in size and are rendered

noticeable by their power of refracting light and consequent lustre. In fact, this peculiarity renders the drops of oil liable to be confounded with endogenous spores—a point on which warnings have been issued by E. Laurent (VII.) and afterwards by O. Seiter (I.). This was also probably the cause of the mistaken opinion formed by Johan-Olsen (I.), whose error was corrected by Alb. Klöcker and H. Schiönning (IV.). In some cases the gemmæ subsequently develop a longitudinal septum, due to the transverse division of the mycelial cells. At a still later period the external layers of the thick cell wall sometimes become mucinous to such an extent as to render the nutrient liquid viscous (*see* vol. i. p. 285). In this manner a culture of *Dematium pullulans* (grown, for instance, in beer wort) will develop into a greenish brown to dark green, thin, but viscous, film, resembling paper, on the surface of the liquid, whilst a deposit of yeast-like conidia and conidial cultures collects at the bottom.

According as the gemmæ are well nourished or the reverse, they either develop into a mycelium from which lateral buds are separated by constriction, or they produce these buds direct.

Dematium pullulans also affords an example of the cell fusion already referred to on p. 6, vol. ii., by the coalescence of two adjoining cells of the same mycelium, one of them penetrating the other. In *Dematium pullulans* more particularly—which, as already stated, exhibits a marked tendency to the formation of budding cells by constriction—there gradually develops, within the invaded cell, or host, a varying number of approximately ellipsoidal cells, which are in turn capable of reproduction by budding. In this way, under favourable conditions, a filament may become filled with cells which an observer unfamiliar with its method of origin may readily mistake for an ascospore (*see* Fig. 189). Such an erroneous impression has already been produced in the minds of several workers: for instance, O. Johan-Olsen (I.) in the case of a fungus he named *Dematium casei*, and also Alfr. Jörgensen and Fr. Weleminsky (I.) with *Dematium pullulans* itself. We are indebted to A. Klöcker and H. Schiönning (IV. and VII.) for the correct interpretation of the phenomenon. This correction destroys the corresponding erroneous classification of *Dematium pullulans* with the group of the *Exoasceæ* or similar low *Ascomycetes*.

As a result of its frequent occurrence on straw, and therefore in the atmospheric dust in cowsheds, *Dematium pullulans* is likely to be often found in milk; and it has actually been detected in that liquid on many occasions by Adametz (III. and IV.). When the milk is curdled, a larger or smaller proportion of the fungus content passes into the curd. Its mode of action in this case requires elucidation, and an attempt to explain it was made by O. Johan-Olsen (I.). This worker discovered in Norwegian "gammelost" (*see* p. 85, vol. ii.), a hyphomyces which he termed

Dematium casei, regarding it as allied to *D. pullulans;* and, in a second communication (II.), he stated that the fungus caused the

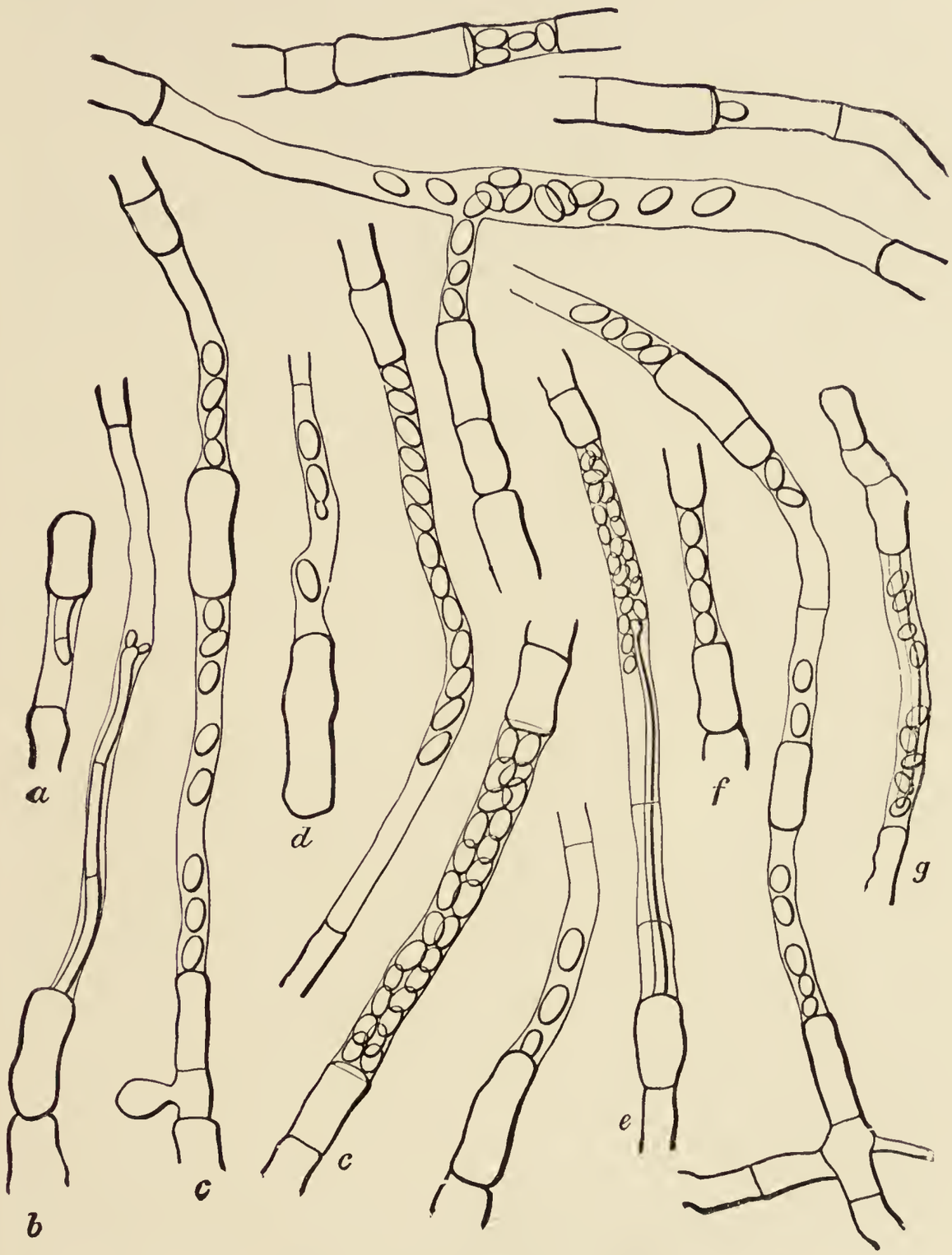


FIG. 189.—Dematium pullulans.

Filaments *a*, *b* and *c* contain fused adjacent cells, some of which are constricting conidia. In *c* the septa at both ends of the cell have developed conidia; *f* might be mistaken for a quadricellular sporangium. In *d* a conidium is budding like yeast. Magn. 500. (*After Klöcker and Schiönning.*)

very bitter flavour of the cheese in question. A repetition of his examination, however, by Klöcker and Schiönning, led to the discovery that the fungus does not belong to the genus *Dematium*, but is more closely allied to *Monilia* or *Oidium*.

SECTION XVI.

GENERAL MORPHOLOGY, PHYSIOLOGY AND CLASSIFICATION OF TECHNICALLY IMPORTANT BUDDING FUNGI OF THE GROUP "FUNGI IMPERFECTI."

CHAPTER LIX.

TORULACEÆ, PINK YEASTS AND BLACK YEASTS.

By Prof. Dr. H. WILL.

Head of the Physiological Department of the Munich Scientific Station of Brewing.

§ 298. Historical, Delimitation, Derivation.

The name *Torula* was applied by Pasteur (XXVII.) in 1862 to a group of fungi, which reproduce by budding, like yeast, and are devoid of a typical mycelium. Plate III. of that authority's *Etudes sur la Bière* gives a very good and characteristic illustration of two species of *Torula* with globular cells, so that no doubt can exist in the case of at least one of the groups of budding fungi that were included by Pasteur under the generic name of *Torula*.

Pasteur also gave six pictures of other forms of *Torula*, of which those he showed in Figs. 1 and 2 of the above work also exhibited globular cells, whereas, as was pointed out by Hansen (LIII.), the one shown in Fig. 6 resembles the budding cells of *Dematium*. It is therefore doubtful whether this form should be allocated to the *Torulaceæ* at all or forms a separate group, though similar, pointed cells occur in many *Torula* forms with mixed cells. Figs. 3 and 6 of the said Plate, on the other hand, depict species in which thin, elongated budding cells appear in association with those of more contracted form (down to globular cells), and these exhibit certain analogies with the budding fungi now allocated to the genus *Mycoderma*. Pasteur also logically classed *Mycoderma vini* with the *Torulaceæ*, the difference between this fungus and the other *Torula* forms consisting, in his opinion, merely in the special structure of the cells and in a

greasiness which causes the cells tc adhere superficially. This feature, however, is not a strong one.

The species shown in Fig. 5 of the Plate in question is morphologically similar to an ordinary yeast (*Saccharomyces*), but probably also belongs to the *Torulaceæ*.

Pasteur himself expressed doubt as to whether all the forms depicted represented an equal number of species, and showed (as illustrated in his Figs. 1 and 2) that the elongated cells could also produce those of the small, globular type. He held the opinion that different varieties could be obtained from a *Torula* species with mixed cells, by sowing the different cell forms. Although he adduced no proofs in support of this, it is nevertheless correct, the more so because—as we now know—there are *Torula* forms which, under the influence of a certain environment, reproduce almost exclusively in the form of small, more or less globular, cells, the elongated cells being comparatively small in number, whilst under other conditions these latter calls becomemore prominent.

Pasteur mentions, as a chief characteristic of the *Torula* forms depicted in his Figs. 1–6, that, like *Mycoderma*, they are unable to set up alcoholic fermentation.

Although Pasteur was able to characterise a series of the chief representatives of the *Torulaceæ* with a fair amount of certainty, notwithstanding that he was not in possession of absolutely pure cultures, the line of demarcation between them and the *Saccharomycetes* is uncertain and imperfect; and among them may be found *Saccharomycetes* with weak powers of fermentation.

It was not until the researches of Hansen (XXIV.) that the two could be more effectually separated. This worker applied the name *Torula* to budding fungi which produce neither endospores nor typical mould vegetations, thus differentiating them from the *Saccharomycetes* on the one hand and from *Monilia*, *Dematium* and other budding *Hyphomycetes* on the other.

By this definition Hansen greatly restricted the morphological circle of the *Torulaceæ*, allocating to the latter only such species as produce cells of a more or less globular shape, although some of his species form elongated, sausage-shaped cells in film vegetations. In any event, this limitation excludes from the *Torulaceæ* all the asporogenic budding fungi resembling *Mycoderma*, as also *Mycoderma* itself. Moreover, whilst Pasteur's *Torulaceæ* set up merely a very weak fermentation, or none at all, the species classified by Hansen exhibit all gradations in this respect, a few of them setting up a fairly vigorous fermentation. Consequently, Hansen's *Torula* species (*see* pp. 9, 10, vol. ii.) cannot be unconditionally united in one group with those of Pasteur.

The faculty of sporulation excludes from the *Torulaceæ* all such of the *Saccharomycetes* as—like the species *Torulaspora Delbrückii* (*see* p. 284, vol. ii.) of P. Linder (XXXVI.), and another

species discovered by him in the mucinous exudation from oak-trees—occasionally form strictly globular cells, each containing a large drop of oil, like those of typical *Torulaceæ*.

Whilst it is true that the diagnostic characteristics set up by Hansen define the *Torulaceæ* more sharply than was done by Pasteur, recent investigations have brought to light difficulties in the way of this delimitation. The *Torulaceæ* are separated from the *Saccharomycètes* by a merely negative characteristic, namely, the absence of sporulation, whereas in other respects they have many points in common, both morphologically and physiologically, with the latter, as also with other asporogenic groups, such as *Mycoderma*. Hansen himself, however, showed that some of the *Saccharomycetes* lose their power of sporulation (*see* p. 260 *et seq.* vol. ii.) under certain conditions of treatment; and though the occurrence of such variations in the natural state has not yet been demonstrated, it is not impossible. In the absence of any information as to their origin, such forms would have to be grouped with the *Torulaceæ*. In the case of many *Saccharomycetes*, sporulation is also known to be of very difficult and rare occurrence, and one that evidently depends on certain well-defined but as yet unrecognised conditions.

The present writer's own researches have shown the existence of typical *Torulaceæ* incapable of setting up fermentation with the ordinary kinds of sugar, and therefore coinciding in this respect with those of Pasteur. He has also become acquainted with forms which, as mentioned above, produce almost exclusively small, more or less globular cells under certain conditions of cultivation, these cells exhibiting all the characteristics of the *Torulaceæ*, and being rarely accompanied by elongated forms. Under different conditions, however, the latter forms become more frequent, and thus constitute an intermediate link with the species forming *pastorianus* cells (*see* p. 116, vol. ii.) in addition to those of oval and globular shape and mycelial agglomerations of same. They also possess fermentative properties, thus differing from the *Mycoderma* species.

On the basis of his own investigations, the writer, in contrast with Hansen, enlarges the morphological circle of the *Torulaceæ* so as to include species like those characterised by Pasteur.

Consequently, the following lines will treat not merely of such budding fungi as have not yet been observed to sporulate—and comprising those forming exclusively more or less rounded or oval cells, with or without the power of exciting fermentation (first sub-group)—but also of those producing mixed cells, developing interchangeably, but distinguished from the *Mycoderma* species by their fermentative power (second sub-group). The *Monilia* are excluded by the possession of a septate typical mycelium (see chapter lxii.). No fundamental reason exists for excluding the so-called pink yeast (or some of them at any rate)

from the *Torulaceæ*, many of them, so far as is known, having many points of resemblance, morphologically, with the *Torulaceæ*, although they have not been studied with any thoroughness. Pigmentation cannot, at least, be regarded as a sufficient reason for their separation, since the researches of Kossowicz (I.), confirmed by R. Schander (I.), show that several of the *Saccharomycetes* which are colourless under ordinary conditions develop a red colouring-matter in certain circumstances, notably in presence of salts of magnesium. On the other hand, some of the typical forms of *Torula* occasionally assume a pink coloration only under certain conditions of growth, such as in films on nutrient liquids and in slant colonies. Pigmentation is by no means a constant feature. Nevertheless, the so-called pink yeasts and other coloured budding fungi will, for practical reasons, be dealt with separately in the present chapter under § 301.

The opinion that the *Torulaceæ* are only stages in the development of other fungi has already been expressed by Hansen (LIII.). It is known that the conidia of certain *Ustilagineæ* (*see* p. 109, vol. ii.) are able to maintain an independent existence, by budding, in suitable nutrient solutions. Budding cells are also met with in various groups of fungi; and possibly similar biological conditions may give rise to the same or similar external phenomena. The *Torula* group is not a natural one, and is merely of a temporary character.

E. Klein and M. Gordon (I.) claimed to have traced the origin of a pathogenic pink yeast to *Puccinia suaveolens*. On the other hand, R. Meissner (II.), in comparing his six species of mucinous yeasts (*see* p. 177, vol. ii.) with the budding cells of *Exoascus* spores, with which they seemed to have some connection, established an important point of difference between them and the budding cells of *Exoascus deformans*. It may also be remarked that Laurent (VIII.) has stated that the budding forms of *Cladosporium herbarum* (*see* p. 378, vol. ii.) are transformed into a pink yeast by insolation. In addition, Winkler (II.) claimed that *Mucor* spores, under certain culture methods, furnished "yeast cells" that were asporogenic, for which reason he proposed to group them, *pro tem.*, with the *Torulaceæ*. Agreeable though the idea may be that these organisms are merely budding forms of the conidia or spores of higher fungi, reports in this connection require to be very critically examined.

In treating of the asporogenic budding fungi mentioned in the literature, under the generic name of *Torula*, or more generally referred to as yeast, white yeast, &c., it is often difficult, nay impossible, to decide if they belong to the group under consideration. On the one hand it must be remembered that the name *Torula* has been, and is still, applied at different times to very different organisms. Originally implying *Hyphomycetes* with conidia arranged in wreaths, with simple or branched chains,

it was used by Turpin in 1838 to denote beer yeast (*Saccharomyces cerevisiæ*), which he named *Torula cerevisiæ*. Cohn even applied the name *Torula* to the wreathed chains formed by the *Micrococcus* bacteria. At present the *Toruleæ* form a sub-group of the *Dematiaceæ*: compare A. Engler and K. Prantl (I.). The species belonging to this sub-group, as, for example, *Torula monilioides*, Corda, found by F. Ludwig (III.) in the mucinous exudations of trees (*see* p. 138, vol. ii.), are outside the morphological circle.

Saccharomycetes greatly resembling certain forms of *Torula* are also known, as stated above. The statement "sporulation could not be detected" affords no guarantee that sporulation would not occur under certain conditions. In the opinion of many observers, all monocellular fungi that reproduce by budding, especially when they also set up fermentation, are yeasts, that is to say, *Saccharomycetes;* and they apply the name *Saccharomyces* to all such species described by them. The descriptions are mostly very imperfect, and it is difficult to decide with any degree of certainty, from the isolated characteristic peculiarities, whether the budding fungi under consideration belong to the *Torula* group or not. Matters are little better in the delimitation of the species that have been more closely examined; and any attempt to arrange them into a system has small prospect of success at present.

For the same reason it is difficult to decide whether the comparatively small group of (presumably asporogenic) budding fungi exhibiting the characteristic property of being able to ferment with sugars (*see* p. 397, vol. ii.), belongs to the *Torulaceæ*. Of this group the following members have been described: the so-called *Sacch. galactocola*, by Pirotta and Riboni (II.); one species by Duclaux (XXIII.); the so-called *Sacch. lactis*, by Adametz (XI.); the so-called *Sacch. Kefyr* and *Sacch. tyrocola*, by Beijerinck (XX. and XXV.); several species by Weigmann (XI.) and Grotenfelt (II. and III.); *Torula Duclauxi*, by E. Kayser (IV.) as well as the so-called *Sacch. lactis*, Adametz, and a species isolated from milk; one species by L. C. Mix (I.); *Lactomyces inflans caseigrana* by Adametz (XII.), in collaboration with W. Winkler, and also by Nicola Bochiccihio (I.); the so-called *Sacch. Kefyr*, by Freudenreich (XI.); *Torula amara* by A. Kalanthar (I.); by Harrison (II.); and by O. Jensen (III.) and P. Mazé (I.), who also included in his examination the *Torula Duclauxi*, the so-called, *Sacch. lactis*, Adametz, and the species described by Kayser. The so-called *Sacch. lactis*, Adametz and *Sacch. tyrocola*, Beijerinck, were subjected to a careful examination by Heinze and Cohn (I.). These two last-named species undoubtedly belong to the *Torulaceæ*, *Sacch. lactis* to the second sub-group and *Sacch. tyrocola* to the first sub-group, so that they ought, correctly, to be named *Torula lactis* and *Torula tyrocola* respectively. On the other hand, it is questionable

whether the kephir yeasts belong to this category, though in other respects they should be classed with the *Torulaceæ;* since, according to the concordant statements of Adametz, Freudenreich and Heinze (III.), they are, of themselves, incapable of fermenting milk sugar. It is highly probable that the kephir granules contain a variety of budding fungi, and the so-called *Sacch. Kefyr*, Beijerinck, is apparently not always present. On the other hand, it is very likely that Boersch (I.), who attributes sporulation to the so-called *Sacch. Kefyr*, was examining a species that is not always contained in kephir granules; for, as far as the writer is aware, the bulk of the budding fungi in these granules do not sporulate. It is doubtful whether all the various forms described represent so many distinct species. Comparative researches by E. Kayser have shown that *Torula Duclauxi*, the so-called *Sacch. lactis*, Adametz, and the species isolated from milk by himself, possess very divergent and constant chemico-physiological properties. *Sacch. Kefyr*, Beijerinck, closely resembles *Sacch. lactis*, Adametz, whilst *Sacch. tyrocola* is probably identical with *Torula Duclauxi*. According to the researches of Heinze and Cohn, little doubt can exist as to the separate identity of *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck.

§ 299. Occurrence, Dissemination and Morphology of the Torulaceæ.

The *Torulaceæ* are very widely disseminated. The frequency of their occurrence in the air depends, however, on certain conditions, chiefly on the way in which the ground is planted, the fruits of vineyards and orchards in particular affording the most favourable environment. Hansen (II.) found them in the air of the open country under fruit trees between July and November, most abundantly in September, whereas they were absent in May, June and December; and this report is confirmed by the investigations of the writer. According to Hansen (II.), their normal winter habitat, like that of the *Saccharomycetes*, is the soil.

The *Torulaceæ* also find a home on field and garden fruits, and indeed on plants of all kinds; and they seem to find a suitable environment both during the decay of these fruits and during the technical processes for preserving same, such as the pickling of gherkins and beans, and the fermentation of sauerkraut.

Possibly the yeasts found in the fermentation of tobacco and tea also belong to the *Torulaceæ*.

They accompany the food into the stomach, and are found there in the human subject during complaints of that organ (fermentation and distension).

During the plague of *Lipara monacha* caterpillars in Bavaria, the intestinal tract of these insects was discovered to be occasionally packed with the cells of various *Torulaceæ*.

These organisms penetrate all kinds of organic substances and develop therein, frequently to a surprising extent. For instance, the thick white to whitish yellow coatings found on stored sausages consist sometimes of these fungi exclusively. Milk, butter and cheese also afford a favourable environment, and they occasionally develop abundantly in bread.

The numerous biological investigations of water, for brewing purposes in particular, have shown that river water is often very rich in budding fungi belonging to the morphological circle of the *Torulaceæ*. Budding fungi are also found in sea water, especially in northern regions; and some of these organisms are evidently *Torulaceæ*.

Establishments where dairying and fermentation industries of all kinds are carried on form a chief habitat of these budding fungi; and they develop enormously not merely in the raw materials and products, but also in the air of all the rooms, in the walls of the fermentation- and store-rooms, and on the utensils employed.

The form and dimensions of the cells vary considerably, more particularly in members of the second sub-group. Cells of one and the same species, grown in a given nutrient medium, often vary but slightly, though sometimes to a not inconsiderable extent, so that purely globular forms are accompanied by oval and more or less elongated types of *pastorianus* cells, especially in very old cultures. In species producing globular cells on the average, a few sausage-shaped or irregularly formed daughter cells are developed; and even those resembling *Sacch. apiculatus* are regularly observed in certain species. The shape of the cells is influenced by the reaction and composition of the nutrient medium; and above all by the presence of certain sugars.

The cell dimensions of members of the first group vary within wide limits; and the cells of certain species may almost be mistaken for those of globular bacteria.

A very remarkable form that appears regularly, not only in the *Torulaceæ*, but also (though to a smaller extent) in the *Saccharomycetes*, is that of giant cells (*see* p. 118, vol. ii.), the dimensions of which greatly exceed the average size. These giant cells, occurring regularly (so far as observation has extended) in the second sub-group of the *Torulaceæ*, are often found in a decrepit condition. Whether they are abnormal, or cells endowed with certain physiological functions, cannot be decided at present.

Still greater variety exists in the form and size of the cells of the second sub-group with mixed cells. Cells agreeing in form and size with those of the first sub-group are accompanied by club-shaped, sausage-shaped and filamentous cells of all grades. Other species develop very thin and graceful cells; and the spindle-shaped cell, tapering off at both ends, is a not infrequent

form. Filamentous cells, measuring up to 40 μ in length and 2 μ in diameter, have been observed. Chains of elongated cells frequently develop numerous rounded and oval cells on the ends of the individual members (in giant colonies).

As already mentioned, the appearance of this elongated cell form is connected in part with certain conditions of environment, so that, for example, the organism inside the nutrient liquid produces mainly or entirely the more compact cell forms, whilst the elongated forms are produced on the surface, as in the film vegetations of *Saccharomycetes*.

In giant colonies of many *Torulaceæ*, on the other hand, the superficial cells are chiefly globular or oval, whilst the under side exhibits numerous elongated, sausage-shaped cells, penetrating for long distances into the gelatin. Other forms, again, develop both within and upon the nutrient liquid, simultaneously with mixed cells.

The cell integument of *Torulaceæ* is of an even more highly diversified character than in the case of the *Saccharomycetes*. It is mostly strong, and in some cases of typical species attains a considerable thickness that is apparently associated with stratification. Still more frequent than in the *Saccharomycetes* (*see* p. 145, vol. ii.) is the phenomenon of sloughing the outer layer of skin. These extremely thick-skinned cells, which are found in nearly all cultures, are possibly resting forms (Chlamydospores). Conversely, the cell integument of many other *Torula* species is very delicate. Sometimes, as in the species depicted by P. Lindner (XXXVII.), and the mucinous yeasts of Meissner (II.), the cell wall develops an almost imperceptible mucous layer, whilst in other species a gelatinous network is clearly visible (*see* p. 178, vol ii.) in the film vegetations on nutrient liquids, and occasionally the cultures transform the entire nutrient liquid into a tough gelatinous mass.

The cartilaginous film vegetations of certain close-growing species on nutrient liquids may be attributed to a peculiar condition of the cell integument. Species that, like *Mycoderma* and *Willia*, produce superficial films very rapidly, imprison air between the cells, a peculiarity favouring the assumption that the cell integument is of a greasy nature, like that of *Mycoderma*. No reports have been published dealing specially with the chemical composition of the cell integument of *Torulaceæ*.

Apart from isolated inclusions, the cell contents, as a rule, have only slight refractive power and remain pale, agreeing in this respect with *Mycoderma* and contrasting with the *Saccharomycetes*. While the cells are young, the contents are homogeneous, but afterwards turn cloudy and frothy; numerous small vacuoles appear, to give place subsequently to a single one (in globular or oval cells) or several (in elongated cells). The contents of older cells are occasionally crumbly and finely granulated, or a number

of highly refractive granules appear. The number of these, however, is usually limited, the highly refractive inclusions forming a very characteristic element of the cell contents. This applies particularly to the typical *Torulaceæ*, the species of the first sub-group.

As a rule, the globular cells contain an oily particle which seems to have been regarded by some authors as a nucleus. It is barely visible in submerged growing cells with homogeneous contents, and comes into prominence only on the appearance and growth of vacuoles, especially when the cells in film vegetations come into contact with the air. Even when the vacuoles have attained considerable dimensions, and the plasma has diminished to a mere stratum lining the cell wall, the oil particle remains coated with a layer of plasma. Usually globular, it is not infrequently flattened in appearance. The presence of an oil particle in the lactose yeasts characterises them as belonging to the *Torula* group. Raum (III.) differentiated one of these in kephir yeast by staining and assumed it to be a *Torula* form in the sense defined by Hansen. The size of the particle increases with the age of the cell and by contact of the latter with air, although, so far as observation goes, it remains small in some species, so that the size of the oil particle may serve as a means for the characterisation of species. The globular and oval cells of some species contain two or more oil particles; and these have probably been confounded with spores in many instances. The elongated cells of the second sub-group of *Torulaceæ* also contain oil particles, distributed in the same manner as those in the cells of *Mycoderma*. Nevertheless they may also be lacking in species with mixed cells, in the rounded and oval cells of which they occur regularly.

Crystalline bodies in the vacuoles (*see* p. 153, vol. ii.) form a highly characteristic inclusion in *Torula* cells. Of regular occurrence in a few species, they appear to be lacking in others with morphologically similar cells. Accordingly, they might serve as a diagnostic feature, in the same way as the varying number of oil particles in the globular cells.

Very old cells of typical *Torulaceæ* frequently contain, like those of the *Saccharomycetes*, a single large globule that is partly of a fatty nature. According to Lindner (XXXVI. *b*) small fatty drops are of frequent occurrence in the small cell species of *Torula*, even under normal conditions, the cells being mostly highly refractive, with a greenish tinge. *Torula pulcherrima* develops large, highly refractive globules.

Glycogen was absent from very few of the species examined by the writer, when grown in beer wort or in neutral yeast-water with 6 per cent. of saccharose. The production of glycogen was also observed by Meissner in certain of his mucinous yeasts. The intensity of the reaction varies considerably, but is generally

faint, the most decided result being obtained with a species of high fermenting power. According to Heinze and Cohn, glycogen is formed by *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, to the same extent as by *Saccharomycetes*, and especially so in young cultures on acid wort gelatin. The red-brown coloration with iodine is given by the *Torulaceæ*, either in the plasma or the vacuoles ; in the latter case it may extend over the whole contents of the vacuole, or be restricted to globular inclusions of varying dimensions.

With regard to the nucleus, the only reliable communication available is that of Guilliermond (V.), Beijerinck's report on *Sacch. Kefyr* leaving it doubtful whether he has not mistaken oil particles for nuclei. Other authors, who may be credited with a full acquaintance with morphological conditions, have asserted that a nucleus can be clearly discerned in cases where the oil particle is apparently alone in question.

The budding of the globular cells may take place at any point on the parent cell, sometimes occurring simultaneously in several places (coronation). The young generations form single, unbranched chaplets; and the order of budding is similar to that of the *Saccharomycetes* producing branched bud chains. Like the *Saccharomycetes*, too, the members of these chains either adhere firmly together, so as to form chains of considerable length, or they separate readily into short lengths of 3–4 cells each. Acid nutrient media stimulate certain lactose yeasts to form extended chains, especially when the degree of acidity is high. A few species also branch extensively, even in distilled water. Not infrequently one is able to observe phenomena that approximate more closely to germination than budding, the cell bulging out in one place with a very broad basis and bursting at the same time, whereupon the growing daughter cell becomes separated from the parent by a broad septum. The production of abnormal cells is frequently observed in budding.

The elongated cells of the second sub-group form either extensive chains of buds, or long mycelial rows with insignificant lateral branchings through shorter or longer cells ; or again, they may produce a large number of globular *Torula* cells.

The giant colonies of the second sub-group with elongated cells are in some cases of very handsome appearance. The surface exhibits mesenteric folds of varying dimensions, though these folds are not always formed in presence of the elongated cells. In this respect, therefore, the giant colonies differ from those of most *Saccharomycetes*, but resemble those of *Willia* and *Mycoderma*, though differing from the *Monilia* species.

The giant colonies of the first sub-group, on the other hand, are usually more or less flat, with slightly dished edges, and exhibiting, at most, faint radial stripings, with numerous smooth or warty excrescences on the surface. These excrescences form a

general characteristic of the giant colonies of this first sub-group, and are not confined to the *Torula* form isolated by M. Hartmann (I.) from a dried yeast purchased in Java. Consequently, the specific name, *colliculosa*, applied to this *Torula* does not by any means characterise this species. Moreover the fact stated by Hartmann, that these excrescences are composed of large cells, is not confined merely to this species.

In many forms the surface of the giant colonies bristles with numerous tufts. The nature of the nutrient medium has no great influence on the form of the giant colonies, the character and colour of which, moreover, are highly diversified. In many cases the colour is characteristic of the species: pale pink, yellow or yellow-brown, both in the film vegetations and in the giant colonies; though sometimes it is confined to, or attains its greatest intensity in, the latter, which thereby acquire increased diagnostic importance. Mostly, the colonies are colourless. They may be mucinous, gelatinous, or more or less dry, dull, semi-matt, or shining like cut glass or mother-of-pearl. Some species produce giant colonies of a waxy character or resembling enamel.

§ 300. Physiology and Chemistry of the Torulaceæ.

Reproduction in liquid nutrient media, like that on solid substrata, depends on the composition, reaction and concentration, as also on temperature and other external conditions, but primarily on the species itself. In a large number of species compared by the writer, the most favourable development took place in neutral yeast water containing 6 per cent. of saccharose, next in order coming the cultures in hopped and unhopped beer wort, and those in saccharine yeast water with an addition of 0.5 per cent. of peptone. Even a small quantity of peptone has great influence on the development, though asparagin also forms a good source of nitrogen. According to Beijerinck (XIX. and XXV.) and J. Schuurmans-Stekhoven (I.), *Sacch. Kefyr* also assimilates succinic acid; and growth is likewise stimulated by ethyl alcohol. Hayduck's nutrient solution is the least suitable food-stuff for the *Torulaceæ*. The *Torula* species examined by the writer also grew well in beer, provided the liquid was not too deep. Meissner's mucinous yeasts throve in Raulin's solution, but less favourable results were obtained with E. Laurent's nutrient solution. All the writer's *Torula* species developed in milk, and in some cases produced a cheesy smell, whilst in isolated instances the milk was coagulated. Among the lactose yeasts which also thrive in this medium, *Lactomyces inflans caseigrana* alone produces coagulation without any important formation of acid. The coagulum is partly reliquefied. Certain of the mucinous species develop very slowly indeed in all the nutrient media examined, growth proceeding exclusively at the bottom of the

vessel at first, whereas other species of the same sub-group reproduce at once and very rapidly, as films on the surface.

The production of a film occurs sooner or later with all the species hitherto examined by myself. Several of them, chiefly from the first sub-group, cover even alcoholic nutrient liquids with a film by the end of twenty-four hours, and develop, like *Mycoderma*, principally on the surface. The external similarity to *Mycoderma* species is the greater when the dry, dull grey films assume, like the latter, mesenteric folds in the course of further development. In certain cases the film remains smooth and delicate. The production of film vegetation takes a long time with some species, and occasionally nothing more than a ring is formed (*see* p. 120, vol. ii.), even at high temperatures. The films then exhibit a moist gloss, resembling that of *Saccharomycetes*, and are occasionally of a thick, mucinous character. Strongly developed films may become coloured (lemon-yellow, rose-red, leather-brown, olive-green).

During development in nutrient liquids, a variety of phenomena characteristic of the species are observed. A cloudiness may set in at first, to subsequently disappear with the formation of pulverulent, flocculent, agglomerate, yeasty, solid or mucinous, ropy sediments; or, as in the case of *Sacch. lactis*, the cloudiness may persist. In other instances the liquid remains perfectly limpid. Two species impart a decided lemon-yellow colour to saccharine yeast water. Other nutrient liquids, such as beer wort and must (*see* p. 224, vol. ii.), are decolorised to a greater or smaller extent. This has been established in the case of beer wort by L. van den Hulle and H. van Laer (II.), Will (XXXI.) and P. Lindner (XXXVII.); and for must by R. Meissner (II.). In Will's experiments, the highest degree of decoloration, determined by the method of C. J. Lintner (II.), was 0.6. Many *Torula* species, on the other hand, seem to darken the colour of beer wort, but whether this also applies to the *Torula Novæ Carlsbergiæ* of Grönlund (II.). must remain an open question owing to the unreliable method of colour determination employed by that worker.

The capacity of many *Torula* species for acclimatisation in highly concentrated nutrient liquids (*see* p. 229, vol. ii.), seems to be very extensive. Thus the writer found one species able to develop and produce a fairly brisk fermentation in a 76 per cent. malt extract. Wehmer's salt yeast remained capable of development for several months in herring pickle, representing a 24 per cent. solution of salt, whereas *Lactomyces inflans caseigrana*, Bochicchio, could not stand saturated solutions of salt for more than 30–40 minutes. An addition of 15 per cent. of common salt to the nutrient solution merely retarded the development of the salt yeast in question.

Acid nutrient solutions—fairly strong, *e.g.*, sauerkraut water

containing nearly 1 per cent. of lactic acid with some species—formed a more favourable medium than neutral for most of the species examined by myself. *Lactomyces inflans caseigrana*, Bochicchio, continued to vegitate in a broth containing 1–2 per cent. of lactic acid, and *Torula amara*, Harrison, even in one with 2.4 per cent. of that acid. A few of the species described by other workers, such as the *Torula* isolated from pine-apple by E. Kayser (V.) proved sensitive toward acids, as did also the so-called *Sacch. lactis* and *Sacch. tyrocola*. All the species examined by Will (XXXII.) were able to stand direct treatment with a 4 per cent. solution of tartaric acid for forty-eight hours at 25° C. (*see* p. 245, vol. ii.).

Some species will even grow in alkaline media, Meissner's muscinous yeasts developing as quickly in alkaline Liebig's meat extract with sugar, as in wine must. On the other hand, the budding fungi—some of which at least must belong to the group now under consideration—discovered by O. Bail (I.) in decaying rhubarb leaves, disappear when the reaction of the leaf mass changes to neutral and alkaline. A series of budding fungi capable of fermenting lactose, isolated by Mazé, induced a far better fermentation in alkaline nutrient media than in those with an acid reaction. Probably the alkali fixes the acids that are liberated during fermentation and retards that process.

Carbon dioxide retarded the development of Meissner's mucinous yeast, without killing them, and the reproduction of these organisms decreases as the alcohol content (*see* p. 239, vol. ii.) of the nutrient liquid rises, ceasing when it reaches 9 per cent. in must, though the cells do not die. The power of resistance is a variable quantity. In the researches of Wirgin (I.) reproduction ceased in the case of a species of *Torula*, when the alcohol in the grape-sugar broth reached 8.5 per cent., addition of ammonia causing rapid reproduction. Sulphur dioxide also influenced the activity and development of the said mucinous yeasts, about 0.1 per cent. being the limit for hindering development. Tannin restricted the growth and reproduction of the mucinous yeasts; and their resistance to acetic acid (*see* p. 246, vol. ii.) was very slight.

The temperature at which the known *Torulaceæ* continue to reproduce occurs between wide limits, nearly all of them growing even at 5°–6° C. The intensity of reproduction varies considerably, but is generally small at low temperatures, in which case, moreover, the character of the nutrient solution greatly influences development. Several of the species examined by myself remained for a month without reproduction, in pure yeast beer at about zero C., though some of them grew, if only to a small extent, in neutral saccharine yeast water; and a few also in hopped beer wort. With one of the species examined by Hansen (LV.), the minimum temperature was also 0.5° C. *Sacch. lactis*, Adametz,

Torula Duclaux, and the lactose-fermentating species isolated by E. Kayser from milk, will not adapt themselves to temperatures of about zero C. In the species examined by Will, the optimum temperature varied between 20° and 25° C.; whereas it lay between 25° and 30° C. for the Hartman's *Torula colliculosa*, *Sacch. lactis*, Adametz, and Duclaux's *Torula* and the lactose-fermenting species of Kayser. In the case of *Sacch. lactis*, Adametz, the optimum fermentation temperature was 37·5°–40° C., whilst *Sacch. tyrocola* preferred lower temperatures, viz., 23°–27° C. The optimum temperatures for development and fermentation do not always coincide. With *Torula colliculosa* the limit of growth was reached at 45° C., whereas Hansen found it to be 36°–37° C. for several of his *Torula* species, and in one case 38–39° C. formed the limit for several of the species examined by myself. *Lactomyces inflans caseigrana*, Bochicchio, grows very rapidly at 40° C.; but growth recedes at 45° C. and the fungus dies in a short time at 50°–60° C. The optimum growth temperature of *Torula amara*, Harrison, is 37° C., the limit being 48°–50° C. As in the case of the *Saccharomycetes*, a considerable divergence in the limits of budding temperature is observed among the *Torulaceæ*, a circumstance capable of affording valuable diagnostic indications.

Growth and reproduction are largely influenced by the admission of air. All the known *Torulaceæ* require free oxygen, a characteristic which certainly stands in causative connection with the predominating tendency of many species to grow on the surface of the liquid. This requirement, however, does not extend so far as to necessitate direct contact with the air, growth proceeding also in fairly high strata of liquid.

Numerous reports are available on the behaviour of the *Torulaceæ* towards the various kinds of sugar, *e.g.*, by E. C. Hansen (XLVI.), L. van den Hulle and H. van Laer (II.), E. Kayser (V.), C. Grönlund (II.), V. Peglion (II.), R. Meissner (II.), A. Kalanthar (I.), O. Bail (I.), M. Hartmann (II.), L. A. Rogers (I.), J. J. van Hest (I.), N. Hj. Claussen (I.), and chiefly by P. Lindner (XXXV.). The writer himself has also carried out numerous fermentation experiments with the *Torula* species he examined. The methods adopted by the various authors differed among themselves. P. Lindner, M. Hartmann and the writer employed the small-scale method (introduced by the first-named) in hollow-ground slides, with yeast water as the nutrient liquid. In any event the nutrient solution used plays a certain part in the fermentation.

Fermentative power is lacking in only a very few of the known species, such as the majority of Meissner's mucinous yeast, and a few of the *Torula* forms examined by P. Lindner and the writer; but, apart from a few lactose-fermenting species, the *Torulaceæ* are not extensive alcohol-formers. Most of them ferment glucose, mannose, galactose and fructose with comparative readiness;

maltose is fermented with difficulty, if at all, whilst the same species are able to split up the other sugars named into alcohol and carbon dioxide. In other cases, *e.g.*, *Torula colliculosa*, the maltose is fermented only by certain cells present in the warty excrescences of the giant colonies, whereas the cells growing in the flat portions of the colonies do not exhibit the least sign of fermentation in presence of maltose. Saccharose is fermented with vigour by a large number of species; but others cannot invert it, though able to reproduce at the expense of this sugar, as they do in the case of others they are unable to ferment. Lactose, trehalose, melibiose and melicitose are not split up into alcohol and carbon dioxide by the majority of species, and in a few cases the same applies to raffinose. *Torula Novæ Carlsbergiæ*, Grönlund, ferments dextrin.

On the other hand, a small group of *Torulaceæ*, including the species mentioned above, is characterised by the property of fermenting lactose, and consequently possesses high practical importance. So far as research has been pushed this group exhibits the same characteristic as the others, namely, that glucose, galactose and saccharose are fermented readily, maltose only with difficulty. When grown in beer wort, the non-sporulating, so-called *Sacch. pinophthorus melodus*, isolated by J. J. van Hest from spoilt beer, generates a gas that burns with a blue flame.

The fermentative power of a given species varies with the kind of sugar employed. Some of them, when grown along with yeast, are able to hinder the fermentation set up by the latter organism, probably in consequence of their transformation products. In some species the amount of alcohol produced is considerable. On the other hand, as shown by Heinze, the presence of 10 per cent. of alcohol in the nutrient liquid completely hinders the development of *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, even 5 per cent. being sufficient to suppress fermentation and reproduction almost entirely. Heinze and Cohn found the remarkable ratio of about 3 : 2 between alcohol and carbon dioxide with the last two lactose yeasts in meat-broth cultures containing lactose. Esters are also produced during the fermentation.

With regard to the enzymes of the *Torulaceæ*, little is known at present. Invertase appears to be excreted by many of them: compare Schuurmans-Stekhoven (I.) and E. Fischer (IV.). According to the researches of H. van Laer (VII.), inversion appears only in certain nutrient solutions. On the occurrence of lactase, which was questioned by Schuurmans-Stekhoven (I.) and E. von Freudenreich (XI.), *see* E. Fischer (IV.). Henneberg (V.) established the presence of catalases, that decompose hydrogen peroxide, in living *Torula* cells.

Gelatin is liquefied by all the species hitherto examined, but the nature of the active enzyme remains undetermined. The

Lactomyces inflans caseigrana of Bochicchio produces a lab enzyme and a tryptic ferment (compare p. 63, vol. ii.), and a fat-decomposing enzyme seems to be produced by several species. Nearly all the *Torula* species examined by myself are able to liberate sulphuretted hydrogen—sometimes to a very considerable extent—when sulphur is present in the nutrient solution (compare chap. lxvi.).

So far as information is available, the production of acid by *Torulaceæ* seems to be inferior to the acid consumption, though the amount produced is fairly considerable in some cases, notably by Kayser's pine-apple *Torula* (which produces acetic acid and small quantities of a higher fatty acid) and by Clausen's *Brettanomyces*. The *Torula* cultivated by Weigmann from bad butter produces about 3.6 per cent. by weight of butyric acid when grown in milk; but, in the researches of Heinze and Cohn, *Sacch. lactis*, Adametz, and *Sacch. tyrocola*, Beijerinck, seldom produced more than 0.3 per cent. of acid. Acid is also formed by Grönlund's *Torula Novæ Carlsbergiæ* and van Hest's *Sacch. pinophthorus melodus*, the amount varying with the kind of sugar. The nature of the acid in these cases is unknown. With the species examined by Will, on the other hand, the acidity of the beer wort varied, both the decrease (with one exception) and the increase being, however, inconsiderable. The development of one species changed the reaction of even strongly acid sauerkraut water to neutral in a month; and alkalinity ensued with a less acid medium. No regular connection could be detected between the diminution of acidity and the rapid production of a film growth on the surface of the nutrient liquid. Bail's rhubarb fungi consumed citric and tartaric acids.

The resistance of the *Torulaceæ* to high temperatures is fairly strong in a few species, but varies according to the species, the duration of exposure and the composition of the substratum. The age and physiological condition of the cells are also important factors. After being grown for eight days in wort, seven of the species examined by Will survived exposure to a temperature of 65° C. for half an hour, whilst the remainder, under the same conditions, were killed by a temperature of 60° C. In most cases the fatal temperature was the same for wort and water tests. Another *Torula*, reported by F. Schönfeld (XI.), possessed still greater powers of resistance, succumbing only when heated to 68°–75° C. in beer for an hour. On the other hand, van Hest's *Sacch. pinophthorus melodus* would not stand heating for five minutes at 65° C. The resistance of Meissner's mucinous yeasts terminates between 54.5° and 61° C., and all died when warmed at 45° C. for two hours. The fatal temperature in the case of the lactose-fermenting *Torula* species is 50° or 55° C. It is therefore evident that, under certain circumstances, the capacity for resisting heat may afford useful diagnostic indications.

Very low temperatures are also withstood well, Meissner's mucinous yeasts, for example, being found alive after exposure to - 22° C. for eight hours.

Several species will also stand desiccation, a fact already reported by Pasteur (XXVII.), who was able to convert his *Torula* forms into the dry state without loss of their powers of development. Meissner's mucinous yeasts were more susceptible, however, dying after five days' desiccation in the air. Harrison's *Torula amara* perished almost as quickly, in the dried state, at temperatures between 15° and 5° C., and Bochicchio's *Lactomyces inflans caseigrana* at 35° C.

Direct insolation had no destructive influence on Meissner's mucinous yeasts.

Considerable longevity is exhibited in liquids by some species, as in the case of the *Saccharomycetes*. Hansen (LI.) found living cells, capable of development, in cultures stored for sixteen years in a 10 per cent. solution of saccharose. In beer wort, some of the species perished in less than a year, whereas others were still living at the end of eight years. The mucinous yeasts found by Wortmann (XVII.) in twenty- and thirty-year old wines (*see* p. 242, vol. ii.), exhibited great longevity.

So far as our knowledge goes at present, the *Torulaceæ* do not seem to be of any practical utility to man, or to play any important *rôle* in the economy of nature, though a number of species are capable of producing objectionable effects in the dairying and fermentation industries. A number of problems in which the action of *Torulaceæ* is concerned are still awaiting solution; and certain pathogenic budding fungi described by physicians belong to the *Torulaceæ*, though they need not be taken into consideration here.

According to the researches of Bail (I.), it is highly probable that certain *Torulaceæ* are causatively connected with the decay of many plants. The constant and abundant occurrence of budding fungi, also belonging to this group, in the excreted juices of preserved food-stuffs—*e.g.*, in herring pickle, the aqueous liquid of sauerkraut, and other food-stuffs and delicacies prepared in a similar manner by processes of fermentation—has raised the question whether these organisms are of importance in the preparation of the desired products or not; but no decision has yet been arrived at on this point.

A certain amount of importance attaches to several species in connection with the fermentation industries—especially in the preparation of beer and wine—on account of the maladies they give rise to in the products, in which they are able to reproduce themselves and live. The flavour of beer, for example, is greatly influenced in this way. The formation of aromatic products, exhibiting the flavour and smell of apples, seems to be a property of many *Torulaceæ*. It has often been asserted that the presence

of *Torula* imparts a full, and even pappy, flavour to beer; and, in certain circumstances, this may well be the case. *Torula* species occur almost invariably in beer worts cooled and aerated in vessels that are not enclosed and protected from atmospheric infection. Experience teaches, however, that these organisms do not develop to any considerable extent, because, as shown by Will, most of them are suppressed entirely, or else greatly checked in their development, by the primary and secondary fermentations. It is a matter of experience that beer maladies due to *Torulaceæ* are extremely rare, and therefore these organisms cannot be regarded as injurious to beer in general. According to Will's researches, the addition of yeast to cultures containing certain mucinous species of *Torula* results in the so-called "boiling" fermentation (*see* p. 184, vol. ii.), in which the usual fine head on the liquid is replaced by a few very large bubbles. According to the concordant reports of N. Hj. Claussen (I.) and H. Seyffert (II.), certain species of *Torula* play an important part in the preparation of English beers. This group, known as *Brettanomyces*, is indispensable for the flavour and aroma developed in English beers by ethereal products formed during secondary fermentation.

Certain *Torula* species are valuable to the cattle-breeding mountaineers of Caucasia, as well as to the inhabitants of Armenia and the nomadic tribes of South-East and Southern Russia, since, in collaboration with certain bacteria, they serve in the preparation of important food-stuffs and delicacies, *e.g.*, kefir, koumiss, and mazun. Further particulars on this point are furnished by E. von Freudenreich (XI.) and A. Kalanthar (I.). Harrison's *Torula amara* imparts a disagreeable, bitter taste to milk and cheese; and, according to L. A. Rogers (II.), tinned butter is endangered by species of *Torula*.

§ 301. Red Yeasts and Black Yeasts.

Small as our knowledge is of the, usually colourless, *Torulaceæ* described in the preceding paragraphs of the present chapter, it is still less as regards the budding fungi that attract the eye by their more or less intense and variously shaded red colour. These are called by different authors "pink yeast" or "red yeast," some even classifying them with the genus *Saccharomyces*, though the majority do not form spores. The earliest attempt at a thorough investigation of these budding fungi was made, at a comparatively recent date, by F. A. Janssens and A. Mertens (I.), with a species described as "red *Torula*."

Red budding fungi have long been known. At first they were described by Fresenius (I.) under the name *Cryptococcus glutinis;* and, subsequently, Schröder and Cohn (I.) grouped similar organisms (termed "pink yeast" by Cohn) with the

Saccharomycetes. *Cryptococcus glutinis*, Fresenius, and *Saccharomyces glutinis* are, however, apparently two different species. It was afterwards shown by Hansen (LII.) that the term *Cryptococcus glutinis* comprises a growth of several species, and that these cannot be properly assigned to the *Saccharomycetes*. One of the budding fungi examined by Hansen (LI. and LII.) is probably identical with Cohn's *Saccharomyces glutinis;* the second is a true *Saccharomyces;* whilst the third is characterised by the production of tubular buds, and is allied to *Cryptococcus glutinis*, Fresenius.

So far as Hansen (LIV.) and P. Lindner (XXXIV.) were able to re-examine the pink yeasts subsequently described by the Koch school and physicians generally, these species are incapable of sporulation. According to Lindner, Koch's pink yeast is identical with one of those drawn by Hansen (L.), the same bizarre outgrowths being exhibited by both. Sporulation is also lacking in Elfving's (II.) red budding fungus.

Red-coloured budding fungi are mentioned, and in part more fully described, by L. van den Hulle and H. van Laer (II.), who discovered one species in the Belgian beer known as Lambic (*see* vol. i. p. 255). The red *Torula* of Janssens and Mertens was isolated from the deposit in English bottled beer. M. Ward (VIII.) refers to *Cryptococcus glutinis* as an alien organism in ginger-beer yeast (*see* vol. i. p. 258). E. Kramer (III.) describes a red budding fungus taking part in the fermentation of must; and a pink yeast, found in fermenting must, is mentioned by V. Peglion (II.) and E. Kayser (XII.). A species occurring on milk and cheese, and named *Saccharomyces ruber* by R. Demme (I.), is regarded by him as the cause of gastric catarrh in children of tender age. It should be mentioned that A. Kalanthar isolated from mazun—a beverage of the kefir type, prepared in Armenia from the milk of buffaloes or goats—an orange-coloured budding fungus and a species the giant colonies of which were initially greenish grey, afterwards turning peach-red. Coloured budding fungi seem to be of common occurrence in milk and butter, Krueger (III.), for instance, having found in cheesy butter a budding fungus which he described as *Saccharomyces flava lactis* (*see* p. 282, vol. ii.); whilst R. Reinmann (I.) discovered pink yeast, along with other budding fungi, in butter.

A. Laschê (I.) isolated two species, *Mycoderma humuli* (from hop leaves) and *Mycoderma rubrum* (from an infected gelatin culture). B. Fischer and K. Brebeck (I.) found a pink yeast in the contents of the stomach of a patient suffering from gastric enlargement and fermentation; and another in the water of the open sea to the south of San Miguel, one of the Azores islands. C. Wehmer (XXXI.) also reports having found pink yeast in herring pickle. It is still doubtful whether certain species, such as the red yeast mentioned by A. P. Swan (I.), belong to the

group now in question; J. C. Bay (III.) contests their claim to be considered *Saccharomycetes*. Some doubt also attaches to the *Saccharomyces japonicus* and *Sacch. keiskeana* (*see* p. 240, vol. ii.) of K. Yabé (VI.). On the other hand, one of the species described by K. Golden and G. C. Ferris (I.) is said to be identical with *Saccharomyces glutinis;* and another species has been allocated to the *Mycoderma* group.

From all the reports, which could be amplified without difficulty, as to the occurrence of red-coloured budding fungi, it appears that these organisms are very common.

The arrangement of these diversified forms, the majority of which have not yet been thoroughly examined, into a system is still more difficult than in the case of the species comprised in the generic name *Torula*. It is equally difficult to decide whether certain of these forms are identical or not.

On the basis of their special method of vegetative reproduction, one of the species described by Hansen, as well as the *Mycoderma rubrum* and *Mycoderma humuli* of A. Lasché, the red *Torula* of Janssens and Mertens, Koch's pink yeast (according to P. Lindner), and the *Blastoderma salmonicolor* of Fischer and Brebeck, may be arranged in one group. A second group might include the forms with more or less globular cells, such as *Saccharomyces glutinis*, Cohn, one of the species described by Hansen, and several others. The latter, as producing the most pigment, might be united with the first sub-group of the *Torulaceæ*, with which they appear to have a good deal in common.

The colour of the cells is usually noticeable only when a large number are in juxtaposition. The shades of colour are numerous: pale red, rose-red, vermilion, coral, yellowish red, and salmon-red. Pigmentation seems dependent on certain conditions in many species, and in some occurs very late, so that it is not a constant feature. The intensity of the colour also varies, and is dependent, *inter alia*, on the reaction of the nutrient medium.

The cells vary in size and shape quite as much as with the *Torulaceæ*.

Like the *Torulaceæ*, too, highly refractive bodies that have undoubtedly often been mistaken for spores, occur in the cells, especially those of old cultures. In Janssens' and Mertens' red *Torula*, the bodies of this kind observed in the vacuoles resembles drops of oil, and are orange-coloured. They, however, consist largely of carotin, but do not appear to contain fat. A uniform reddish tinge is often visible in the vacuoles in old cultures of *Blastoderma salmonicolor*. In other respects there is no information available regarding the seat of the pigment in red budding fungi.

The nature of the pigment varies, being sometimes soluble in water, and disappearing under the influence of acids and alkalis; whereas, on the other hand, the red *Torula* of Janseens

and Mertens gives a clear, deep red extract only with carbon disulphide.

According to reports by Laurent (X.), and also by Brault and Loeper (I.), the red budding fungi produce glycogen.

Janssens and Mertens described a globular nucleus, with a nucleolus, in their red *Torula*.

In one and the same species budding may proceed in different ways. Sometimes it resembles the same process in the *Saccharomycetes*, with the modifications exhibited by the *Torulaceæ*. Raum (III.) found a parent cell carrying up to five and more daughter-cells at the same time. In the case of a fixed cell of his species belonging to the second group, Hansen (LII.) observed a considerable number of new cells gradually formed at the same place.

In addition to this method of budding, the red yeasts of the first group also exhibit cell outgrowths in the form of "tubular buds" or "promycelia." Usually the oval cells throw out simple or branched, filamentous lateral growths, which, in association with the sterigmata, impart a strange appearance. The budding of these "tubular buds" results in the production of rounded cells resembling conidia, or, in the case of *Blastoderma salmonicolor*, pear-shaped, plum-shaped or reniform cells. This type of germination forms a highly characteristic feature of the first group of red species, and, so far as is known, is not exhibited by any other group of budding fungi.

Films are produced by all species of red yeasts, and on the most divergent nutrient media, such as beer wort, beer (except in the case of *Mycoderma humuli*), milk, whey, &c. The films are partly smooth and mucinous, partly tough and greatly imbricated (*e.g.*, *Blastoderma salmonicolor*). The film of the red *Torula* of Janssens and Mertens is more strongly pigmented in the dark than in the light, and the cells are larger, but the resisting power is smaller. The film grown in the light resembles woolly felt, many of the filaments projecting above the surface of the liquid. Probably also hairs and tufts are formed, as in the case of *Monilia candida*, certain *Torula* species of the second sub-group, and occasionally also with *Saccharomycetes*. The cells in this case are smaller, but more resistant.

Reports on the giant colonies are few in number. P. Lindner (XXXVI.) has described those of two species, each of which exhibited a slight, mealy "bloom," a distinctive feature of the one being the production of delicate "white" aerial hyphæ. A notable feature is the formation of secondary colonies in plate- and streak-cultures, the more so because this phenomenon occurs in two species belonging to the group that germinates by promycelia and forms cells resembling conidia. Janssens and Mertens explain the phenomenon in the case of their red *Torula* by stating that the liquefaction of the gelatin is accompanied by the libera-

tion of a gas, which forces the liquefied gelatin through the colonies and scatters it over the surface, a number of cells being carried off at the same time. Fischer and Brebeck, on the other hand, attribute the appearance of secondary colonies to the conidial cells being liberated by slight vibrations and then settling down in the vicinity of the original colonies.

The requirements of the red budding fungi in respect of organic nutrient materials have not been specially investigated, though it has been reported that starch paste forms a good medium for certain species. According to Hansen's (LIV.) researches, Elfving's red budding fungus will reproduce in purely inorganic media, a fairly strong light being essential. Hence, in this case at least, the red pigment plays an important part in the physiology of nutrition, though the possibility of saprophytic nutrition is not precluded. The red *Torula* of Janssens and Mertens is also influenced by light, and behave like green plants, respiration being also apparently more pronounced in the light than in the dark. The researches of Went (III.) with *Monilia sitophila*—in which the formation of carotin is dependent on light—indicate that the abundant production of carotin protects the enzymes of the fungus from strong light. Little is known as to the enzymes of the red budding fungi, though the action of catalase was observed by Henneberg (V.). Fermentation is absent, at least among the *Mycoderma*-like species of the second group, and appears to be only imperfectly developed in the members of the first group. E. Kramer's red budding fungus ferments dextrose, maltose and saccharose, which it previously inverts, but does not attack lactose. Fermentation for eight hours in sugar solution furnished 4.5 per cent. of alcohol, by volume, the solution at the same time acquiring an agreeable fruity aroma, indicating the formation of esters. The fermentation proceeded more actively in acid media than in those with an alkaline reaction, even 1.5 per cent. of tartaric acid being more stimulating than restrictive. Lindner (XXXV.) failed to obtain fermentation with any of the red yeasts examined. On the other hand, Kalanthar's greenish mazun yeast (p. 402, vol. ii.) possesses fermentative power.

Very little has been published on the behaviour of the red budding fungi toward acids. The red *Torula* of Janssens and Mertens produces only small quantities of acids, which are exclusively non-volatile.

The optimum temperature of growth is about 20° C., as with many species of *Torula*. The vital activity of Janssens' and Mertens' red *Torula* is impaired by a temperature of 30° C. A red *Torula*, isolated by Schmidt-Nielsen (I.) from the surface of the deep-water shrimp (*Pandalus borealis*) furnishes a luxuriant culture on potato slices in fifty to sixty days at zero C. E. Kayser's pink yeast withstands heating to 45° C. in the damp state.

So far as our knowledge of the red budding fungi extends at present, none of them possesses any great practical importance, except the case mentioned by Demme (*see* p. 402), though they are able, occasionally, to give rise to very unpleasant phenomena. Will (XXXIII.) cites an instance where such organisms coloured a whole batch of green malt red, their reproduction having apparently been greatly stimulated by peculiar circumstances. When dried, the malt turned a dirty brown colour, and the cured malt had an unsightly, discoloured appearance. The infection was traceable to the water used for steeping the barley. Beer wort is partially decolorised both by the pink yeast observed by L. van den Hulle and H. van Laer in Lambic, and also by the red *Torula* of Janssens and Mertens, the former yeast also imparting a sour taste to the wort. These properties, however, are of little practical importance, the red budding fungi and *Torula* species being suppressed by the rapidly multiplying and fermenting beer yeasts; and, even if they survive the fermentation process, the extent to which the water is decolorised by the beer yeast itself cannot be increased very much by the action of the red budding fungi.

Black yeast has also been reported upon occasionally. The fungus isolated by C. Marpmann (VI.) from milk, and termed *Saccharomyces niger* by him, forms round to oval cells, measuring 1.5–3 μ, and reproducing by budding. No mycelial filaments are produced in saccharine nutrient solutions. On gelatin, the fungus forms velvety black herbages, and in nutrient solutions black deposits. Saccharose and lactose are not fermented, though grape sugar is to a small extent. According to B. H. Buxton (I.), the fungus does not contain either diastase, maltase, invertase, lactase or inulase. Hansen (LIV.) has demonstrated that *Saccharomyces niger* does not sporulate, and is therefore no true *Saccharomyces*. According to him, the dark-coloured budding fungi belong to various species, all of them agreeing in being asporogenic and incapable of fermentation. He considers them to be, probably, budding forms of *Cladosporium* or *Fumago* species; and this view is supported by P. Lindner (XXXIV.) who states that, whilst the young cultures of black yeast grown in Koch's laboratory formed a pad consisting of bud cells, they subsequently developed into dark green herbages composed of hyphæ. Apparently Marpmann's black yeast differs from that of Koch. The *Torula nigra* of Guilliermond (IV.) grows luxuriantly on carrots, so that, twenty-four hours after sowing, the substratum is covered with a sticky, blackish green mass, composed entirely of oval and slightly elongated bud cells, held together by a mucinous mass exhibiting isolated black, solid particles. After a few days the less damp portions of the nutrient medium exhibit a thin mycelium, arising out of the black mass of yeast, and assuming the shape of a grey, matted felt. In Guilliermond's

opinion, this fungus is probably allied to *Dematium*. P. Lindner (XXXVI. *b*) mentions a black yeast, isolated by Zeidler, and having ellipsoidal cells, measuring 0.6 μ in length. On wort gelatin it develops with a damp surface and mesenteric folds, covered by a scanty growth of wool. Hansen states that black yeasts are not infrequently found in atmospheric dust, but he does not credit them with any practical importance. G. Grotenfelt (II. and III.) gives black yeast as the cause of blackening in cheese.

CHAPTER LX.

MYCODERMA.

By Prof. Dr. RICHARD MEISSNER,
Principal of the Royal Würtemberg Institute for Viticulture at Weinsberg.

§ 302. Species of Mycoderma.

The film yeasts (*see* pp. 120 and 387, vol. ii.) comprise numerous species, of which comparatively few have, as yet, been thoroughly examined. They are all unicellular budding fungi, which reproduce either by budding and sporulation, or by budding only, and are therefore in part true *Saccharomycetes* of the genera *Pichia* and *Willia* (*see* pp. 287 and 289, vol. ii.), and in part non-*Saccharomycetes*. The latter may be divided into three groups, two of which belong to the *Torulaceæ* (p. 386, vol. ii.), the third comprising the various typical species of *Mycoderma*. These last alone will be treated in the present chapter to the exclusion of such species as were regarded as *Mycoderma* by earlier workers, but must be allocated to the pink yeasts or *Torulaceæ* on account of their fermentative power, oval cell form, or other peculiarities. These excluded forms comprise, for example: Heinze's *Mycoderma cucumerina*, Aderhold; the *Mycoderma* species mentioned by Lasché, *Myc. rubrum*, Lasché's *Myc. humuli*, Henneberg's two *Mycoderma* species, and the sporogenic film yeasts of Fischer and Brebeck. It may be mentioned here that the *Torulaceæ* and *Mycoderma* species have a number of properties in common; their distinguishing characteristics will be found on p. 385, vol. ii.

To the *Mycoderma* species belong, *inter alia*, a species of film yeast examined by Will (XIII.); certain film yeasts described by Meissner (XI.); others described by Hansen, A. Petersen, Grönlund, Jörgensen, Lindner, Prior, Bélohoubek, Kukla, Forti, Seifert, Lafar, Koch, Wortmann, E. Rist and J. Khoury, and others. Like the true wine yeasts, these various species of *Mycoderma* have their natural habitat in the soil, from whence, as shown by the researches of Hansen, Müller-Thurgau and Wortmann, they are conveyed to their appropriate nutrient solutions by insects, rain or wind.

Even as recently as 1871 we find Trécul expressing the view that proteid materials can change themselves into bacteria or direct into beer yeast, these again into *Mycoderma*, and the latter in turn into *Penicillium* (*see* p. 107, vol. ii.); and similar ideas are found in a treatise by Hoffmann (VII.) in 1869. At the same period, however, Adolf Mayer (X.) disputed the alleged genetic relation between yeast and *Mycoderma*, and between yeast and *Penicillium;* and Reess (IV.), in 1870, denied the identity of *Penicillium*, wine yeast and *Mycoderma*.

Moreover, the old assumption that the so-called *Sacch. Mycoderma* (*see* p. 271, vol. ii.), *Mycoderma vini* and *Myc. cerevisiæ* were one and the same species, has been disproved by the numerous researches of later workers. The paths by which this knowledge was attained are identical with those pursued by Hansen in establishing the existence of the different races of beer yeast, namely, by the pure culture of the organisms, and by accurate morphological and physiological investigation.

There is no difficulty in obtaining material for the pure culture of different races of *Mycoderma*. Bottled wine, fruit wine, beer, &c., low in alcohol, is taken, half the contents of each bottle being poured out, and the remainder shaken up once or twice, after which the bottles are plugged with cotton-wool and are left to stand for several days at about 20° C. This treatment admits a sufficiency of oxygen to the bottles and liquids, so that the *Mycoderma* species and other organisms present therein are enabled to develop. Pure cultures of these organism scan then be prepared by the method recommended by Hansen (*see* p. 278 *et seq.*, vol. ii.).

The various races of *Mycoderma* can be differentiated by the size and shape of their cells, their rate of reproduction growth in giant, stab and streak cultures, the character of the superficial vegetation, and by the attendant phenomena of the same. Physiological examination also reveals differences between the various species that may also indicate racial peculiarities.

§ 303. Form, Dimensions and Contents of Mycoderma Cells.

Attempts to identify single or mixed races of *Mycoderma* by microscopical examination alone meet with exactly the same difficulties as are encountered in the corresponding investigation of beer and wine yeasts, the problem being still further complicated by the fact that the development of *Mycoderma* in must or other nutrient media is accompanied by numerous morphological changes, whereas the true wine yeasts, for example, retain their form practically throughout such treatment. *Mycoderma* species, on the other hand, and especially while young, sometimes vary in form to such an extent that the observer might be led to think the cultures had become contaminated, if he had not, by

constantly following the development of individual cells into chaplets, convinced himself of the morphological variability of the cells.

This phenomenon has been observed by different workers at various times. Winogradsky (XI.) attributes the variation, not merely to the specific nutrient medium, but more particularly to the greater or smaller supply of oxygen available for the growing vegetation. Thus, one and the same species will produce cells similar to those of yeast when oxygen is present, whereas, in the absence of that element the growth is mycelial in character. It was also found by Will (XIII.) that the form of the *Mycoderma* species examined by him varied between wide limits, as did also the dimensions and contents of the cells; and Meissner (XI.)

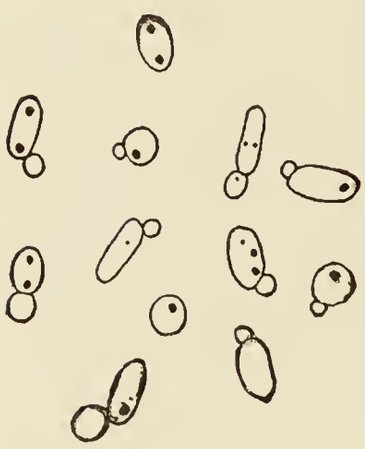


Fig. 190.
Mycoderma from Eltville red wine. Pastorianous and round cell forms. Magn. 600.

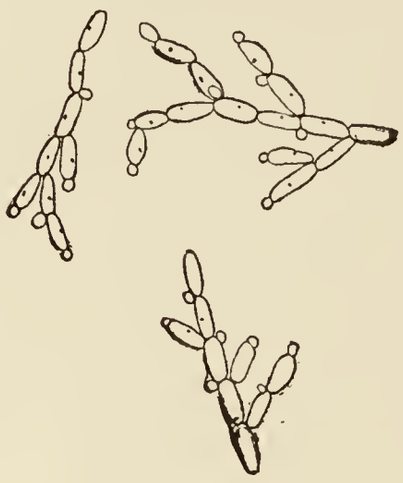


Fig. 191.
Mycoderma from bilberry wine (West Prussia). Pastorianous cell forms. Magn. 600.

Fig. 192.
Mycoderma from Rüdesheimer wine. Irregular cell forms. Magn. 600.

arrived at a similar result. In the races subjected to mophological examination by him, the largest cells measured 4.6 μ by 19.2 μ, those of the other races remaining below this level. According to Will (XIII.), the typical *Mycoderma* cells measure 8–11 μ in length (the mean being 9 μ), and 5 μ in breadth.

The cell form is pastorianous, with rounded edges (Figs. 190 and 191), though, as pointed out by P. Lindner (XXXI.) irregular half-moon and pear-shaped cells (Fig. 192) are also found now and then. Will (XIII.) gives precise data respecting the integument and contents of the cells. According to this worker, the young cells exhibit only faint refraction after their contents have been shrunk by the action of glycerin. The membrane is usually thinner than in yeast cells; and even in the slender cells, occurring regularly in older cultures, the integument is pale and seems to remain so always. On the other hand, *pace* Will (XIII.), the slightly oval, tough cells, appearing in older cultures, are distinguishable from the other cells by their strong membrane, bounded by a broad outline. Treatment with 1 per cent. osmic

acid stained the cell wall dark brown, a result that MEISSNER (XI.) did not succeed in obtaining in the case of wine *Mycoderma*. This behaviour on the part of the membrane led Will to conclude that substances behaving like fats or oils were stored up in or upon the membrane. The same worker (XI.) has also pointed out that the microscopical examination of Mycoderma cells has revealed the presence of individuals characterised by a certain lustre, accompanied by a bluish sheen. This peculiarity he ascribes chiefly to an enveloping stratum of air; and this conclusion was confirmed by MEISSNER (XI.). The higher lustre of the cells may also be the result of their content of glycogen, which varies considerably in the individual cells of one and the same culture. As is the case with the cells of *Torulaceæ*, the contents of young *Mycoderma* cells have a low refractive power (*see* p. 391, vol. ii.), and consist, according to WILL (XIII.) of a somewhat fluid substance that is stained by iodine. They must, therefore, contain a large proportion of water. The vacuoles, of which at first there are three, four or more in each cell, afterwards coalesce to form one or two. No highly refractive bodies (oil particles) can be observed in the very young cells; but when these cells are treated with iodine, deeply stained, dense granules become visible in the places where the refractive bodies are found in more mature cells. At the end of forty-eight hours these granules can be perceived without the aid of the reagent. By the third day they number from one to three, and are situated either at the ends of the cells, or one of them is in that position, whilst the other lies between two vacuoles at one side or in the middle. At the same time the vacuoles are more clearly visible, and exhibit a denser plasmal integument. At the end of forty-eight hours the cells will give a faint glycogen reaction with iodine. In aged cells crystalloids are gradually developed in the vacuoles, the oil particles come into being and attain considerable dimensions (2 μ in diameter). These latter are stained a blackish brown by osmic acid. According to Will the oil particles differ from those in old yeast cells by remaining unstained when treated with concentrated sulphuric acid, though, as with yeast cells, the oil is expelled from the cells on the addition of the acid. Further particulars on this point, and on the position, structure and subdivision of the cell nucleus, will be found in chapter xlvii.

§ 304. Reproduction of Mycoderma in and upon Various Nutrient Media.

As already stated on p. 408, vol. ii., all the *Mycoderma* species reproduce by budding, which process has been described on p. 9, vol. ii. At present we will merely deal briefly with the formation of the bud aggregations, characteristic of the pastorianous

Mycoderma cells, and is easily distinguished from that occurring in the case of typical, oval yeast cells.

If a pastorianous *Mycoderma* cell be sown in a nutrient solution—*e.g.*, wort or grape-juice—and its development be followed under the microscope, it will be found to bud at one end in exactly the same manner as true yeast (*see* Fig. 193 *a*). As soon as the daughter cell (2) is completely formed, it buds (*b*) in the direction of its longitudinal axis, whilst the parent cell throws out a new daughter cell (3) at one side of the place whence the first cell (2) made its appearance. These new daughter cells in turn bud longitudinally (*c*), whilst their parent cells again develop lateral buds (*d*). Eventually the assemblage of buds assumes a form similar to that of a pine-tree, the central stem and lateral branches of which continue to grow in their initial direction, whilst branching out regularly every year. In the case of *Mycoderma* cells, this branching occurs about every two hours in a good nutrient solution; and as, when grown in wort or grape-juice, the assemblage of buds is not broken up by ascending bubbles of carbon dioxide, it frequently consists of hundreds of cells.

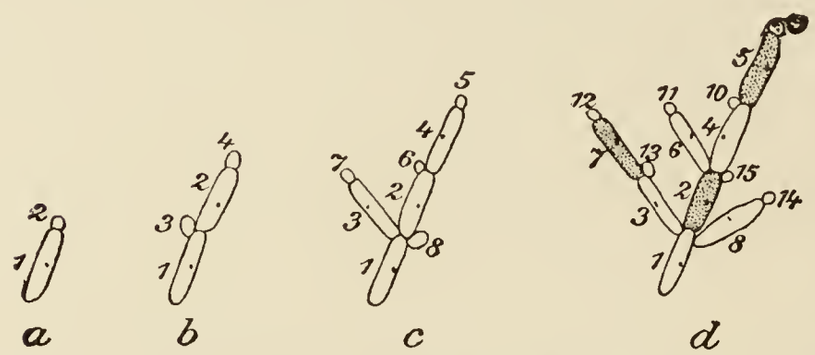


Fig. 193.—Aggregation of buds from Mycoderma. Cells 2, 5 and 7 are surrounded by an envelope of air. Magn. 600.

When a nutrient gelatin is employed as substratum, in which the cells are compelled to develop *in situ*, the cells commence to bud in the same manner; but since the daughter cells are unable to spread out uniformly, as is the case in or upon a liquid, a compact, spherical colony is formed, like those of beer or wine yeasts.

With regard to the giant colonies (*see* p. 393, vol. ii.), Lindner (XXXI.) rightly calls attention to the fact that their variability of form is greater in this case than with any other group of budding fungi. According to that worker, "In some cases the colonies form a dull grey to yellowish grey mass without any surface markings, or else veined, like leaves; in others a number of delicate and closely set concentric rings are exhibited, or wedge-shaped strata proceed from the centre of the colony, spreading out and usually assuming a dry, mealy appearance, or the entire surface becomes covered with innumerable fine or coarse wrinkles. In still other cases the colony takes the form of a hill, with a number of circular walls thrown up on the slopes; or it resembles a

mountain peak with a number of dichotomous branches running down into the plain; or again, a miniature volcano, whose uniform slopes, covered with powdery white dust, exhibit a number of supplementary craters in the form of small, warty excrescences. Other colonies are broad and round, like cakes, with superficial fissures through which the pasty mass is exuding in the form of a round protuberance; or again, like a cake the substance of which has subsided along radial lines." These descriptions of Lindner's, however, are only applicable to a few typical *Mycoderma* species. MEISSNER (XI.) classes the giant colonies of the *Mycoderma* races examined by him into four different types, according to their habit of growth. Type No. 1

FIG. 194.—Giant colony of Mycoderma from Geisenheim currant-juice. Nat. size.

FIG. 195.—Giant colony of Mycoderma from Gau-Algesheim grape-juice. Nat. size.

comprises smooth colonies, the members of which differ in their lustre, the fluting at the edges, the extent to which they grow into and liquefy the gelatin medium, and in a smaller degree by their size and colour. Type No. 2 includes the circular, compact colonies, which differ in size and superficial markings. In type No. 3 the colonies are also compact, but exhibit more extensive markings on the surface (Fig. 194). In type No. 4 the colony is raised in the centre, sinking thence, with a concave slope, to a ring concentric with the original cell. Radial lines extend from this ring or wall to the edge of the colony; and between these lines, irregular depressions and excrescences (Fig. 195) can be observed. As regards the microscopical examination of the cells, it will be noticed—as shown by WILL (XIII.) and MEISSNER (XI.)—that the cells around the edge of the colonies are larger than those nearer the centre.

In the case of stab cultures, WILL (XIII.) showed that the species examined by him—which grew to a depth of 60 mm. into the gelatin—was able to bear a certain degree of deprivation of air. The streak cultures of Will's species failed to exhibit any characteristic features at the end of 15 days.

§ 305. Superficial Vegetations and their Attendant Phenomena.

All *Mycoderma* species share the property of forming superficial vegetations on the nutrient liquids to which they gain access; beer, wine, beer wort, grape- and fruit-juice, fruit-wine, the residues from the distillation of beer, wine, &c. This circumstance at once gives rise to the question, Why should this peculiarity be general among *Mycoderma* species, but only shared—and in less decided degree—by a few races of the morphologically similar wine yeasts?

The explanation accepted at one time was that *Mycoderma* species require oxygen, and therefore form superficial vegetations; but this theory is imperfect. Lindner (XXXI.) and Will (XIII.) are of opinion that the cells of *Mycoderma* repel water (*see* p. 392, vol. ii.), and enclose air in their intercellular spaces, or attract air; and that it is probably this property that enables them to remain so easily on the surface. The researches of Meissner (XI.) in this direction show conclusively that air alone is the support of the film vegetations, which are themselves specifically heavier than grape-juice, for instance. This air is firmly retained in the bud aggregations, which often contain many hundred cells, and are extensively branched into brush-shaped masses. In the case of wine yeasts, on the contrary, the bud aggregations, previous to the commencement of fermentation, consist of comparatively few cells. It should also be remembered that, during alcoholic fermentation, in the words of Wortmann (XV.), "the small yeast cells are whirled about in a giddy dance, and prematurely torn apart, by the ascending tiny bubbles of carbon dioxide that are soon liberated extensively, with effervescence, by the yeast itself." The bud aggregations of *Mycoderma*, on the other hand, are able to develop in quiescent liquids—hence their larger number of cells.

When a cell of any species of *Mycoderma* is sown in grape-juice, beer or wine, it appears from the exhaustive researches of Meissner (XI.) that the phenomena of film-formation proceed in the same way as with many of the film-forming *Torulaceæ*. In a very short time the surface of the liquid is found to carry a vegetative growth, formed either by the coalescence of originally separate islands, or by progressive growth from the walls of the vessel. In the first stages of development, the film growth is delicate, flat and very elastic. This dull or partly lustrous surface exhibits a varying number of white spots of different sizes, more or less clearly visible, distributed irregularly or in curved lines, and representing accumulations or cells retaining air between their aggregations of buds. A peculiarity of many species of *Mycoderma*, to which attention has been drawn by Lindner (XXXI.)

and Will (XIII.), is that the films produced by these fungi are often perforated in the earlier stages, these open spaces closing in, however, during the subsequent growth of the cells. This phenomenon still lacks explanation.

In consequence of the progressive growth of the cells, the smooth, colourless film, as in the case of many film-forming *Torulaceæ*, becomes veined, folded and wrinkled, this taking place sooner with some races than others. The initial folding differs with the various races. According to the researches of Meissner, the following four groups are well defined: the first group exhibits broad veins, protruding upward like bubbles (Fig. 196). In the second and third groups the width of the veins decreases

Fig. 196.—Mycoderma from Eltville red wine. First stage of film formation. Slightly reduced.

Fig. 197. — Mycoderma from Guben cider. First stage of film formation. Slightly reduced.

Fig. 198.—Mycoderma from Berlin white beer. First stage of film formation. Slightly reduced.

(Figs. 197 and 198). The fourth group exhibits similar veins, but finer and of a decorative character. Under certain conditions, more especially when isolated portions of the liquid are left uncovered either in the mass of the film or against the edge of the culture vessel, the veining of the film may be preceded by the formation of parallel folds. As the film continues to grow, the veining passes over into the mesenteric form or becomes linear. In the former case the mass assumes the appearance of a loosely woven fabric, the threads of which are all entangled. These threads may be either coarse or fine. In the linear form the lines proceed either from an eccentric point in the film, or from a point on the glass wall (up which, in all cases of *Mycoderma*, the cells climb a short distance), or again from several central points on the film or from one of the open spaces thereon. The lines running in one direction may also be uniformly distributed on the film. During the further progress of growth, alterations take place in the folding, cultures which were mesenteric at first exhibiting wrinkles of various depths. According to Meissner (XI.), there are five different types of corrugation: No. 1, resembling cauliflower; No. 2 having shallower corrugations; No. 3 more uniform and finer; No. 4 still finer; No. 5 showing very fine wrinkles.

The colour of the film also varies with the stage of growth and with the race. While the film is still quite thin, it has no particular colour, only a few spots (as already mentioned) showing up white. These are the places where the film has already become somewhat thicker and contains a good deal of air enclosed between the individual cells. In proportion as folding (*i.e.*, cell growth) progresses, the colour turns white at first, owing to the inclusion of air ; and when the film has thickened, it becomes whitish grey, whitish yellow, whitish violet-green, violet, yellow, yellowish red, and so on. In some species a very thick film is produced, in others it remains thin, the rugous films being usually the thickest, and the more finely wrinkled forms correspondingly thinner.

With regard to the attendant phenomena in connection with the production of films, when the latter are formed on a nutrient liquid the latter may either remain limpid or become cloudy after a time, or else cloudiness may set in as the film is being formed. The cloudiness is due to the *Mycoderma* species concerned forming loose bud aggregations, the cells of which are readily detached and dispersed throughout the liquid, and the degree of cloudiness depends on the extent to which this dissemination can take place. In the case of certain species of *Mycoderma*, the detaching of the cells does not occur for some little time ; and here, again, we meet with two well-defined types : either (1) large masses, or (2) small aggregations of buds, being detached from the film. On reaching the bottom of the culture vessel, the *Mycoderma* cells do not perish immediately ; in fact, they are very tenacious of life, and put forth fresh bud cells from time to time. Wortmann (XVII.) succeeded in isolating living *Mycoderma* cells from wine that had lain in bottle for 25–33 years, tightly closed with the original corks.

The starving cells at the bottom of the culture vessel may also be carried up again into the liquid by the gas bubbles rising from time to time from the deposit. These cells, being in an emaciated condition, are but little heavier, specifically, than the surrounding liquid, so that the latter occasionally remains cloudy for some considerable time.

When *Mycoderma* species are grown on grape juice or wort, the liquid may be decolorised thereby (*see* p. 395, vol. ii.). This was observed by Will (XIII.) in the case of beer wort, and confirmed by Meissner (XI.) in the case of grape juice. The latter, however, observed that the pale yellow colour of grape juice may turn to a dark brown when this juice serves as a habitat for certain races of *Mycoderma*, owing to the formation of alkaline substances which neutralise the acids in the juice and finally render the liquid alkaline.

§ 306. The Destruction and Production of Acid in Nutrient Liquids by Mycoderma.

This matter has been thoroughly investigated by MEISSNER (XI.), who obtained the following important results: The previous researches of other workers, such as KOCH (V.), WORTMANN (XV.), and WILL (XIII.), on the physiological behaviour of *Mycoderma* revealed the fact that these fungi do not invariably lessen the acidity of grape juice, wine, beer, &c.; but that some races in particular produce acid, often in considerable quantity. MEISSNER (XI.) explains this phenomenon by stating that *Mycoderma* are able to form acids, as well as to destroy them, both processes going on concurrently. The preponderance of the formative or destructive action depends both on the powers of the various races and on external conditions, such as the amount of oxygen admitted to the cultures, the quantity of nutrient solution present, &c. If the acid-forming capacity prevails, the total effect is an increased acidity of the medium, and *vice versâ;* whilst if the two powers be equal, the quantity of acid remains unaltered.

In order to obtain a satisfactory insight into the nature of the process whereby a reduction of acidity is effected in liquids inhabited by *Mycoderma*, MEISSNER (VI.) closely examined the behaviour of these fungi when grown on artificial nutrient media (containing the necessary mineral ingredients) with different organic acids as the sole source of organic matter. The effect on malic acid was insignificant in certain races, but very strong in others. One species, isolated from Colmar wine, consumed within thirty-five days 5.72 grms. per litre (*i.e.*, 73 per cent.) of the malic acid originally present in the artificial medium, the growth of the organism increasing in luxuriance with the extent of decomposition attained. Tartaric acid proves generally ill adapted for the building up of *Mycoderma* cells, and is therefore consumed to only a small extent by the fungus. The same results were obtained by SEIFERT (II.). The case is different with lactic acid, MEISSNER (VI.) finding that six out of nine stocks examined consumed this acid extensively, the other three to only a small degree corresponding with their scanty growth. One race from Silesian perry reduced the lactic acid content of the solution (originally 0.7633 per cent.) to 0.0673 per cent. Citric acid and succinic acid were also attacked, sometimes extensively. In the former case, the acid was almost entirely consumed in one-third of the tests performed, whilst with the other acid, a similar result was obtained in one-fourth of the tests: *see* MEISSNER (VII.). In one-half of the same worker's experiments (VII.), acetic acid was vigorously attacked, but only slightly in three instances, whilst in three other cases the sowings failed to develop at all. H. VAN LAER (VIII.) in examining one race of *Mycoderma* found

that it ceased to grow in wort in presence of 1.25 per cent. of acetic acid, though vigorous growth occurred in the presence of 1 per cent., four-fifths of the acid being consumed in ten days at 30° C.

The simultaneous formation of acid by *Mycoderma* has been demonstrated by the total acidity of the medium being maintained in several of the nutrient media tried, notwithstanding the consumption of the organic acids supplied. In other instances the reduction of total acidity was slight, although energetic growth of the vegetation was observed.

The various *Mycoderma* produce different volatile acids, which can be identified by the odour of the nutrient liquids. According to Wortmann (XVII.), many mouldy wines smell strongly of rancid butter, owing to the presence of butyric acid formed by the action of the *Mycoderma* on different constituents of must and wine. As long ago as 1893, Lafar (III.) isolated a film-producing budding fungus, containing *Mycoderma cerevisiæ* in the involutions of the film, from the cask sludge of a brewery where difficulties were of frequent occurrence. The beer on which the cultures were grown exhibited an agreeable, fruity smell, and, after turning sour, a pleasant taste recalling that of wine vinegar. Will (XIII.) also noticed extensive production of acid in the pale Munich beer on which his *Mycoderma* cultures were grown. "On opening the culture vessel (he says) one noticed a sour smell, at first similar to that of acetic acid, but afterwards more difficult to define, being something like baked apples. The beer had a decidedly sour taste; but not of acetic acid." It should be mentioned that Will (XIII.) cites the authority of Raymond and Kruis for the statement that formic acid and acetic acid were discovered in a culture of *Mycoderma* that had been standing for a year at 20° C.

In addition to volatile acids, the *Mycoderma* produce fixed acids and esters, as is shown by the fact that the volatile acids formed are insufficient in quantity to account for the difference in the total acidity when an increase occurs in the acidity of the nutrient liquid. Graf (II.) mentions that the *Mycoderma cerevisiæ* examined by him causes increased acidity in sterilised wort, the acid content rising in twenty-eight days from 5.7 c.c. to 8.5 c.c. in terms of decinormal baryta water. The Egyptian beverage "Leben," of the kephir type, contains a *Mycoderma lebenis*, discovered by Rist and Khoury (I.), that produces fixed acids and acetic acid. Will (XIII.) assumes that the production of acid is the cause of the decoloration of top-fermentation beer when contaminated by large quantities of the *Mycoderma* species examined by him.

§ 307. Destruction and Formation of other Organic Substances by Mycoderma.

In addition to the organic acids, the alcohol in beer, wine and fruit wines is subjected to complete destruction by *Mycoderma* fungi, being, on the one hand, oxidised to carbon dioxide and water, and on the other employed by the fungi as an organic structural material. This last circumstance was deduced by A. Schulz (I.), who expressed the view that "the film fungus is capable of itself forming its constituent organic compounds, and requires only ammonia and alcohol for that purpose." This opinion was confirmed by Meissner (XI.). Schulz employed in his experiments an artificial nutrient solution, containing magnesium sulphate and alcohol, in addition to potassium phosphate and lime. In one case ammonium nitrate was added to the solution, as the source of nitrogen, asparagin being used in another, and ammonium tartrate in the third. The fungus thrived in all three cases, and consumed a large portion of the alcohol, thus indicating the capacity of *Mycoderma* to supply their nitrogen requirements from ammonium nitrate and to utilise alcohol in the building up of their cell contents. It must, however, be mentioned that Schulz did not work with pure cultures, and that the race used by him apparently belonged to the genus *Pichia*. An important complement of these experiments was afforded by the investigations of Meissner, as also by the previous experiments of Winogradsky (XI.), which were afterwards confirmed by A. Kossowicz (III.)—*see* p. 209, vol. ii. These showed that pure cultures of *Mycoderma* also exert the activity first recognised by Schulz. In addition to the aforesaid nitrogenous food-stuffs, Meissner (VIII.) used ammonium phosphate and chloride, which he added to an artificial nutrient solution along with the necessary ash constituents. The energetic growth demonstrated that *Mycoderma* can be supplied in nitrogen by these substances as well, and consequently part of the alcohol in the nutrient solution is oxidised, a part being utilised in building up the cell body.

The sugars are attacked by the various *Mycoderma* species in a different manner and with varying intensity. H. van Laer (VIII.), for example, reported that dextrose is not attacked in Nägeli's nutrient solution by *Mycoderma;* whereas in yeast water it forms a better food-stuff than alcohol. Maltose and saccharose are attacked in very different degree; and in this case also it appears that the degradation depends entirely on the nature of the nurient material. "When different sources of carbon are added simultaneously to the nutrient solution, the one that is most readily assimilable is degraded first; and it is not until the alcohol has disappeared that the disaccharides are attacked." When maltose, saccharose and dextrose are added to the yeast

water, the last-named sugar is attacked first, the disaccharides not being degraded at the start. No invertase or maltase could be detected in the *Mycoderma* cells. The saccharose and maltose were oxidised direct to water and carbon dioxide. Finally, Meissner's *Mycoderma* species, which were grown on sterile grape juice, partly oxidised dextrose and lævulose, but also formed acids from these sugars. On artificial nutrient solutions, containing dextrose or saccharose as the sole organic substance, in addition to the requisite mineral food-stuffs, the *Mycoderma* oxidised the sugars, though utilising a portion of the same in the formation of new cells, and also forming acids therefrom. *Mycoderma lebenis*, Rist and Khoury (I.), grew admirably well in glucose and maltose, the glucose being transformed into acid and the alcohol oxidised.

According to further researches by Meissner (VII.), glycerin and tannin are also consumed by *Mycoderma* in the same way as alcohol, acids and sugars. A species of *Mycoderma* found by Eitner (I.) on mimosa bark also decomposed tannin.

Mycoderma species, however, in addition to decomposing glycerin, are also capable of forming the same from other organic substances. W. Seifert (II.) reports that his *Mycoderma vini I.* produced 0.152 per cent. of glycerin in Pasteur's nutrient solution by the end of 14 weeks, the whole of the alcohol having disappeared. *Mycoderma vini II.*, on the other hand, formed only 0.016 per cent. of glycerin, the alcohol diminishing concurrently from 4.8 to 4.1 per cent. by volume.

§ 308. Influence of other Factors on the Vitality of Mycoderma.

Highly interesting observations of the longevity of *Mycoderma* in wort cultures and in the dry state were made by Will (XIII.). Cultures that had been stored for $4\frac{1}{2}$ years in wort were found to contain living cells when re-inoculated in fresh wort, thus demonstrating the longevity of *Mycoderma* cells in wort. Will also showed that *Mycoderma* can survive for a long time in a dry state—at least two years in the case investigated by him. Low temperature favours the maintenance of vitality in the dry state, and the content of water in the dried cells probably also plays a principal part.

Will (XIII.) also investigated the power of old and young *Mycoderma* cells to resist the action of heat in liquids, the heating being applied in water, then in wort and finally in sauerkraut water. The cultures were of various ages. The duration of heating was half an hour, not reckoning the preliminary warming. The results showed definitely that the degree of resistance offered by the cells is influenced by the character of the substratum. For the species under examination, the critical temperature in

the heating in water test was found to be 50° C., whereas after heating for half an hour at 55° C., *Mycoderma* films were developed in all the check inoculations heated in wort. Older cultures proved better able to stand the heat than younger ones, the difference being 5° C. The formation of resting cells is not considered to account for this, Will's explanation being that the older and more strongly developed cells possess greater powers of resistance than such as are younger and more delicate. Seifert gives 0°–40° C. as the limits of temperature between which *Mycoderma* cells are capable of development, the presence of alcohol narrowing the range—for instance, down to between 2° and 33° C. in wine containing 8 per cent. of alcohol by volume. A continuous exposure of five minutes to a temperature of 60° C. is sufficient to destroy the vitality of *Mycoderma* in wine.

With regard to the influence of chemical agencies on the life of *Mycoderma* cells, the investigations of Holm and Jörgensen show that the development of the cells is accelerated by the addition of small quantities of fluorides. Siebel found that neither yeast, *Mycoderma* nor bacteria will develop in beer that has been treated with a solution of formalin (40 per cent. solution of formaldehyde) in the proportion of 1 : 10,000; whilst in solution of 1 : 50,000, yeast and *Mycoderma* were able to grow, but not bacteria. According to Seifert (II.), the various *Mycoderma* differ considerably in their power of resisting the influence of alcohol, development ceasing in presence of 13 per cent. of that substance by volume. In the case of furfurol, 0.5 per cent. was fatal to Will's *Mycoderma*. Sulphur dioxide is also known to be very poisonous to *Mycoderma*, and is therefore used in curing beverages that have been attacked by these organisms. Wesenberg (I.) investigated the action of antigermin, mikrosol, afral, mycelicide and antiformin on *Mycoderma cerevisiæ*, to ascertain the amount of a fatal dose. On immersion in a 2 per cent. solution of the antiseptic, the cultures, 4 days old, were killed by antiformin in ¼ hour, by antigermin in 1 hour, by mikrosol in 8 hours, and by mycelicide in 9 days, whilst afral merely retarded their growth. In a 1 per cent. solution of the poison the *Mycoderma* perished in ¼ hour with antiformin, 5 hours with antigermin, and 8 hours with mikrosol. The results were very different, however, when the antiseptics were added in definite quantity to beer wort, the growth of *Mycoderma* being then arrested by the following degrees of concentration: antigermin, 1 : 1000; mikrosol, 1 : 5000; antiformin, 1 : 20. The most powerful antiseptic, as regards the restriction of development, was undoubtedly antigermin, which, according to Wesenberg, is from 3 to 10 times as strong as mikrosol.

CHAPTER LXI.

SACCHAROMYCES APICULATUS.

By Prof. Dr. H. MÜLLER-THURGAU,
Director of the Swiss Experimental Institute for Fruit Wine and Horticulture, at Wädenswill near Zurich.

§ 309. History, Distribution and Morphology.

Ripe, soft fruit is often found to be infested with a budding fungus, to which the name of *Saccharomyces apiculatus* has been given on account of its tapered ends. This is the fungus described by Kützing under the name *Cryptococcus vini;* and we are indebted to Reess (I). for its closer examination and for the introduction of its present name into the literature. This worker found it, associated with various *Saccharomycetes*, in fermenting fruit juices and wine musts, but never succeeded in inducing it to form ascospores. The reason why Reess in this instance left out of consideration the characteristic on which he founded the genus *Saccharomyces*, namely, the production of endospores (*see* p. 274, vol. ii.), and nevertheless applied the generic name *Saccharomyces* to this species, was on account of "its known morphological peculiarities and its physiological behaviour as an alcoholic ferment," as also "in the expectation that its power of producing ascospores will be revealed by some other method of cultivation." It was not until quite recently, however, that such a method was discovered. Hansen (IX.) tried to find it in vain, as did also Klöcker (IV.) with reference to the expression of an adverse opinion by Beijerinck (XVIII.); and consequently the so-called *Saccharomyces apiculatus* had perforce to be excluded from the family of the *Saccharomycetes* for the time being. The only reason for retaining the name bestowed upon it by Reess was a disinclination to rechristen a well-known organism.

Engel (II.) claimed to have discovered an entirely new form of fructification of this fungus, analogous to that of *Protomyces* (*see* p. 108, vol. ii.), for which reason he conferred on it the new generic name, *Carpozyma*. No one else, however, not even E. C. Hansen (IX.)—who repeated Engel's experiment—has been able

to observe the form of fructification in question. The systematic position of the fungus therefore remained undetermined; and on this account the organism was separated from the rest of the *Saccharomycetes* in arranging the material for the present work.

Since that time, however, P. Lindner (XXXVIII.) has succeeded in obtaining monosporous cells in beer-wort cultures of an *Apiculatus* yeast isolated from Robinia blossoms. The drawing illustrating these cells is not very convincing, especially when it is remembered that, under certain conditions of environment, large, isolated fat globules, that can readily be mistaken for spores, are often formed in *Apiculatus* yeast. Even Reess (I.) depicted *Apiculatus* cells, each containing a round, highly refractive body resembling a spore, and expressly referred to the same as possible sporulation. Lindner's statement that only one spore occurs in each cell conflicts with an earlier communication by Beijerinck, according to whom the *Apiculatus* cells swell up to asci containing 4–6 ascospores in each. Neither Beijerinck nor Lindner succeeded in prevailing on the "spores" to germinate. The last named himself mentions this deficiency, and points out that some such preparation is necessary to ensure the germination of *Apiculatus* yeast as is the case with the seeds of the carob-tree, which have first to be passed through the alimentary canal of some animal. Bearing this idea in mind, A. Röhling (I.) cultivated vigorous *Apiculatus* yeast for twenty-four hours in sterilised grape juice and then used it for gypsum-block cultures, in which a "body resembling a spore" made its appearance in many of the cells by the tenth day (temperature not stated). The germination of a spore was thereafter observed in a decoction of horse-dung, mixed with 5 per cent. of grape sugar. This experiment needs repetition, from the circumstance that only one cell gave the said result, and also on account of the manner in which germination is said to have proceeded. The writer has tested four different *Apiculatus* races exactly in the same way as described by Röhling, but failed to obtain spores.

Lindner and Röhling, on the basis of their researches, concluded that *Apiculatus* yeast does sporulate, and therefore really belongs to the genus *Saccharomyces* of Reess and Hansen, but as it constitutes a peculiar type, Lindner (XXXII.) considers that a new genus should be established, for which he proposes the name *Hansenia* (*see* p. 284, vol. ii.).

The specific name, *apiculatus*, well expresses the characteristic that distinguishes this budding fungus from all others. The (otherwise ovoid) cells are pointed at both ends like a lemon (*see* Fig. 199), which form, however, predominates only during the first stage of development in a nutrient solution, whereas later on, when the conditions of nutriment are less favourable, a considerably larger number of ovoid cells make their appearance, and the lemon shape is less noticeable.

As already stated, reproduction in this fungus is effected entirely (or mainly, if sporulation indeed takes place) by budding. The progress of this operation was described by Reess and Engel, and it was more closely investigated by Hansen, whose observations show that the budding of a lemon-shaped cell proceeds in the following manner (*see* Fig. 199). The lower pointed extremity (*a*) of the cell swells up (*a'*) and grows there until it attains normal dimensions (*a''*). The two cells then separate, each of them acquiring the hitherto lacking second tip. From *b-b''* in the Fig. it will be seen that a bud can be put forth at

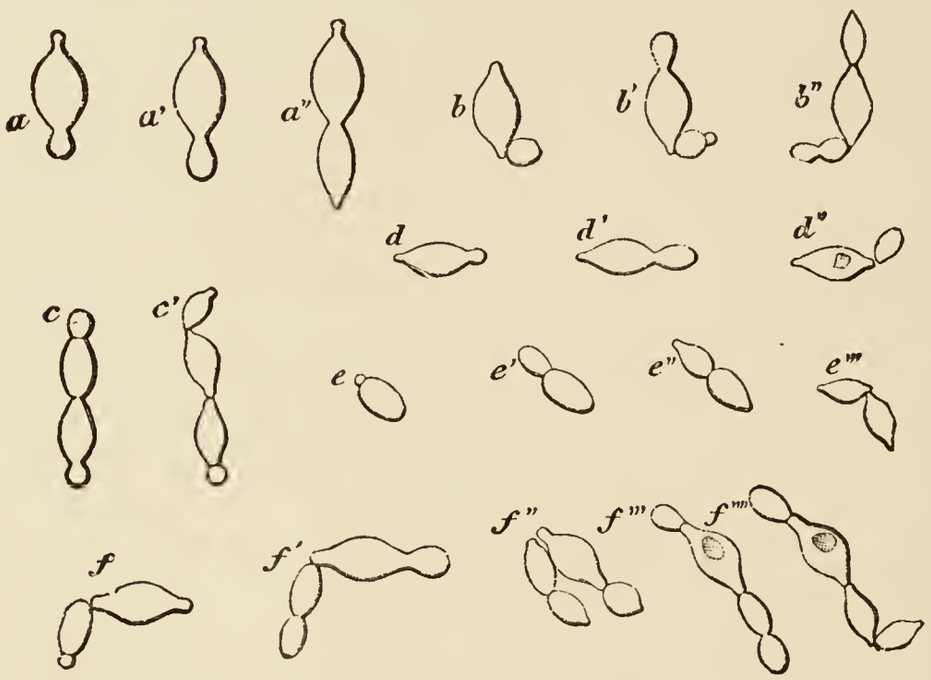


Fig. 199.—Saccharomyces apiculatus.
Typical Form. Reproduction of the cells by budding. Magn. about 950.
(*After Hansen.*)

each end simultaneously. Figs. *c-c'* represent in each case an aggregation of four lemon-shaped cells. The question whether a lemon-shaped cell can be produced from an ovoid one by budding, may be answered in the affirmative. In this case the parent cell (*e*) puts forth a bud at the one extremity, whilst the other becomes pointed (*e'*), the daughter cell rapidly increases in size (*e''*), then separates, and, together with the parent cell, acquires a point at the second extremity. R. Meissner (IX.) regards the oval shape as the normal form, and the apices of the pointed cells as incipient buds. This, however, can hardly be accepted, since it evolves the assumption that the majority of cells cease growing just when they have begun to reach the budding stage.

A peculiar shape is exhibited by the *Sacch. apiculatus*, var. *parasiticus*, Lindner, discovered by Lindner (XXXIX.), and infesting cochineal insects. In most of the cells, one end tapers out to a long point, by means of which the eggs are infected in

the body of the parent insect, and propagation is ensured in the offspring.

In addition to the oval and lemon-shaped cells already described, this fungus produces sausage-shaped growths, as shown

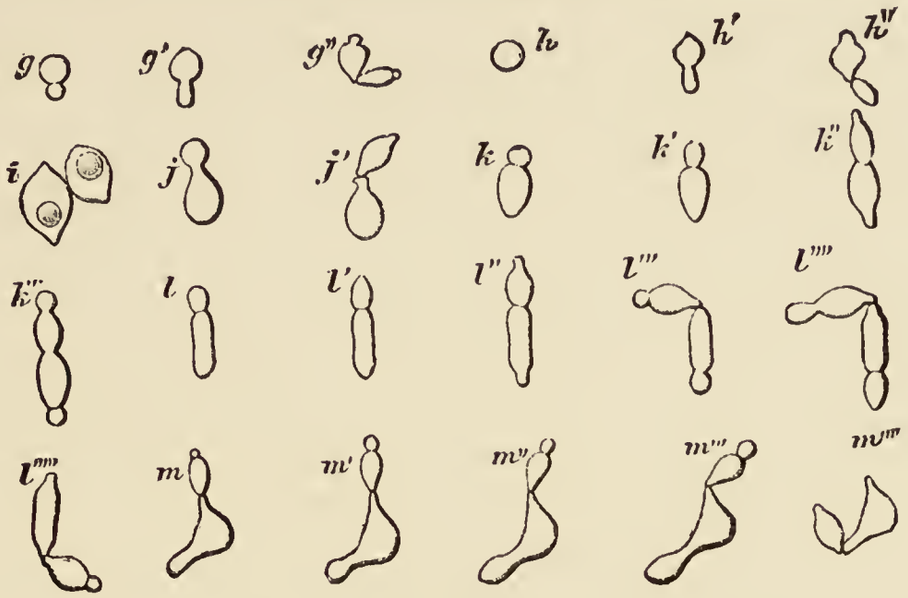


FIG. 200.—Saccharomyces apiculatus.
Abnormal cell forms. Magn. about 950. (*After Hansen.*)

in Fig. 200; but the external conditions under which these are formed have still to be elucidated, Reess's statement that they appear towards the close of fermentation needing confirmation, since he did not work with pure cultures. No mycelial growth has yet been observed, nor do the cells collect in long aggregations, the daughter cells separating quickly from the parent cells after bending in a peculiar way—a phenomenon that was first described by REESS (I.).

FIG. 201.—Cells of Sacch. apiculatus and Sacch. cerevisiæ.
Magn. about 950. (*After Hansen.*)

As in the case of the true *Saccharomycetes*, the dimensions of the cells vary considerably, even in the same culture, some of them measuring 2 μ, whilst others are four times that length. In the majority of instances the length is 7 μ, so that the cells are far smaller than those of beer yeasts, as can be seen from Fig. 201, where a mixture of *Sacch. cerevisiæ* and *Sacch. apiculatus* is depicted at a magnification of about 950. The first-named can be easily recognised from the greater dimensions and oval shape of the cells. On the other hand, the *Apiculatus* cells exhibit a feature that is often observed in this fungus, especially when in

an unfavourable environment, namely, a large vacuole. Nothing has been published hitherto with regard to any further morphological peculiarities of the structure of the cell membrane or contents differentiating the *Apiculatus* yeasts from the other *Saccharomycetes*.

If plate cultures of a mixture of *Sacch. apiculatus* and *Sacch. ellipsoideus* be started in must gelatin, the colonies of the former usually make their appearance only after those of *Sacch. ellipsoideus* have already attained considerable size; and they remain smaller than the latter throughout. This difference is due, not to any slower rate of reproduction on the part of the first-named yeast, but chiefly to the smaller size of the individual cells, which diminish as the colony increases. The gelatin surrounding the *Apiculatus* colonies is liquefied at an early stage, and the latter seem also to excrete substances that restrict the further reproduction of the yeast cells. A similar result is observed in the development of streak cultures of *Apiculatus* yeasts, so that, for the most part, these exhibit merely a delicate, filmy appearance, at a time when the yeast streak in cultures of *Sacch. ellipsoideus* of the same age has already grown to a thick white strip. Even after a considerable time the former do not usually show any vigorous development; and this applies also to the giant colonies, which fail to exhibit any decided, special morphological characteristics, and quickly sink in the liquefied gelatin. Further particulars on this point will be found in the next paragraph.

§ 310. Racial Differences.

Sacch. apiculatus, var. *parasiticus* (*see* p. 424, vol. ii.), must be considered as a separate variety from the *Apiculatus* yeast collaborating in the fermentation of beer and wine, since it differs from these by its strictly parasitic habit, even more than by the shape of the cells. This variety cannot be grown either in fruit juices or in artificial nutrient media. Moreover, the *Apiculatus* yeasts indigenous on fruit and playing a regular part in vinous fermentation belong to different races and are not of uniform stock, a discovery for which we are indebted to K. Amthor (I.) in 1888. Owing to the absence of sporulation and to the great morphological variability of the cells, characteristics of a chemico-physiological nature have to be mainly relied upon in demonstrating the difference, chief among them being the kind and quantity of the metabolic products furnished by cultures of different origin when grown under identical conditions. Thus, Amthor was able to prove the racial divergence of two cultures of *Sacch. apiculatus*, one of which was isolated from red Heilbronn must, and the other from a white wine must from Rhenish Hesse. Whereas the former race produced 3.65 per cent. (by weight) of alcohol and 365 mgrms. of glycerin per 100 c.c. of the

grape must it was employed to ferment, the other race, under the same conditions, produced only 2.58 per cent. of alcohol and 311 mgrms. of glycerin. On the other hand, it formed a larger quantity of volatile acids, namely, 127 mgrms. per 100 c.c. as compared with 103 mgrms. in the other case. Müller-Thurgau (XXIV.) tested seven different races of *Sacch. apiculatus*, isolated by him, in grape juice, as well as in pear juice and currant juice, and found that, in the first-named medium, the production of alcohol varied between 2.5 and 3.8 per cent. by weight. The same serial order was obtained, on the basis of the rapidity of fermentation, in all three culture media; and in every instance a high proportion of volatile acids was obtained—viz., with race 8, for instance, in grape wine 93 mgrms. per 100 c.c., and in perry 123 mgrms. (calculated as acetic acid), whereas the elliptical yeast Steinberg 1 produced only 53 and 47 mgrms. respectively in the same media. Schander (II.), who afterwards compared pure cultures of twenty-four *Apiculatus* yeasts, also observed morphological differences in the cells. In some races the cells are short, thick, and of the typical lemon shape, whereas in others they are thin and elongated, the lemon shape being less noticeable. Different races are also distinguishable by the cell dimensions. Whilst old cultures of *Apiculatus* yeasts do not generally produce a film on the surface of fruit juice or grape juice, a growth of this kind, though slight, could be detected in certain races. Racial differences were also exhibited in the form of the streak cultures and giant colonies, though not to any very decided extent. More pronounced differences became apparent in the quantity and character of the sediment and in the percentage of the fermented wines, the alcohol content being 1.44 grms. per 100 c.c. in the case of the weakest race, and 4.53 mgrms. with the strongest. Another point of racial difference consisted in the consumption of acid, and still others will be referred to in the two following paragraphs.

§ 311. Conditions of Growth and Nutrition.

In liquid media, such as beer wort and fruit juices, the reproduction of *Apiculatus* yeasts proceeds rapidly in the primary stage of fermentation, in many instances more quickly than with the ferments in technical use; but this superiority soon disappears, owing to the susceptibility of *Apiculatus* yeasts to alcohol. It is most particularly noticeable on comparing the rapidity of reproduction of *Apiculatus* and *Ellipsoideus* races in very dilute must, where the amount of alcohol formed is insufficent to retard growth, or when only the initial reproduction—up to the point when 1 per cent. of alcohol has been formed—is taken into consideration in ordinary must. No precise observations of this kind, or with regard to the amount of alcohol sufficing to stop growth under

various other conditions of enviroment, have, however, been published. An idea of the difficulties encountered in determinations of this kind may be gathered from § 268. The rapid reproduction of *Sacch. apiculatus* in comparison with low-fermentation beer yeast is shown by certain researches performed by E. Hansen (IX.); and these also show how one of these budding fungi can retard the growth of the other when present simultaneously in the same nutrient solution. Thus, for instance, three Pasteur flasks were charged with beer wort; (*a*) being sown with 22 cells of *Sacch. cerevisiæ* per unit volume, (*b*) being sown with the same number, along with 19 *Apiculatus* cells, and (*c*) with 20 cells of this latter only. At the end of 13 days (at 8°–10° C.) the number of cells per unit volume amounted to: (*a*) 242 of *Sacch. cerevisiæ* (the alcohol content being 6 per cent. by volume); (*b*) 240 of *Sacch. cerevisiæ* and 45 of *Sacch. apic.* (alcohol 6 per cent.) (*c*) 791 of *Sacch. apic.* (alcohol 0.5 per cent.). Hence, in the pure culture, *Sacch. apiculatus* reproduced more than three times as much as *Sacch. cerevisiæ*, but was greatly retarded by the latter in the mixed culture. These figures of course do not afford any criterion of the rapidity of growth, since, for a given increase of growth, more than three *Apiculatus* cells must, as a rule, be formed for each one of the far larger cells of the beer yeast. This harmonises also with the circumstance that, in spite of the larger number of cells, the sediment in the fruit juices fermented by *Sacch. apiculatus* is much smaller, in weight and volume, than that in the juices fermented by *Sacch. ellipsoideus*. Consequently, in order to decide whether the protoplasm of *Apiculatus* yeast has a lower fermentative energy than that of *Sacch. cerevisiæ* or *Sacch. ellipsoideus*, the activity per unit weight of the yeasts must be compared, and not that of an equal number of cells.

The influence of alcohol on the growth of several *Apiculatus* races was investigated by Röhling (I). In harmony with the general low powers of resistance of these yeasts against injurious influences, a small percentage of alcohol in the culture liquid is sufficient to restrict reproduction considerably. When the number of yeast cells per unit volume of grape must, previous to fermentation, was = 1, it had increased to 514 in one of the races at the close of fermentation without any addition of alcohol; but, when the must contained 2.86 per cent. of alcohol, by volume, at the outset, the final number was only 192, and when the initial addition of alcohol was 4.62 per cent., only 88 cells were obtained.

The particularly frequent occurrence of *Apiculatus* yeast on berries might lead to the supposition that the juice of these fruits is specially suitable for the growth of the fungus; but comparative experiments have shown that such is not the case. According to Müller-Thurgau (II.), it matters little to the development of *Sacch. apiculatus* whether malic acid or tartaric acid preponderates in the fruit juice.

On the other hand, the growth of this yeast is stimulated by the admission of free oxygen (*see* p. 252, vol. ii.). This is apparent from the behaviour of stab cultures in must gelatin, where vigorous reproduction of the yeast cells is found to be confined to the upper portion of the stab. More accurate information is afforded by an experiment of Röhling's (I.), in which five different races of *Apiculatus* yeast were sown in grape juice, one portion of the latter being aerated in the fermentation vessels, the other not. A difference in favour of the aerated yeast was already apparent on the second day; and at the close of fermentation the number of cells in the aerated samples had increased from 3.3 to 9.3 times as great (according to the race) as those in the unaerated samples. This appears to harmonise with the observation that the percentage of *Apiculatus* cells is occasionally much higher in the superficial strata, and especially the head, of red wine must fermenting in open vessels than it is in the lower layers that are poor in oxygen. This need of oxygen is, of course, satisfied when the fungus is grown on the surface of solid nutrient substrata; and it is not impossible that *Sacch. apiculatus* should gain the upper hand in consequence of these specially favourable conditions. Considerable accumulations of *Apiculatus* yeast, practically in the state of pure cultures, are not infrequently found in the wounds of burst or gnawed grapes, or in the tunnels eaten out in core fruit by apple-roller larvæ.

The special conditions of nutrition of *Apiculatus* yeasts have not been closely investigated to any extent. Its requirements in respect of carbon are chiefly fulfilled at the expense of the hexoses in the nutrient medium, but the fungus is incapable of fermenting disaccharides, such as saccharose, or of utilising them for structural purposes. More on this point will be found in the following paragraph. The circumstance that the non-volatile organic acids are consumed to a larger extent in cultures of *Apiculatus* yeast in fruit juices than is the case with *Sacch. ellipsoideus*, for example, induces the idea that these acids may also be utilised as a source of carbon for building up the cells; but, on the other hand, it is equally possible that the tartaric and malic acids in the instances observed may have been decomposed by fermentation rather than used for the purpose in question. In respect of nitrogen assimilation, *Apiculatus* yeasts do not seem to differ from others; at least no statements have been published on this point.

Sacch. apiculatus appears to be more susceptible to injurious influences than the beery yeasts and the races of *Sacch. ellipsoideus* occurring in wines. Its high susceptibility toward alcohol has already been mentioned (p. 417, vol. ii.); but though a small percentage of alcohol is sufficient to retard growth, and apparently a small additional quantity will arrest fermentation, a considerably higher alcohol content would seem to be necessary in order to kill the cells. In this connection, however, considerable differences are

exhibited by the various races. Apart from this, *Sacch. apiculatus* is much more sensitive than *Sacch. ellipsoideus*, the cells of the former being generally found dead in the deposit from fermented wine. The fact that individual cells—probably of a more vigorous race—occasionally exhibit considerable longevity, was proved by a discovery of R. Braun (II.), who found living cells of *Sacch. apiculatus* in beer containing about 8 per cent. of alcohol after at least five years. Müller-Thurgau (XXIV.) also found, in his attempts to discover suitable methods of obtaining a purer fermentation of fruit and grape wines, that *Apiculatus* yeasts are killed by a quantity of sulphur dioxide (in the must) that is innocuous to the elliptical wine yeasts. Both Hansen (LVII.) and Kayser (III.) mention *Sacch. apiculatus* as particularly susceptible to the influence of desiccation; though, on the other hand, according to Will (XXXIV.) the various races differ in this respect. A. Berlese (II.) regards the fungus as offering a powerful resistance to the action of direct sunlight. The resistance of *Apiculatus* yeast to high temperatures was examined by Müller-Thurgau (XXV.), who found, moreover, that the various races differ in this particular. One of them proved to be far more susceptible to this influence than the others under examination, being killed by an exposure of ten minutes to 50° C. in grape juice, whereas the others were able to withstand ten minutes at 55° C., and thus closely approximated to the elliptical wine yeasts in this respect. The point whether the growth of the races of *Sacch. apiculatus* is dependent on temperature in a different manner from that of the true wine yeasts has not yet been investigated, though the matter is one of some importance to the conduct of the fermentation (*see also* p. 254, vol. ii.).

§ 312. Fermentation Phenomena of Apiculatus Yeast.

The fermentation set up by *Saccharomyces apiculatus* is invariably of the bottom fermentation type. In fruit and grape juices it is often confined to a merely slight turbidity, owing to the low general fermentative activity of this yeast, and also—as pointed out by Reess (I.)—because the cells being detached and not aggregated, do not offer suitable points of attachment to the bubbles of carbon dioxide.

The various races exhibit a certain uniformity in point of fermentation phenomena and metabolism, though they differ considerably from the true beer and wine yeasts. In this connection, however, the first-named have not been examined very closely. The races described by Hansen set up a vigorous, though not very extensive, fermentation in a nutrient solution containing dextrose (*d*-glucose), the resulting "head" being composed of numerous fine bubbles, and not attaining the same dimensions as that thrown up by *Sacch. cerevisiæ*, for instance. The same behaviour toward dextrose is exhibited by Amthor's

races, and that of L. Boutroux (V.). According to M Cremer (I.), lævulose or *d*-fructose is also fermented by *Sacch. apiculatus ;* and the same applies also to mannose or seminose. On the other hand, F. Voit (I.), E. Fischer and H. Thierfelder (I.) are unanimous in stating that a fourth hexose, *d*-galactose, is unaffected by this fungus. So far as our knowledge extends, not a single member of the disaccharide group is fermented by *Apiculatus ;* a fact demonstrated by E. C. Hansen in respect of saccharose and lactose, and by this authority and Amthor (I.) in respect of maltose. According to these workers, *Sacch. apiculatus* is capable of forming only small quantities of alcohol in beer worts, namely, from the hexoses contained therein.

The fact that this budding fungus ferments hexoses, but not disaccharides, justifies the assumption that it cannot secrete inverting enzymes like invertase and maltase; and this has been confirmed, so far as invertase is concerned, by the experiments of Hansen (IX.). On the other hand, when the disaccharides are inverted—for instance, by heating with a little acid—they come within the sphere of action of this fungus, as is also the case when the latter is associated in the nutrient liquid with a budding fungus that secretes invertase.

In order to utilise the aforesaid behaviour of *Sacch. apiculatus* toward sugars, for the purposes of the analytical chemist, K. Amthor (I.) proposed to employ it in cases where small quantities of dextrose have to be determined in presence of disaccharides, *e.g.*, in beer wort, a definite quantity of the previously boiled sample being inoculated with *Apiculatus* yeast. This latter consumes only the dextrose, the percentage of which can then be estimated by the amount of alcohol (or carbon dioxide) formed. A modification of this method was proposed by A. Bau (I.), who defended it in subsequent papers (II., V. and IV) from certain objections urged against it by H. Elion (II). Nevertheless, in view of the susceptibility of this ferment to alcohol, he was constrained to admit that the method can only be used when the dextrose content of the sample is low, and that, even in such event, one cannot be certain whether the whole has been fermented. Even with this limitation, however, the method is useless to the practical analyst, owing to the sluggish fermentation of the hexoses. This is evident from the statements of Amthor (II.), who inoculated sterile wort with *Sacch. apiculatus* and found the production of alcohol amounted to 0.66 per cent. by volume at the end of twenty-seven days, 0.79 per cent. after a further fifty-four days, 1.2 per cent. nine months later, and 1.5 per cent. after a further nine months, *i.e.*, twenty-one months in all. Hence, even assuming—as has not yet been proved—that all the hexose is eventually fermented, the method must be regarded as far too slow for practical purposes. The trouble bestowed on its elaboration, however, has not been wasted, since it affords physiological

confirmation of the previous discovery of certain chemists, *e.g.*, H. Bungener and L. Weibel (II.), that beer worts contain a larger proportion of fermentable sugars, other than maltose, than had been hitherto supposed, amounting probably to one-fourth or one-third of the total sugar.

On the basis of experiments, which we cannot go into more fully here, E. Dubourg (I.) formed the conclusion that yeasts can be induced, by habituation, to utilise certain sugars that they are normally incapable of attacking. A yeast, for instance, that is unable to invert saccharose may acquire this capacity by being grown in a mixture of glucose and saccharose, and then transferred to a solution of saccharose containing suitable yeast foods. Unfortunately, the yeast was not properly identified in the report. A. Klöcker (IV.) repeated Dubourg's experiment with a number of yeasts characterised by the peculiarity in question, among them being the one with which we are more particularly concerned at present, namely, *Sacch. apiculatus* (*see* p. 259, vol. ii.). The results, however, were entirely negative, our budding fungus being unable to ferment saccharose after this preparatory treatment and therefore—contrary to Dubourg's hypothesis—incapable of secreting invertase.

Despite the initially rapid reproduction of *Apiculatus* yeasts in grape and fruit juices, the fermentation they induce produces but little alcohol, proceeds slowly, and therefore usually extends over a considerable period of time. This is evident, for example, from the series of experiments recounted by Müller-Thurgau (XIV.). At 14° C., the quantity of carbon dioxide liberated amounted, during the first ten days of fermentation in grape juice, to 6.8 grms. per litre; in 20 days, 9.4 grms.; in 40 days, 12 grms.; in 80 days, 14.6 grms.; in 100 days, 15 grms.; in 130 days, 16.8 grms., and in 205 days, 18 grms.; consequently the *Apiculatus* races are all weak ferments, though differing considerably in relative degree. Under ordinary conditions of fermentation, the quantity of alcohol finally produced (*see* p. 426, vol. ii.) by the races hitherto described varies between 2.5 and 4.5 per cent. by weight; though two of the races recently tested by the writer formed 6 per cent. of alcohol from grape juice. The final content of alcohol also differs correspondingly in pear juice and grape juice, even when the same yeast is employed in both cases, Müller-Thurgau having found that *Apiculatus* race 8, for instance, furnishes 2.8 per cent. of alcohol in grape juice, as compared with 3.5 per cent. in pear juice. It may be assumed that with this budding fungus, as with others, the activity as well as the growth of the individual cells will be influenced by the conditions of nutrition and general environment, the relative speed of fermentation and the attenuation obtained with liquids of different constitution being affected both by the number of active yeast cells and the fermentative activity of the individual cells. It is,

however, not impossible that, in certain circumstances, only one or the other of these two factors will come into play; but at present no systematic investigations have been carried out to decide this question.

It has already been mentioned (p. 429, vol. ii.) that the reproduction of *Apiculatus* yeasts is stimulated by the admission of free oxygen or air; and all that now remains is to deal with the influence of the oxygen supply on the fermentation process. Röhling (I.), who experimented with several races, found them to be powerfully stimulated by the admission of oxygen, and enabled to produce much larger quantities of alcohol in grape juice. In the absence of a supply of oxygen the final percentage of alcohol varied between 2.27 and 3.03 per cent. by weight, rising to between 5.01 and 5.76 per cent. when oxygen was supplied. Since only 0.2 per cent. of unfermented sugar remained in the sample furnishing the maximum quantity of alcohol, the yeast in question would probably have been able to produce still more alcohol in a stronger juice. These experiments show that the production of alcohol was doubled on the average by the provision of a supply of oxygen, the vital energy and power of resisting alcohol being considerably increased, to a greater extent than had hitherto been observed with elliptical wine yeasts. When oxygen is supplied, the fermentation proceeds more rapidly from the start, and, despite the higher final content of alcohol, is terminated sooner than in the check experiment without oxygen.

Certain chemical substances met with in vinous fermentation, such as acetic acid, sulphur dioxide, and tannin (*see* pp. 246–248, vol. ii.), restrict the growth of *Apiculatus* yeasts, and also probably exert a direct lowering influence on the fermentative energy, in the same way as they do in respect of beer yeasts and wine yeasts. According to the experimental results communicated by Röhling (I.), even 0.1 per cent. of acetic acid exerts a decided influence on fermentation; 0.5 per cent. restricts the fermentation to about one-third the normal, and 1 per cent. practically arrests it altogether. In the case of sulphur dioxide, as little as 0.025 per cent. suffices to stop the fermentative activity of *Sacch. apiculatus* almost completely; and, indeed, the earlier discoveries of Müller-Thurgau (XXIV.) show that even 65 mgrms. of this substance per litre, *i.e.*, 0.0065 per cent., will bring about the same result. Tannin is less energetic, no considerable restriction of fermentation being observed until the tannin content reaches 0.5 per cent.

During the fermentation of fruit and grape juices by *Apiculatus* yeasts, the non-volatile or fixed organic acids, *i.e.*, tartaric acid and malic acid, are also involved in the process of metabolism (*see* p. 205, vol. ii.). This was demonstrated by Müller-Thurgau (XIV.), in whose experiments the races under investigation reduced the amount of non-volatile acids in grape juice from 0.883 to 0.669 per

cent.—that is to say, by about 24 per cent. of the original quantity—and in pear juice from 0.450 to 0.265 per cent., a reduction of about 40 per cent. (In the latter case, also, more alcohol was produced.) Elliptical yeasts, employed with the same juices for the sake of comparison, consumed a smaller proportion of fixed acids; and the greater activity of *Sacch. apiculatus* toward these acids was also apparent in mixed cultures. Nevertheless, since various acids are present in fruit and grape juices, and acids (*e.g.*, succinic acid) are also formed during fermentation, these experiments, though of great technical interest, are incapable of affording a complete solution of the behaviour of *Apiculatus* yeasts toward acids. This is more likely to be obtained by fermentation experiments with liquids containing only a single organic acid, and of simple, known chemical constitution. Schukow (II.) showed, in a single experiment, that *Apiculatus* consumed a larger quantity of acid than the beer and wine yeasts, when grown in an artificial nutrient solution containing both tartaric acid and malic acid. Additional researches in the same direction would furnish valuable results. Of late the behaviour of various fungi toward lactic acid has been investigated by Meissner (X.), *Sacch. apiculatus* being also borne in mind. These experiments, however, were performed with artificial solutions, lacking fermentable sugars, so that, possibly, the behaviour of the organisms would be different from that in fermenting liquids more favourable to development. Whereas, in the solution containing mineral substances, peptone and lactic acid, various species of wine yeasts decomposed 70 per cent. and more of the lactic acid, the diminution produced by one of the *Apiculatus* yeasts—which exhibited only very slight reproduction—was only 0.018 per cent., or 1.5 per cent. of the initial quantity. Two other races proved incapable of growing at all in the solution. In fermented wine the decrease of lactic acid under the action of an *Apiculatus* yeast was less, on the average, than with the elliptical yeasts, no doubt on account of the greater restrictive influence of alcohol on the former.

The consumption of acid may also be accompanied by a production of acid (both volatile and fixed), in which the *Apiculatus* yeasts likewise play some part. Of the fixed acids, succinic acid has long been known as a fermentation product, and is also produced by *Sacch. apiculatus*. In the case of the two races examined by him, Amthor furnished definite proof that they produce considerable quantities of fixed acids during fermentation, the one forming 0.37 per cent. (calculated as tartaric acid), or three times as much as Pasteur found in fermentation with ordinary yeast. To this must also be added the amount eliminated by decomposition processes. Meissner has also shown that *Sacch. apiculatus* can produce lactic acid from succinic, malic, and citric acids, in which respect it is but little inferior to the wine yeasts. Nevertheless,

since the acidity of the liquid is diminished, rather than increased, by the conversion of the aforesaid organic acids into lactic acid, this cannot explain the considerable increase of the total fixed acids reported by Amthor. Unless one is disposed to assume that the newly formed acid is succinic acid exclusively, it must be concluded that fixed acids of some other kind are also formed during fermentation by *Apiculatus* yeasts.

The well-defined power of *Sacch. apiculatus* of producing volatile acids in large quantities has already been mentioned on p. 427, vol.ii., together with the figures of production given by various workers. In the opinion of Müller-Thurgau (XI.), these volatile acids constitute a weapon by which this fungus is able to restrict the development of other yeasts. The nature of these volatile acids, however, cannot yet be precisely stated. In any case, as Amthor (I.) showed, by the preparation of the silver salt, they consist only partly of acetic acid; and moreover, according to Müller-Thurgau (XVII.), the wines do not exhibit the characteristic flavour of acetic acid. In experiments with a sterilised nutrient solution containing ammonia salts, dextrose, and invert sugar, and inoculated with *Sacch. apiculatus*, Amthor (II.) detected in the distillate from the fermented liquid both acetic acid and formic acid, together with traces of an acid boiling at 120°–125° C. The fixed acids consisted of succinic acid and lactic acid. The first-named probably combine in part with the alcohol to form esters, which do not taste or smell sour in the liquid, but are decomposed in distillation so that the acid is available for determination. These esters, which *Sacch. apiculatus* is capable of producing to a larger extent than other yeasts, are the chief cause of the fruity flavour exhibited by the musts, worts, and fruit juices fermented by means of this fungus. According to the experiments of W. Seifert (IV.), *Sacch. apiculatus* produced a larger quantity of volatile esters (together with 0.064 per cent. of volatile acids) from one and the same grape must than was furnished by six pure-culture yeasts. The ester content, expressed in c.c. of decinormal alkali per 100 c.c. of wine, was 10.8; whilst in the case of the other yeasts it ranged between 1.32 and 4.4. P. Lindner (VII.) observed an extensive production of fruity ethers by an *Apiculatus* yeast, more particularly when the liquid under fermentation was vigorously aerated and contained a sufficiency of dextrose. In addition to the sweet-smelling esters, *Apiculatus* yeasts may also produce other kinds of odorous and flavouring substances under certain conditions. In fact, H. Will (V.) succeeded in effecting a means of differentiating the various species isolated by him, as pure cultures, from wort, beer, grapes, &c., according to the character of the smell they produce. One series is distinguished by the mouldy, fusty smell of the cultures, whilst another exhibits a very decided bouquet (fruity smell) resembling amyl ether. In saccharine yeast water they

produce acetic ether, more particularly when the nutrient solution is aerated continuously. According to Schander (III.), some races of *Sacch. apiculatus* are to be numbered among the yeasts that produce sulphuretted hydrogen, and its accompanying disagreeable taste, in wine.

As another metabolic peculiarity may be mentioned Henneberg's (VI.) discovery that the cells of this yeast are only able to store up small quantities of glycogen. On the other hand, the abundant secretion of proteolytic enzymes is demonstrated by the rapid liquefaction of gelatin in cultures of various *Apiculatus* yeasts. Finally, Amthor (I.) mentions, as a special property of these budding fungi, that it exerts a powerful decolorising action when employed to ferment wine must, which observation was confirmed by Schander (II.) in the case of the races examined by him.

§ 313. The Importance of Saccharomyces apiculatus in Wine-making.

Reess (I.) found *Sacch. apiculatus* to be a constant member of the abundant fungoid flora on ripe grapes and fruit; and this discovery has since been confirmed by Pasteur (XXVIII.) and numerous other workers. Hansen (IX.), in his researches on the life cycle of yeasts, showed whence and how this yeast finds its way on to grapes, the easily recognisable cell form of *Sacch. apiculatus* being specially adapted for this purpose. The particulars have already been given in § 271. Cells of *Sacch. apiculatus* are only found occasionally on unripe fruit, on which they soon perish, owing to the unfavourable conditions, especially drought; but as the fruit ripens, the conditions of existence become more suitable for the fungus. It is first found on cherries, these ripening earlier than any other fruit (in Central Europe); soon afterwards it is met with on gooseberries and currants, then on plums, and, finally, on grapes. Strawberries, raspberries, and sorb apples come in in their proper turn. Where two kinds of fruit that ripen at different times are met with, even side by side, such as currants and grapes, only the earlier one will be found infested with the fungus at first, the other exhibiting none at all or only a few isolated cells. Thus, in a vineyard at Geisenheim-on-Rhine, where a plantation of early Burgundy grapes lay side by side with one of late grapes of the same class, Müller-Thurgau (XXVI.) observed on August 23 large numbers of yeast cells on the earlier grapes, that were just ripe, whereas none could be found on the adjoining (unripe) late grapes. Apart from the fact that the wind-borne yeast cells found a more suitable habitat on the ripe grapes, and were able to reproduce extensively thereon, especially when they could gain access to the juice through any aperture, it is certain that—as discovered by Müller-Thurgau—a considerable part is played by insects, which prefer ripe fruit, and,

in visiting the latter, transfer yeasts from wounded berries to sound ripe ones, which latter they also wound in many cases, and thereby infect. It was at one time considered that the transference of budding fungi in this way was chiefly effected by the legs, maxillary organs, and the hairy parts of the insects; but A. Berlese (II.) showed that yeasts—especially *Sacch. apiculatus*—are present in the alimentary canal of various insects, and also reproduce abundantly therein, so that the organisms are transferred to the fruit in the excrement. In fact, Berlese regards this as one of the most important means of disseminating the fungus in question.

Considerable divergence exists in respect of the numerical ratio in which the various organisms occur on the ripe fruit, and subsequently in the expressed juice therefrom; and as this greatly influences the progress of fermentation and the character of the fermented product, it is desirable that the conditions determining this ratio should be more closely examined. Thus, in view of the great influence exerted by the *Apiculatus* yeasts, it is highly desirable to trace out the factors by virtue of which the fruits used in wine-making are infested one year by a relatively large number of these fungi, whilst another year there are comparatively few; and also why the ratio between elliptical and apiculate yeasts is favourable at one time and unfavourable at another, according to the external conditions of environment. At present we know very little about this matter.

Reess, who verified the frequent occurrence of *Sacch. apiculatus* on grapes, found experimentally that in many cases this fungus assumes the duty of starting the primary fermentation of wine must, being afterwards displaced by *Sacch. ellipsoideus*, which is then in a state of vigorous growth and carries the fermentation to completion. The fungoid flora of a large number of grapes from different districts was investigated by Martinand and Rietsch (II.). In one case they found *Sacch. apiculatus* exclusively on eight varieties of grapes; and in three others 20 per cent. of *Sacch. ellipsoideus*, with 80 per cent. of *Sacch. apiculatus* and *Mycoderma* In another case they allowed crushed grapes to ferment, and found only mould fungi and *Sacch. apiculatus;* and it was only after repeated experiments that a few colonies of *Sacch. ellipsoideus* could be detected. Musts obtained from Marcobrunner grapes contained 80 per cent. of *Sacch. apiculatus*, those from Johannisberg grapes containing 25 per cent., whilst in a few others the proportion was lower. Of course it must not be forgotten that the constitution of the fungoid flora on packed grapes is liable to alteration if transmitted to a distance. Müller-Thurgau (XXX.), however, has found that fresh grapes are frequently infested with very large quantities of *Apiculatus* yeasts, accompanied by only small numbers of elliptical yeasts. Thus, *Sacch. apiculatus* alone was found on grapes from Bernegg (Rhine

valley) and Winterthur; and to the extent of 93 per cent. on grapes from a vineyard at Brestenberg (Aargau); though in other instances the proportions were more in favour of *Sacch. ellipsoideus*. The above figures refer to single specimens of grapes; but of course the fungoid flora may vary on grapes from adjoining stocks or even from the same vine, so that there is no instance on record of wine must being imperfectly fermented owing to the absence of any other yeasts than *Sacch. apiculatus*. Such cases, however, have been known with currant wine; though why they should occur with this class of wine and not with grape wine is unknown. *Sacch. apiculatus* is also frequently present in abundance in the fermentation of cider and perry, at least at the outset, to a greater extent than in grape must.

The influence of *Sacch. apiculatus* in collaboration with *Sacch. ellipsoideus* (mixed sowings) in the fermentation of wine has been studied by Müller-Thurgau (XXX. and XVI.). Various wine yeasts were sown in flasks containing equal quantities of previously sterilised must, the yeasts being used alone in one series, and conjointly with *Sacch. apiculatus* in another. The number of yeast cells in each sowing was approximately the same, so that the flasks with mixed sowings contained about twice as many cells as those sown with wine yeast or *Apiculatus* yeast exclusively. The amount of carbon dioxide liberated, and consequently the progress of fermentation, was ascertained daily by weighing the flasks. The results obtained in this way with two of the yeasts during the early stages of fermentation have been plotted in Fig. 202, the abscissæ expressing the duration of fermentation in days, and the ordinates the total amount of carbon dioxide (in grms. per litre of the liquid under examination) liberated—as determined by the loss in weight—up to the corresponding days. The line marked by crosses shows the progress of fermentation with Steinberg yeast No. 1, a vigorous white wine yeast from the Rheingau, whilst the dotted line represents the work done by a weak red wine yeast from Karthaus, near Ittingen (Canton of Thurgau), and the unbroken line the progress of fermentation in the sample inoculated with *Sacch. apiculatus* (3) only. The small circles correspond to the carbon dioxide determinations. Other particulars can be gathered from the chart itself, especially the difference in the progress of fermentation with the two yeasts, and the considerable extent to which their fermentative activity was impaired by the *Apiculatus* yeast. Even so vigorous a yeast as Steinberg No. 1 was greatly retarded by the pointed yeast at the outset, so that, on the 19th day, for instance, the total disengagement of carbon dioxide amounted to 38.1 grms. with Steinberg No. 1, 6.8 grms. with *Apiculatus*, and to only 11.6 grms. in the sample with both yeasts, nothwithstanding the double sowing. In proportion, however, as the percentage of alcohol increases, the *Apiculatus* yeast is weakened, and

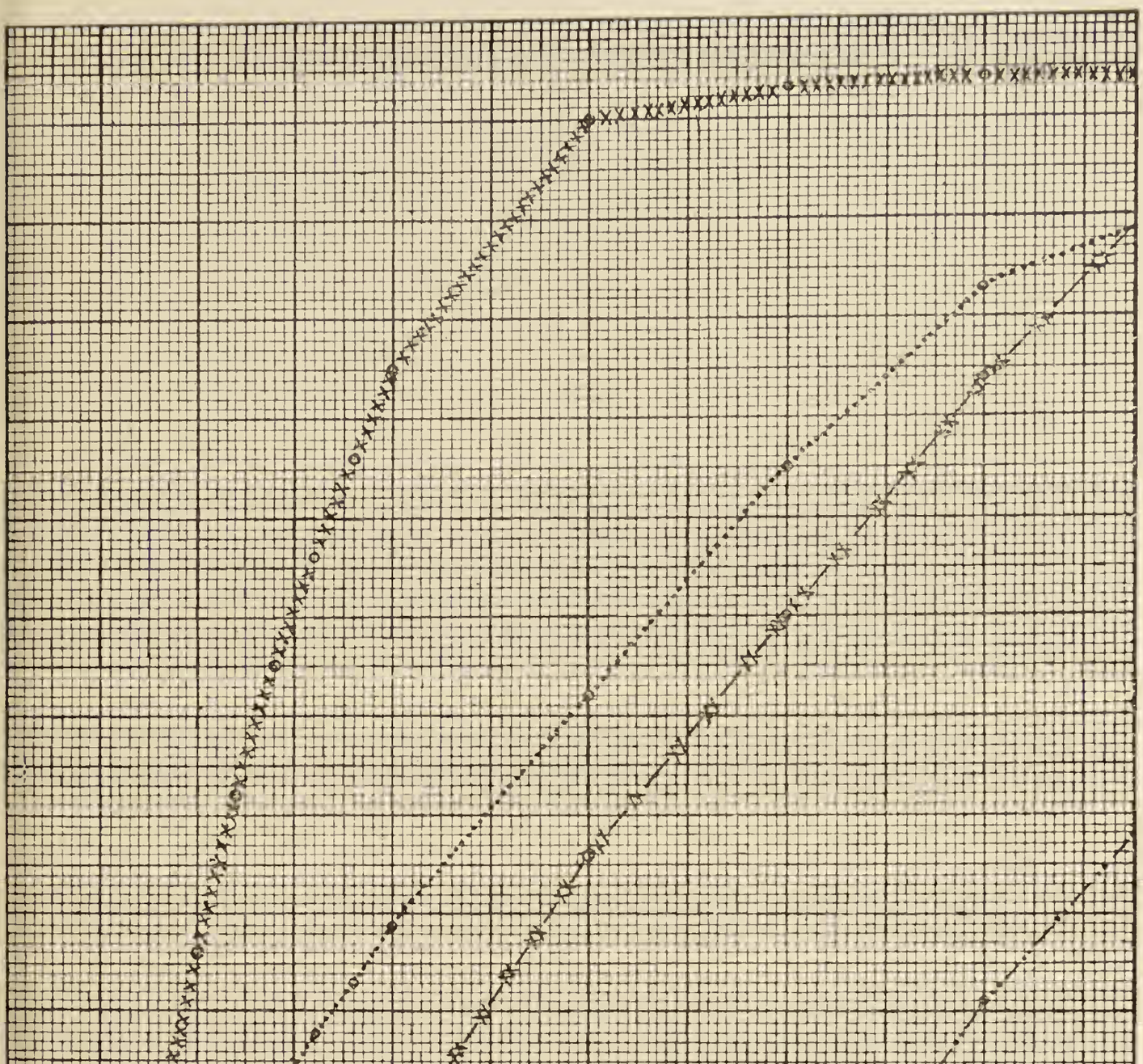


FIG. 202.—Influence of Saccharomyces apiculatus on the fermentative activity of two kinds of wine yeast:

- Steinberg yeast only.
- Karthaus yeast only.
- S. apiculatus only.
- Steinberg yeast + S. apiculatus.
- Karthaus yeast + S. apiculatus.

2 mm. = 1 day = 1 grm. of carbon dioxide.

its injurious influence on the wine yeast diminished. This is clearly evident from the chart. The influence of *Apiculatus* on the weaker red wine, Karthaus yeast is still more adverse than with the Steinberg yeast, and is more clearly evident from the chart. On the 20th day the amount of carbon dioxide liberated was 39.6 grms. with the Karthaus yeast alone, 9.4 grms. with *Apiculatus*, and 10.8 grms. with the two together, so that the wine yeast had hardly come into action at all by that time. However, as soon as the proportion of alcohol reached 1 per cent. by weight, the fermentation increased rapidly, more especially from the 40th day, when the alcohol content had attained about 2 per cent., the wine yeast being then able to free itself from the *Apiculatus* enfeebled by the alcohol.

The cause of the restrictive influence of the *Apiculatus* yeasts on the fermentative activity of wine yeasts has not yet been definitely ascertained. Müller-Thurgau (XXX. and XVI.) has shown that when *Apiculatus* acts in conjunction with wine yeast in fruit must or grape must, smaller quantities of yeast are formed than when the latter is acting alone. It is, however, probable that the former restricts the fermentative activity of the individual cells, as well as their growth, either by lowering their fermentative energy or shortening their period of activity, or both.

The active agent in restricting the other yeasts probably consists chiefly of volatile acids. True, as Müller-Thurgau (XXX.) asserts, the quantity of these acids is smaller than would correspond to their powerful adverse influence, at least if they consisted entirely of acetic acid ; but the more powerful formic acid is also present, and probably other substances capable of retarding growth and fermentation. When the fermentative action of *Sacch. apiculatus* is restricted by the liberated alcohol, the further production of these injurious substances ceases ; and at the same time the progress of fermentation indicates that the injurious substances already present are either destroyed or converted into others of less power (*e.g.*, the volatile acids into esters).

Experiments prove that the more vigorous the elliptical yeast the less is it affected by *Apiculatus* yeast, on the one hand because of its greater power of resisting injurious influences, and also because it produces more rapidly the requisite quantity of alcohol for checking the enemy On this account the difficult period of the struggle will be traversed more quickly when the proportion of *Sacch. ellipsoideus* to *Sacch. apiculatus* is greater in the sowing. In fact, experiments performed on this point by Röhling (I.) showed that when a large proportion of elliptical yeast is sown along with a small quantity of *Apiculatus* yeast, the latter is kept in the background and fermentation is scarcely hindered at all, though retardation occurs when even a small extra additional quantity of volatile acid is formed. The best flavoured

wines are those in which the number of elliptical yeast cells in the sowing was high in comparison with those of the apiculate cells.

The collaboration of *Sacch. apiculatus* in the fermentation of wine is always a drawback, not merely because fermentation is retarded, the fixed acids destroyed in an uncontrollable manner (and one that may proceed too far in certain beverages that are low in acid), and volatile acids and other malodorous and bad flavoured products, or such (esters, &c.) that may alter the character of the beverage, are formed; but also because in many instances the attenuation is unsatisfactory. For example, Müller-Thurgau (XVI.) has found that both fruit and grape wines fermented with an elliptical wine yeast and a race of *Apiculatus* mostly contain a large residue of unfermented sugars of the kind the latter yeast cannot attack. In the case of the experiments detailed on p. 437, vol. ii., the amount of these sugars left in a grape wine at the close of primary fermentation was 10 per cent. with *Sacch. apiculatus*, 0.05 per cent. with Steinberg yeast, 0.075 per cent. with Steinberg and *Sacch. apiculatus*, 0.189 per cent. with Karthaus yeast, and 0.396 per cent. with Karthaus and *Sacch. apiculatus*. Moreover, it is well known that wines low in alcohol are more liable to maladies like turning (acetic taint), and more especially ropiness, when they also contain an appreciable residuum of sugar capable of furnishing the corresponding pathogenic organisms with material for nourishment or fermentation.

The wine and fruit wine industries are in the unpleasant position of having to make the best of the presence of *Sacch. apiculatus*. In the case of apples and pears a large proportion of the indigenous yeast can be removed by washing, but this is impracticable, for several reasons, with berries and especially with grapes. The purification of grape must by filtering or centrifugalising is attended with insuperable difficulties at present, and pasteurisation is mostly impracticable under the existing conditions of the wine industry. On the other hand, the growing employment of pure culture wine yeasts affords a suitable means for rapidly suppressing the injurious influence of the *Sacch. apiculatus* present in the indigenous yeast. Whenever it is anticipated that fruit must will be strongly infected with the pest, or the presence of the latter has been discovered in the microscopical examination of grapes or the freshly expressed juice of same, it is advisable to employ more than the usual quantity of pure yeast, and to add it to the must as early as possible. In making cider and perry the fermentation of the beverage will be purer and the content of volatile acids smaller if the fruit be washed thoroughly before putting it through the mill, and the juice be then pitched with a sufficient quantity of vigorous pure yeast. Even in these circumstances *Sacch. apiculatus* will have some opportunity during the must stage and at the commencement of

fermentation of running riot for a short time. This may, however, be prevented, and a purer fermentation achieved by adopting the suggestion of MÜLLER-THURGAU (XXIV.), viz., killing the more susceptible fungi (*Apiculatus* yeast included) with sulphur dioxide, and starting fermentation with a vigorous pure yeast that has, if necessary, been habituated to that reagent. NATHAN (I.) claims to have prevented the development of *Apiculatus* yeast in berry juices by an initial addition of 2 per cent. of alcohol, though it stood the ordeal better when treated with 10–15 per cent. of fermented grape or berry wine immediately after pressing. This recommendation, however, does not seem to have found application.

Wines made by the pure-fermentation process are not always preferred by consumers, at least in the case of fruit wines, many liking the more strongly flavoured and odorous fruit ethers and esters generated by *Apiculatus* yeasts, especially in beverages otherwise poor in bouquet. Probably this accounts for the conflicting opinions expressed in the literature with regard to the influence of these budding fungi on the flavour of fruit wines. The fact that wines fermented with the powerful aid of *Apiculatus* yeasts give, on analysis, high volatile-acid values without being sour, forms a matter of considerable interest to the foodstuff chemist.

CHAPTER LXII.

THE MONILIÆ AND OIDIA.

By Dr. H. WICHMANN,
Deputy Manager of the Austrian Research Station
and Academy of Brewing, Vienna.

§ 314. Monilia, Sachsia and Chalara.

Among the organisms now classed as fungi imperfecti (p. 26, vol. ii.), the species assigned to *Monilia* have a particular interest for the fermentation technologist, the species in question forming a connecting-link, morphologically speaking, between the mould fungi and the budding fungi.

The members of the genus *Monilia* lack, in the first place, the complete mycelium exhibited by the mould fungi, *e.g.*, *Penicillium;* and, though divergent and branched hyphæ are by no means uncommon, the structure of the mycelium is very loose. For this reason the films produced by certain of the species when grown on liquid media are easily disintegrated, and exhibit a greater resemblance to mould films. On the other hand, the bud mycelia, the usual form of growth in this genus, show a more extensive polymorphism than is ever found among the true budding fungi; and it is this peculiarity that forms the characteristic feature of the genus. The bud mycelium, especially when aged, mostly exhibits all the forms observed in budding fungi from globular cells, resembling *Torula*, to elongated cells like those of *Mycoderma*, and even tubular cells of remarkable length, these being interspersed by cells analogous to those of *Oidium* and radial hyphæ of typical structure. These mycelia, in addition to appearing in nutrient liquids, constitute the normal form of growth on solid, moist substrata, so that, *e.g.*, the giant colonies on wort gelatin resemble yeast rather than moulds. Another regular phenomenon is that the vegetations of one and the same species on different nutrient media exhibit such a great divergence of cell form that no one would attribute them to the same species.

Another feature equally of diagnostic value is the absence of characteristic organs of fructification. In most species it is

impossible to speak of fructification at all; and even in the few species, like *Monilia sitophila*, which throw up hyphæ resembling conidiophores, the difference between vegetative cells and reproduction cells is very slight and confined entirely to the shape. Thus, in *Monilia sitophila*, both the conidia and the mycelial cells are of a uniform orange-yellow colour. In some species, *e.g.*, *Monilia variabilis*, the tubular or *Oidium* cells occasionally display unevenly distributed tubercles or points, on which the yeast-like conidia are sessile, and which might be regarded as sterigmata; but this is all. Hence, in *Monilia*, we have merely to deal with vegetative yeast conidia (*see* p. 21, vol. ii.), which do not differ materially from the cells of the bud mycelium either in shape, contents or origin.

A comparison of the budding cells of a *Saccharomyces* and a *Monilia* easily reveals a remarkable difference in the appearance of the protoplasmal contents. In *Monilia* these are more delicate, homogeneous, so that the cell is lighter in appearance, and the large vacuoles, invariably present, contain a spheroidal granule that is in constant rapid motion. According to A. Guilliermond (VI.), these bodies are identical with Babes' metachromatic granules or Butschli's red granules, and are similar to the chromatin granules in bacteria. Hansen and Guilliermond state that a cell nucleus is present; but, as in most cells, it is not visible.

Monilia candida (Bonorden), Hansen, affords the finest examples of the typical forms of growth. E. C. Hansen (XLVI.) investigated this fungus, and identified it with a species described by Bonorden (I.). The morphological variations of this species are highly diversified. When grown on sweet fruits or fresh cowdung, it appears as delicate mycelial filaments, whereas in saccharine liquids and on solid media a yeast-like growth predominates (*see* Fig. 99). This latter form is seen at its best in hopped beer wort, where the globular to ellipsoidal cells produce buds actively, so that, as in the case of top yeasts, small aggregations of cells are formed, containing characteristic elongated buds that are at once noted by the experienced observer. These cell forms are chiefly found in the sediment, whilst the quick-growing film, though initially of the same type of cells, afterwards consists of a vegetation resembling mould with greatly elongated, radial hyphæ that partly develop into numerous yeast conidia and partly disintegrate like *Oidia*. The mycelium of the film consists therefore of an intricate mixture of true hyphæ, aggregated buds, bud cells and *Oidium* cells. On solid media like wort gelatin, the colonies resemble those of yeast, with a puffy, corrugated centre and flat, fibrous rim, the growths resembling yeast cells being situated in the central portions of the colony and the mycelial forms in the outer portion. *Monilia candida* is characterised by considerable enzymatic power, and for a long time served as a

typical example of a fungus capable of fermenting saccharose direct, that is to say, without the assistance of an inverting enzyme, until it was discovered to possess an endoenzyme of this class, namely, *Monilia* invertase (*see* chap. lxv.), by Emil Fischer and P. Lindner (III.), as also by E. Buchner and J. Meisenheimer (I.). The alcoholic fermentation is most active in glucose solutions, and weakest in saccharose; in beer wort the production of alcohol amounted to 1 per cent. by volume in 14 days, and to 6.7 per cent. in 26 months. Pure maltose is fermented very readily, and completely so in a yeast-water solution; and the fact that, in addition, a true dextrin is decomposed that beer yeast is unable to attack, explains A. Bau's (XXI.) discovery that beer wort can be more completely fermented by *Monilia candida* than by beer yeast, though the operation proceeds more slowly. The fermentation is accompanied by the formation of volatile by-products that restrict the process. Fermentation in grape must furnished 6 per cent. by volume of alcohol (as compared with 14 per cent. in the case of true wine yeast) in about 3 weeks, and —as reported by E. Mach and K. Portele (III.)—the resulting wine had a decided peculiarly fruity flavour. The fermentation temperature is relatively high, the maximum being about 40° C. For the vegetative processes the maximum temperature is 42°–43° C., and the minimum at 6°–4° C., the fungus being therefore apparently a lover of warmth. According to Hansen, the metabolic products include acids (lactic acid ?) and nitrites, the latter having been found in barely detectable quantities by A. Maassen (I.). This species is of very widespread occurrence. W. Bräutigam (II.) found it as the chief fungus in sugar refinery waste and brewers' grains, and also in the dung of cattle fed on the first-named material. Other, morphologically similar, fungi are often classed as *Monilia candida*, even though they do not exhibit all the characteristic features of same. Thus, Adametz (XIII.) describes an example of this species from arable soil, Marpmann (VII.) reports its occurrence in cheese, and Harz (I.) in Allgau cheese and also on hay, dried plums and drum figs. According to Aderhold (V.), it is found in pickled gherkins; and Behrens (III.) observed it in the preliminary fermentation of tobacco. It should also be mentioned that B. Fischer and Brebeck (I.) observed "endogenous cell formation" in *Monilia candida*, and wished to classify this fungus with the genus *Endo-blastoderma* (*Blastoderma, see* p. 405, vol. ii.) of their system: a proceeding that cannot be sustained in view of the criticisms of Lindau and Lindner.

Monilia variabilis, Lindner, is a species characterised by extensive polymerism, and was discovered by P. Lindner (XII.) on Berlin white bread, as greyish white, mealy patches, resembling *Oidium lactis*, but mostly consisting of torulaceous cells. These form heaps of larger or smaller dimensions, between long,

cylindrical, almost empty cells, supporting small tubercles carrying isolated torulaceous conidia. These latter, which measure 1.8–4 μ in diameter, swell up in beer wort to as much as 8 μ and over in diameter before germinating, and usually develop a branching chain of ellipsoidal cells. The terminal cells frequently become filamentous, divide and disintegrate like *Oidium*. It is worthy of note that, in small-drop cultures, these filaments grow on the surface of the drops, and produce aerial, torulaceous conidia, which frequently mask the *Oidium* cells completely (Fig. 203). In surface cultures the torulaceous cells predominate, whereas when air is excluded, cells resembling yeast and *Dematium* are formed. Hence, in its various stages of development, this *Monilia* exhibits all the different cell forms found in the budding fungi: *Dematium* forms, *Oidium* forms, *Saccharomyces* forms and *Torula* forms; and consequently, in any given case, any cells of *Saccharomyces cerevisiæ* accidentally present could not be distinguished from the cells of *Monilia*. On beer wort, *Monilia variabilis* quickly produces a dry, loose, mealy film, which is readily disintegrated, but in time grows to a thickness of about 1 cm. and acquires considerable strength. It consists chiefly of torulaceous cells, together with long aggregations resembling *Oidium*, and puts forth a number of tufted growths extending downward in the liquid. Aerial hyphæ can be observed on the surface. At the same time a considerable sediment is formed, which, as already mentioned, mainly consists of yeast-like cells of various shapes and sizes. The growths obtained by inoculating beer wort from the film and sediment respectively, differ so greatly as to

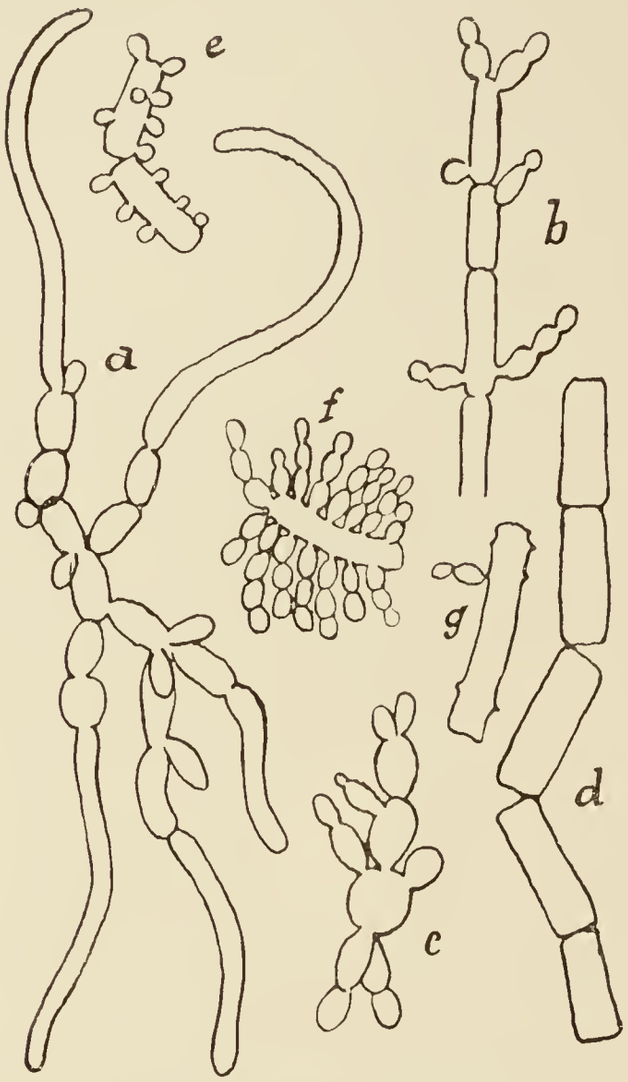


FIG. 203.—Monilia variabilis.

a, young bud mycelium, with terminal cells elongated to filaments; *b*, older filaments with yeast conidia; *c*, yeast-like bud mycelium; *d*, Oidium-like disintegration of aged hypha; *e*, Oidia with torulaceous conidia; *f*, same conidia germinating—"aerial cells"; *g*, Oidium, after shedding the conidia, showing basidial tubercles. Magn. 600. (*After Lindner.*)

give rise to the impression that they are cultures of dissimilar organisms; and these variations remain constant for several generations. In respect of its physiological behaviour, *Monilia variabilis* belongs to the fermenting group of the genus, since it ferments glucose, fructose, galactose, trehalose, saccharose, lactose (doubtful), raffinose and dextrin, though leaving intact mannose, which, on the other hand, is fermented by *Monilia candida*. Alpha-methylglucoside and β-methylglucoside are also fermented, which latter, according to LINDNER (XL.) is attacked by only one other micro-organism, namely, *Sachsia suaveolens* (*see* p. 450). HEINZE and COHN (I.) give *Monilia variabilis* as a true lactose ferment. In any case the production of alcohol is small, being only 1.4 per cent. by weight in beer wort after five months.

Despite their extensive fermentative capacity, the *Monilia* described above have no technical importance; whereas the two now to be mentioned find application in the preparation of foodstuffs in Eastern Asia.

Monilia javanica is the name given by F. A. WENT and PRINSEN-GEERLIGS (II.) to a fungus occurring, in association with others, in Ragi (*see* p. 91, vol. ii.). It forms dense filamentous masses, interspersed with, usually globular, cells (so-called yeast conidia). When grown on solid, artificial nutrient media, such as agar-agar or rice (*Oryza glutinosa*), the edges of the colonies exhibit septate filaments, on which account the discoverers of this species regarded it as the sterile condition of a higher fungus, a conclusion which more recent investigations have failed to confirm. The species thrives well in saccharine nutrient liquids, on which it first forms a film before commencing to produce alcohol—a circumstance pointing to very feeble initial fermentative activity, Glucose, fructose, saccharose, maltose and raffinose are fermented but not lactose. Owing to the presence of volatile fermentation-products, the alcohol formed (maximum 5 per cent.) has a disagreeable flavour and smell, so that the arrack furnished by this fungus is of inferior quality. Dextrin and glycerin are also utilised as foodstuffs, the former being also fermented to some extent as well.

Monilia sitophila (Mont.), Saccardo, is said by WENT (IV.) to be used by the natives in West Java in the preparation of a sweetmeat known as "ontjom," composed of the seeds of the ground-nut or earth-nut, (*Arachis hypogœa*). The ground-nuts, which are thoroughly permeated by the fungus, are made up in the form of small, orange-coloured cakes, the surface of which is covered with the conidia, whilst the interior is both chemically altered and loosened in structure by the mycelium. In the interior of solid media and nutrient liquids, the fungus develops into a plentifully branched mycelium of radial hyphæ, whilst the hyphæ projecting above the surface of the medium produces numerous conidia on short stalks. The oval to cylindrical conidia,

measuring 5–14 μ, are produced by the formation of numerous septa in branches of the aerial hyphæ (regarded as conidiophores by Went), the individual cells thereafter becoming rounded and beginning to detach themselves. Simultaneously there occurs an additional reproduction, by the budding of the conidia, so that branched chains and groups of conidia are produced (Fig. 204). The conidia separate in a peculiar manner, recalling to some extent the isthmus formation observed in the *Penicillia*. The conidia and that part of the mycelium which is in direct contact with the air, exhibit a pale orange-yellow coloration, due to a pigment similar to carotin. The colouring-matter is mostly distributed throughout the protoplasm as barely visible fine yellow drops, though sometimes collected into visible globules. In addition to the formation of conidia, Went also observed the production of small brown structures, comparable with young perithecia, but which could not be induced to develop completely. They commence with a spiral convolution of the hypha, which puts forth numerous branches and grows to a compact ball. The food requirements of *Monilia sitophila* have been thoroughly investigated by Went (V.). The fungus is very rich in enzymes, none of the more important ones being lacking, whilst even several of the rarer members of the group are present. Consequently, the organism was rightly looked on as omnivorous; and it is even able to thrive on filter-paper. It is almost insensitive to the general reaction of the nutrient medium, a high degree of acidity (10 c.c. of decinormal sulphuric acid per 100 c.c. of nutrient solution) alone being able to arrest development, whilst on the other hand large quantities of alkali have no important influence. The species grows vigorously even out of contact with air; and it is only when oxygen is completely excluded that it languishes. Small quantities of alcohol are formed in both anaerobic and

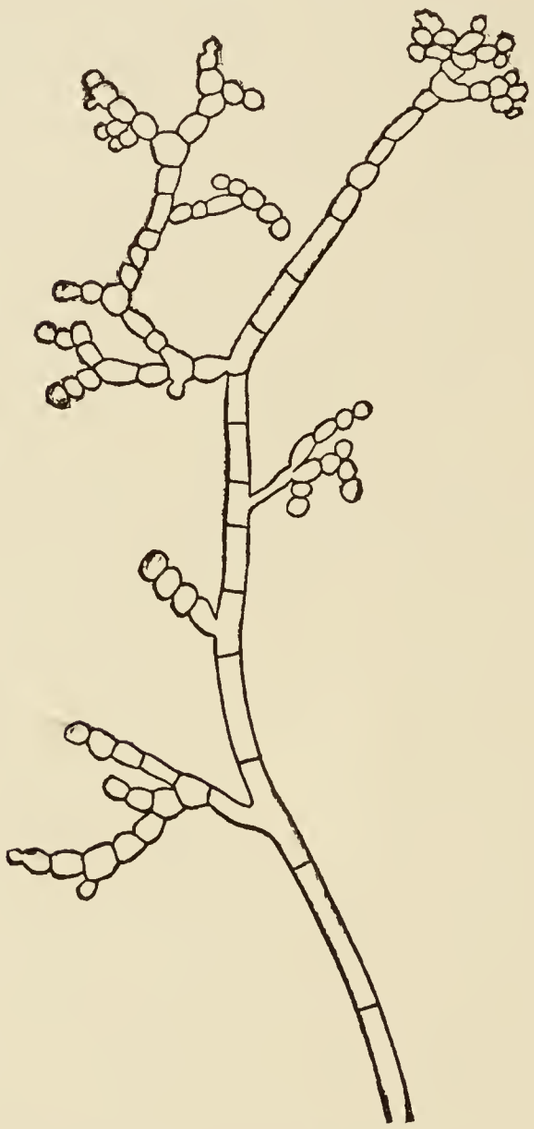


Fig. 204.—Monilia sitophila.

Aerial hyphæ (conidiophores) with budding yeast conidia. Magn. 220. (*After Went.*)

aerobic cultures. One remarkable feature is the abundant formation of esters, which are also produced when protein constitutes the sole foodstuff. Went found this species growing on the spathes of sugar-canes in Java; and Saccardo observed it in wheaten flour and on dough at Lyons. Morphologically, it differs considerably from the other fermentation *Monilia*, and resembles the parasitic species (*Sclerotinia fructigena* and *Scl. cinerea*) in the structure of the mycelium and the restriction of conidia to the aerial hyphæ. According to Went, the enzymatic effects are the most important consideration in the technical utilisation of this fungus; the filaments bore through the cell wall of the ground-nut seeds, and loosen the cells so that they fall apart under gentle pressure, the proteids in the seeds are peptonised, the oil is decomposed, the small quantity of starch present is saccharified, and, finally, some slight importance must be attached to the esterification that is set up.

Monilia albicans (Robin), Zopf (X.), the pathogenic "thrush" fungus, which has also been described under the synonyms *Oidium albicans*, Robin, and *Saccharomyces albicans*, Reess, coincides exactly, morphologically, with *Monilia candida*. Possibly, the gemma-like formations observed by Grawitz might serve as a distinctive characteristic, as also the very moderate fermentative power, traces of alcohol being formed only after a very long time. This fungus is the cause of "thrush" on the mucous membrane of the mouth and throat in very young infants, puppies and kittens, as well as the corresponding disease in fowls; though it is probably associated with other organisms in these diseases. In Nature the fungus is of frequent occurrence on dead, rotting plants, and especially on dung, &c.

Both the fermenting and pathogenic species of *Monilia* are widely distributed, so that the technico-mycological literature contains numerous reports of forms resembling the species described above. Owing to their great morphological similarity, differentiation is often difficult; and owing to the omission of important morphological and physiological properties from the descriptions, it is seldom that the fungi described can be clearly identified with previously known species. On the other hand, many of the *Monilia* have probably been described by different authors as spherical yeasts, film yeasts, mould fungi, &c. The majority of the forms referred to simply as *Monilia* were found in wine, cheese, Chinese yeast, decaying fruit and concentrated fatty substances for feeding cattle.

Sachsia albicans, Bay, is the name given to a fungus accidentally discovered by J. C. Bay (IV.). On the surface of solid and liquid nutrient media it develops a snow-white mycelium, from which numerous cells, resembling those of *Mycoderma*, separate by constriction. When submerged, the mycelial buds bear a greater resemblance to *Dematium* or *Monilia*, and the detached bud cells

are yeast-like, globular, ellipsoidal or pear-shaped (Fig 205, *a*, *b*) Morphologically, this species coincides more nearly with *Monilia*, though the absence of alcoholic fermentation constitutes a difference that was pointed out by Bay himself.

Sachsia suaveolens, the mould fungus of wine bouquet described by P. Lindner (XL.), was discovered in the fermentation vessels of a distillery. It forms a brilliant white aerial mycelium on wort gelatin, whilst in wort it produces large flakes of threads that bud abundantly and readily fall apart into separate cells (Fig. 205, *c*, *d*), the whole of the wort being finally occupied almost completely with masses of the fungus. At high temperatures fermentation is set up at the same time, a very high final attenuation being eventually attained. An agreeable odour, resembling that of Moselle wine, is produced during the fermentation; but the flavour of the fermented liquid, which is rather acid, is too strongly aromatic to be pleasant. This species ferments glucose, mannose, galactose, lactose, maltose and dextrin, as well as raffinose and β-methyl glucoside. Mucinous masses are formed in some of the sugar solutions; and old cultures exhibit isolated greenish mycelial filaments, the cells of which contain large numbers of fatty drops. The faculty of developing a bouquet is utilised in the preparation of a non-alcoholic beverage, for which a patent was taken out by Mirsch and Eberhard (I.).

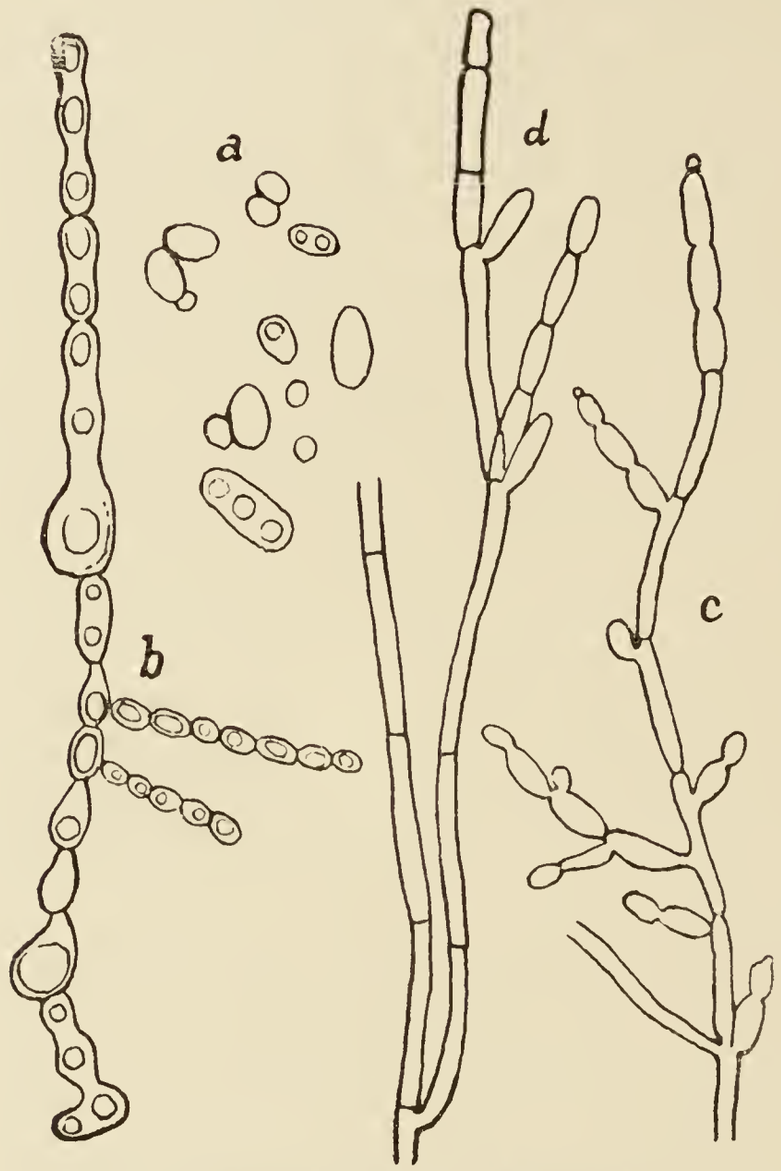


Fig. 205.—Sachsia albicans and Sachsia suaveolens.

a, budding cells (magn. 775); *b*, normal mycelium (magn. 325) of Sachsia albicans; *c*, mycelial filaments of Sachsia suaveolens in the act of budding; and *d*, fully septated (magn. 300). (*a* and *b*, *after Bay*; *c* and *d*, *after Lindner*.)

This fungus closely resembles the *Monilia*, both in the development of the vegetative organs and in respect of its fermentative capacity.

Chalara mycoderma, a name first applied by Bonorden (I.), was afterwards bestowed by L. Cienkowski (III.) on a fungus discovered by him in the films on organic liquids (wine, milk, fruit juices, sauerkraut liquor, &c.). This species, which was afterwards discovered and depicted by E. C. Hansen (LIX.), is mentioned here in connection with the *Monilia* because the film it produces on the surface of nutrient liquids is similar to that of *Monilia candida*. The extensively branched mycelium of the former organism, however, consists of rafter-like aggregations of elongated buds, from which globular to ellipsoidal conidia separate by constriction at the points of contact of the cells. These conidia, which measure about 4–6 μ, are often on short stalks or sterigmata; and they may also be developed on the individual cells into which the mycelium frequently disintegrates after the manner of **Oidia**. Being closely packed with protoplasm and therefore highly lustrous, these conidia are readily distinguished from the delicate, and apparently empty, elongated cells, so that *Chalara* presents a characteristic appearance under the microscope.

§ 315.—Oidium lactis and Allied Species.

The widely distributed *Oidium lactis*, the chief representative of the whole clan, is classified with the *Basidiomycetes* by the systematic mycologist, on account of the peculiar forms of vegetative reproductive cells known as *Oidia*, first observed in their fullest development in *Oidium lactis*. Their form, origin, and importance have already been mentioned on p. 23, vol. ii.; and it should also be stated that the rectangular contour of the conidia is so marked that it is very difficult to mistake an *Oidium* cell for any other kind. So long as the *Oidium* fruit cannot be satisfactorily assigned to the second typical, basidiomycetous fructification, these *Oidium* species may be grouped with the fungi imperfecti, since they are connected with many species of this group by transition forms. We have also retained the old and characteristic name, *Oidium*, because it is well known in all the literature of fermentation, including the present work, and because, in the present indefinite systematic position of the genus, it would be inadvisable to employ the proposed new name, "Oospora."

The species of the genus *Oidium* are characterised by a typical mycelium, consisting of septated, irregularly branched hyphæ, which disintegrate—mostly at the ends, though sometimes in the middle as well—into short cylindrical cells of nearly rectangular contour, only the corners being rounded off a little. Budding is only exceptionally observed with this genus.

Oidium lactis, Fresenius, a fungus widely distributed in Nature and in the fermentation industries, is generally known as milk mould, being almost invariably found on sour milk, and also on the surface of unclean dairy utensils, cheese, &c. It is of such regular occurrence in butter that Laser (II.) proposed to utilise this circumstance as a biological test for that substance. Other common habitats of the fungus are the surface of packages containing pressed yeast, on pickled gherkins, commercial starch, green malt, and in breweries, where it occurs on the sludge-filter bags, wooden utensils, and storage casks (as a white efflorescence), also in waste waters, the dung of domestic animals, &c.

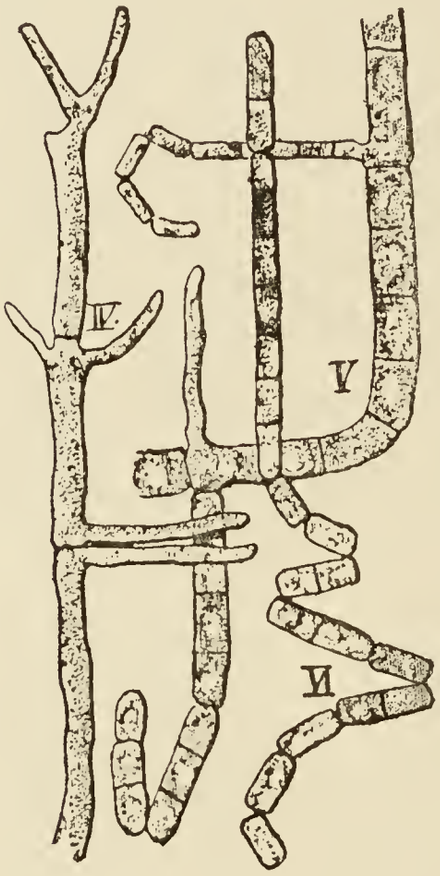


Fig. 206.—Oidium lactis.

IV, hypha with a number of lateral branches; *V*, a branched thread in course of septation; *VI*, disintegration into oidia, the chain bending into the zigzag form. Magn. 600. (*After Lindner.*)

When viewed by the unassisted eye, the fungus has the appearance of a delicate, white down composed of fine threads, though sometimes it is mealy and dry—rarely yellow and mucinous—whereas in artificial cultures on various nutrient media it always forms a uniform snow-white, closely matted, furry covering. The appearance is exactly the same on nutrient liquids, so that an *Oidium* culture can always be identified at the first glance. Old cultures exhibit isolated upstanding masses of hyphæ, resembling *Basidiomycetes*. Lindner (XL.) described similar forms, owing their origin to the fact of the film plectenchyma in giant cultures being perfectly gas-tight, and consequently forced upward, like bubbles, by the carbon dioxide liberated during fermentation, and being prevented from escaping at the sides owing to the colonies adhering firmly to the nutrient medium at the margin.

The mycelium in the fungoid mass, which is often extensive, consists of septated and very irregularly branched hyphæ, the members of which are comparatively long (Fig. 206). In young mycelia, especially during the germination of the conidia, the cells are tubular, septation only occurring later; and the conidia are difficult to distinguish from the short cylindrical cells. The conidia, which have the typical *Oidium* form, are developed most completely when one of the hyphæ raises itself above the level of the substratum and divides into short cells by septation, when growth at the apex is concluded. The various cells soon become

detached, without becoming rounded to any extent, this separation being accompanied for the most part—as observed by Lindner—by spasmodic movements of the whole thread. In nearly all cases the separation is so far incomplete that two or more oidia hang together by a corner, and thus form zigzag chains. A filament in this state is particularly characteristic, though even when unbroken the conidial chains present a remarkable appearance owing to the uniformity of shape and dimensions of the members. After their detachment the conidia preserve their rectangular contour for some time, and become rounded only just before germinating. When these isolated oidia are mixed with yeast cells, *e.g.*, those of pressed yeast, they are readily distinguishable from the latter, both by their shape—the longitudinal walls are perfectly parallel—and contents, these showing up in strong relief and being interspersed with numerous vacuoles and granules. Moreover, the conidia are not merely produced on special hyphæ—this, indeed, being the exception—but the central portion of a hypha may frequently be observed to separate into oidia—especially on very moist nutrient media—and not infrequently the entire mycelium disintegrates into its component cells. This behaviour brings *Oidium lactis* and its congeners into close relation with *Monilia*. Young mycelia, placed in a little water and allowed full access to the air, exhibit in a very marked degree the phenomenon of cell fusion or "internal conidiation" (*see* p. 6, vol. ii.).

The physiology of *Oidium lactis* has been already commented on in several parts of the present work; and these remarks may be briefly summarised here. Although, in comparison with *Monilia*, *Oidium* may be considered as poor in enzymes, it nevertheless possesses a by no means unimportant fermentative activity, which is manifested by a considerable liberation of gas. The quantity of alcohol thus formed is, however, slight, merely traces being produced, according to Hansen (LVIII.), in beer wort and glucose yeast-water, Weidenbaum (I.) giving the amount as 0.6 per cent. in a fortnight, and Brefeld 1.2 per cent. in three months. Lang and Freudenreich (I.) found 0.55 per cent. in ten days, or 1 per cent. in five weeks; the fermentation, according to these workers, proceeding less vigorously in solutions of saccharose or maltose than in those of glucose or lactose. No invertase could be isolated. Lactic acid (*see* p. 320, vol. i.) is oxidised by this fungus, the acidity of sour milk being thereby reduced. The presence of proteolytic enzymes is indicated by the liquefaction of gelatin, which is facilitated by an acid reaction, a peculiarity regarded by Weidenbaum as a means of differentiation from *Oidium* (*Monilia*) *albicans*. Lang and Freudenreich also state that a strong smell of soft (Limburg) cheese is developed in peptonised meat broth containing lactose and maltose, and that a considerable (probably complete) decomposition of casein is effected in sterile milk Henneberg's observation that *Oidium lactis* is

injurious to yeast, the cells of which it kills, is probably attributable to this powerful action on proteids. On the other hand, *Oidium lactis* itself offers very strong resistance to external influences. For instance, Lang and Freudenreich report that growth is not appreciably retarded below 60° C. The statements of various authors on the influence of temperature, however, differ considerably, Hansen (LVIII.) giving 37.5° C. as the maximum and 0.5° C. as the minimum, whereas, according to Weidenbaum (I.) the optimum temperature of vegetation is 20° C. According to

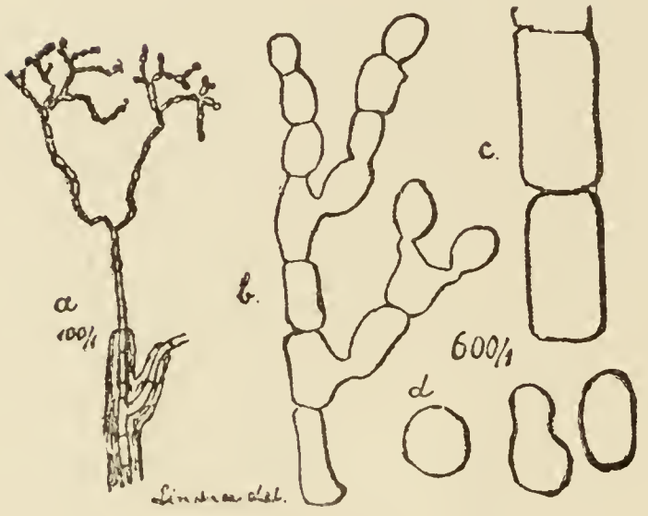


Fig. 207.—Oidium lupuli.

a, Aerial hypha, branched and separating into oidia; *b*, end of a branch of same; *c*, oidia from the central portion of a wide hypha; *d*, isolated oidia before germination. Magn. of *a*, 100; of *b*–*d*, 600. (*After Lindner.*)

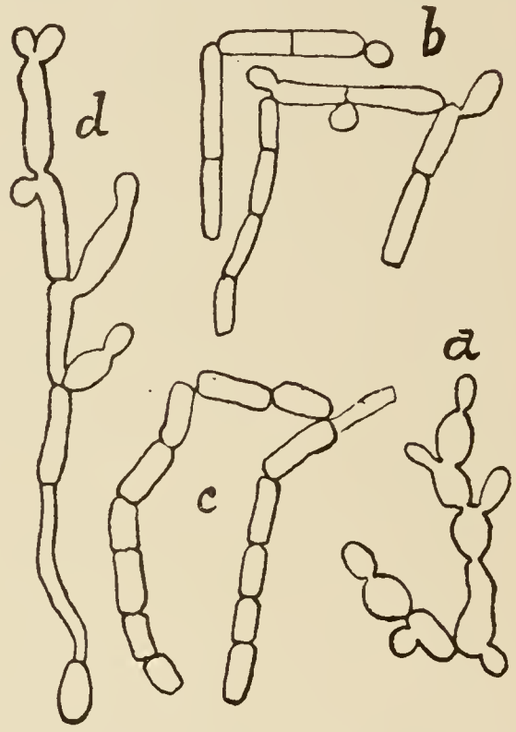


Fig. 208.—Oidium pullulans.

a, budding yeast-like cells; *b*, budding oidial cells; *c*, formation of oidia; *d*, monilial hypha. Magn. 600. (*After Lindner.*)

Hansen, the maximum temperature for film formation is 36.5°–37.5° C., and the minimum 3° C., which clearly expresses the character of *Oidium lactis* as a true film-producer. The species also seems to oppose considerable resistance to antiseptics, even a 1 per cent. solution of corrosive sublimate being insufficient in some cases, whilst 1 : 1000 of formaldehyde failed, and 2.5 per cent. of carbolic acid required thirty seconds to prove fatal. Though growing well on all nutrient media, the fungus develops preferably on those with an acid reaction.

Oidium lactis is the representative of a group of species or varieties, a fact demonstrated by the divergent reports on the production of alcohol, the influence of temperature, &c. On the basis of morphological and physiological differences in the appearance of the colonies, in the size and shape of the oidia and in the power of peptonisation, M. Grimm (I.) set up a number of species,

whose divergences are most clearly apparent in cultures on potatoes and casein.

Oidium lupuli, Matthews and Lott, is occasionally observed, as a reddish dust, on hops that have been stored damp, the dust consisting of the conidia of this fungus. In artificial cultures—according to Lindner (XLI.)—it forms a quick-growing superficial mycelium, the richly branched aerial hyphæ of which fall apart as oidia (Fig. 207). They are mostly oval, swelling up to a nearly globular shape before germinating. The colour is red at first, afterwards turning yellow.

P. Linder (XL.) also assigns to the genus *Oidium* a fungus discovered in samples drawn from the storage casks at the Berlin experimental brewery and occupying a morphological position between *Oidium* and *Saccharomyces*. This species—*Oidium pullulans*, Lindner (*see* Fig. 208)—not only exhibits the oidium-like disintegration of the mycelial filaments, but also budding growths resembling yeast cells. In some small-drop cultures these yeast cells are formed exclusively, without any tendency to septation of the hyphæ, but in other cases there is an abundant formation of mycelial threads with budding cells. On wort it quickly forms a thin film with a strong ring; and on wort gelatin, yellow-brown colonies, resembling those of yeast and with a dull lustre. Lindner did not succeed in observing any fermentation. The close relationship of this fungus to *Monilia* is unmistakable, and it would probably be advisable to include it in that category.

SECTION XVII.

THE ENZYMES AND ENZYME ACTIONS OF YEAST.

CHAPTER LXIII.

ALCOHOLASE.

By Dr. RUDOLF RAPP,

Chief Pharmacist of the Munich Municipal Hospital.

§ 316.—Historical Introduction.

After the vegetable nature of yeast had been demonstrated by Cagniard-Latour (II.) and T. Schwann (I.), and the theory of its fermentative action had been thereby diverted into new channels, the succeeding decades witnessed the rise of a series of theories which endeavoured to assign the fermentative property of yeast to actions that were partly physical, partly chemical, and partly physiological. The most important of these theories have been already discussed in vol. i. (pp. 12–23).

The fact that, until recently, Pasteur's theory on this subject found the largest number of adherents was due to its being the most intelligently developed and to the circumstance that the systematic investigations of that authority first laid the foundations of the chemistry of fermentation. Moreover, Pasteur based his work on the labours of Schwann, whose disciple he professed himself—*see* Delbrück (VII.)—so that he was not really the founder of the new epoch.

In the present paragraph we will deal chiefly with the theories in which fermentation is ascribed to the action of enzymes, to which class belong the hypotheses of M. Traube, J. Liebig and Hoppe-Seyler. Traube (I.) wrote: "Yeast acts as a chemical ferment, which transmits the oxygen present in combination in one chemical substance to another substance," that is, it is capable of acting as a reducing agent on the one hand, and as an oxidising agent on the other. This view was quickly shared by M. Berthelot (V.) and was repeated by Traube (II.) in 1874, after Brefeld (XIII.)—on the basis of a somewhat doubtful method of experiment—had come to the not very expressive conclusion that

yeast reproduction and alcoholic fermentation seem to be two distinct phenomena. As far back as 1839, J. Liebig (I.) classed fermentation and putrefaction as the results of the transmission of chemical motion, proceeding from a proteid ("ferment") substance in a state of incipient decomposition. At a later date (1870) this worker (II.) expressed the opinion that this chemical ferment was a nitrogenous and sulphurous body present in the yeast cell in a state of decomposition and transformation; and that the sugar of the fermenting liquid combined with the proteid substances in yeast to form a solid protein saccharate, alcohol being then separated in consequence of the rearrangement of one or more constituents of the ferment. A similar view was strongly supported by Hoppe-Seyler (VI.), who wrote: "Lower organisms are undoubtedly producers and carriers of ferments just the same as higher ones. In my opinion, the possibility of the production of an alcoholic ferment by germinating yeast is established in principle by the separation of invertase from the yeast cell."

Although the existence of a fermentative enzyme was assumed by celebrated scientists, and the possibility of the production of such a substance by budding beer yeast was brought within the bounds of probability, for example, by the discovery of Fiechter (I.) that hydrocyanic acid destroys the vitality and development of yeast completely, though not necessarily its action as a ferment, an important difference still existed between the enzymes already known and that concerned in alcoholic fermentation. The former were able to perform their functions even when separated from the living cell, whereas the hypothetical yeast enzyme could not at first be isolated from the living cell. The question arose, given such an enzyme in beer yeast, why cannot it be isolated, and since experience teaches that enzymes act only when in a dissolved condition, why is the yeast cell alone able to set up the fermentative action? This question became particularly important when Berthelot and Liebig succeeded in isolating invertase from the yeast cell.

All the attempts made by workers at different times to separate an alcoholic ferment from the yeast cell proved futile. Thus, the experiments of Berthelot (V.), for recovering the enzyme from yeast by maceration, failed. Mitscherlich (IV.), Helmholtz (I.), Dumas (V.) and others filtered the yeast liquid, or tried to separate the yeast from the dissolved constituents by diffusion through a fine membrane, but in none of these cases was any fermentation set up so long as reinfection with yeast cells was carefully precluded. Even wort that is in full fermentation soon ceases to ferment when the yeast cells are removed by filtration and the access of new yeast cells is prevented. This experiment was recently repeated by Wroblewski (V.), who employed sandstone as the filtering medium. A different result was obtained by Colin (I.) and afterwards by Fleck (II.), but their

experiments were valueless, not having been conducted under conditions guaranteeing sterility.

Other workers proposed a different method of separating the fermentative enzyme from the yeast cell, it being hoped that fermentation would be excited by the cell contents when liberated by the disruption of the cell membrane. Experiments on this line were undertaken by Lüdersdorff (I.) and C. Schmidt (at Dorpat), as also by M. Manassëin. The first-named triturated yeast on a ground glass plate by means of a glass pestle. C. Schmidt (I.) consumed six hours in grinding down 1 grm. of yeast, but failed to obtain any fermentation with the product. M. Manassëin (I.) attempted to kill the yeast cells used for the fermentation experiments by the aid of boiling temperature, without destroying the enzyme.

The fact revealed by all these communications is that the existence of an alcoholic enzyme in beer yeast could not be demonstrated by experiment, but that the presence and reproduction of living yeast cells are essential to the inception and continuance of alcoholic fermentation.

Though this state of things apparently controverted the hypothesis of the existence of a fermentative enzyme, further researches and observations contributed to prevent the enzyme theory from stagnating, the chemists, in particular, strongly maintaining the existence of an enzyme. The failure to isolate from yeast an active enzyme capable of decomposing sugar into alcohol and carbon dioxide was generally ascribed to the circumstance that the method of treatment pursued probably altered the composition of the enzyme and rendered it inoperative. Even if alcoholic fermentation had not yet become an accomplished fact, there was no justification for assuming that it would not some day be realised, when a method should be discovered of preparing this enzyme without impairing its activity.

These opinions on the existence of an alcoholic enzyme necessarily acquired a more stable foundation when P. Miquel (VII.), in 1890, succeeded in demonstrating that the fermentation of urea is effected by the intervention of an enzyme, urase, (*see* vol. 1, p. 336), separable from the bacteria present, and not by the vital activity of these bacteria themselves, and when E. Fischer and P. Lindner (II.) isolated from *Monilia candida* (*see* p. 445, vol. ii.), by triturating the cells with powdered glass, a substance capable of decomposing saccharose in a manner analogous to invertase. The probability of this view was further heightened when E. Fischer (V.) applied the stereochemical method to enzyme action and showed the previously assumed difference between the chemical activity of the living cell and the action of chemical agencies with regard to molecular asymmetry to be non-existent; additional confirmation being afforded in course of time by the growing number of communications to the effect that, under certain conditions, the formation of alcohol,

either alone or associated with carbon dioxide, could be demonstrated in the case of higher plants and in fruits in the absence of yeast cells. Under this category may be ranked the publications of Schunck (I.) and Duclaux, the former of whom found that alcohol, succinic acid and carbon dioxide occur during the so-called fermentation of madder—though, as the author himself admitted, the collaboration of living micro-organisms was not precluded. The observation of Duclaux (XVIII.) respecting the production of alcohol in an alkaline sugar solution has been mentioned in vol. 1, p. 25. We may also refer here to the observations of Will (XXXV.), who desiccated yeast at a low temperature and detected fermentation phenomena, but not growth, on examining the samples after nine years' storage.

Thus, during the prolonged discussion on the nature of fermentation previous to the year 1896, opinion had gradually veered round again in favour of the enzyme theory. The experimental proof, however, that the fermentative agent is separable from the living cell, and that consequently the decomposition of sugar into alcohol and carbon dioxide should be regarded as a purely chemical reaction, must nevertheless rank as a new and important fact. This proof was afforded in a simple and precise manner by E. Buchner (II.) towards the close of 1896, in that, by purely mechanical means, after breaking down and removing the cell membranes and the protoplasmal envelope, he obtained the cell contents by themselves, in the form of juice capable of decomposing sugar, and in a practically cell-free condition. Although this discovery has become of the greatest theoretical importance and roused considerable attention in the fermentation industry, it does not seem adapted for direct technical application, so long as the yeast cell has to be drawn upon as the source of the enzyme, owing to the roundabout character of the method. This view was also adopted by Wortmann (XVIII.) in connection with the fermentation of wine, in which the entire metabolism of the living yeast comes into play. E. Fischer (VI.) expressed great approbation of the discovery. The practical application of the method and the preparation of permanent yeast are described in § 320; and the method of preparing the yeast juice by crushing and pressing the cells has found suitable application through the labours of H. Hahn (II.), Mazé (II.), Takahashi (I.), Weinland (I.), Kohnstamm (I.), Czapek (V.), Stoklasa (III.) and his colleagues Cohnheim (I.), Krasnosselsky (II.) and Maximow (I.).

§ 317.—Preparation of Expressed Yeast Juice.

The method of preparing expressed yeast juice was originally elaborated at the Munich Hygienic Institute, valuable assistance being afforded by M. Hahn, who recommended the use of kieselguhr and the hydraulic press. The operation is divided into

five stages: (1) Washing the beer yeast; (2) Draining the washed yeast; (3) Mixing with quartz sand and kieselguhr; (4) Grinding to a pasty mass; (5) Pressing—these two latter stages being repeated. On account of their importance several of these stages will now be described in greater detail.

The washing of the brewery yeast was found to be an essential feature. It is preferably performed in an apparatus designed by Hagenmüller (I.). Before mixing, the yeast must be well drained to free it from water, for which purpose it is placed in a stout, unstiffened cotton cloth (*e.g.*, watertight tent canvas) and pressed for five minutes under a pressure of 50 atmospheres. The resulting yeast cakes, containing 70 per cent. of water, are next mixed with fine quartz sand (passed through a sieve with 200 meshes to the sq. cm.) and kieselguhr or diatomaceous earth, in the proportion of 1000 grms. of yeast to 1000 grms. of quartz sand and 200–300 grms. of kieselguhr, the whole being sifted through a coarse sieve (9 meshes per sq. cm.). The grinding is effected in small quantities—300–400 grms. at a time—either in a mill or in a mortar with a loaded pestle provided with a pestle guide, the operation being continued until a pasty mass is formed, that "balls" and becomes detached from the walls of the mortar. The pasty masses are united, placed in a damp press cloth, of the type mentioned above, and subjected to a pressure, rising gradually to 1280 lb. per sq. inch, in a hand-operated hydraulic press. In order to increase the yield, the press cakes are divided into small portions and triturated over again in the mortar. At first, water was added at this stage, but subsequently it was omitted as superfluous. The exuding yeast juice is allowed to trickle down on to a folded filter, and thence into a vessel cooled with ice.

The yield of expressed juice obtained by this method varies between 450 and 500 c.c., so that after deduction of the cell membrane about 60 per cent. of the total cell contents is recovered. Wroblewski (V.) obtained a yield of 72 per cent.; Macfadyen, Morris and Rowland (I.), working with a pressure of 200–350 atmospheres, obtained 5–87 per cent.; Lange (II.)—with 15 per cent. of added water—44 per cent., and Ahrens (I.) 70 per cent. After this treatment the press cakes are not exhausted, a further quantity of fermentative material being recoverable by repeated trituration and pressing after a small addition of water. Indeed, according to Buchner and Rapp (VII.), these later fractions mostly exhibit a stronger fermentative power than those from the first operation. Will (XX.) also states that the residual press cakes still contain considerable quantities of zymase. Presumably the fermentative enzyme is dissolved by a further addition of water.

When the expressed yeast juice is properly prepared, only a few unbroken yeast cells can be discovered in it under the microscope; but it may be stated at once that these cells are not capable

of producing the fermentation phenomena exhibited by the expressed juice. The sediment is also free from micro-organisms after standing 3–12 days in the ice chest, nothing but a protein coagulum being discernible under the microscope.

A bacteriological investigation of expressed yeast juice by Buchner and Rapp (II.), yielded 50–100 bacterial cultures on meat-water gelatin, and 4 yeast colonies on beer-wort gelatin, per 1 c.c. of juice. The press cakes were microscopically examined by H. Will (XX.), who found :

	Empty skins.	Bruised cells.	Intact cells.
In the first experiment .	55.0 per cent.	28.0 per cent.	17.0 per cent
In the second experiment	15.6 ,,	81.0 ,,	2.7 ,,

In the case of such an important new discovery, it is desirable to obtain confirmation by other workers; and this has been afforded by the labours of H. Will (XXXV.), M. Delbrück (VII.), J. R. Green (II.), and Martin and Chapman (I.). After all these workers had obtained negative results, the three first-named were successful on a subsequent occasion, so that only Martin and Chapman really failed to obtain positive results. On the other hand, very satisfactory fermentations with expressed yeast juice were obtained by Wroblewski (I.), Ahrens (I.), A. Stavenhagen (I.), Macfadyen, Morris and Rowland (I.), and by Harden and Young (I.). Ahrens wrote : " When once we had acquired the technique of the method, we invariably obtained a highly efficient juice; and whereas, at first, we could not get any useful, positive results, we never failed afterwards."

In addition to the Buchner-Hahn method there are several others. In the first place, E. Buchner (X.) himself tried to recover the juice by the aid of liquid air, or by trituration with solid carbon dioxide, equal quantities of yeast and solid carbon dioxide being triturated for half an hour, and the liquid drawn off by aspiration. A yield of 20 per cent. was obtained. Albert (I.) also obtained a fermentative extract from permanent yeast by trituration with sand, kieselguhr and with an addition of water containing 10 per cent. of glycerin, followed by pressing. A new method of preparing yeast juice was worked out by Macfadyen, Morris and Rowland (I.), by subjecting the yeast to a number of rapid shocks in presence of particles of silver sand in a special apparatus. By this treatment none of the cells remain unbroken. The yeast was kept cool with the aid of brine at a temperature of -5° C A yield of 35 per cent. of juice was obtained. Martin and Chapman (I.) recovered the juice by centrifugalising, instead of by pressure.

Other workers endeavoured to obtain extracts rich in protein or extractives, instead of those with fermentative power, and attained their end by means of energetic plasmolysis. Thus, C. J. Lintner (III.) recovered cell juice with salts, M. Hahn and

L. Geret (I.) with chloroform, Dormeyer (I.) with ether and chloroform, and J. de Rey-Pailhade (V.) with 22 per cent. alcohol. In order to recover a yeast preparation similar to meat extract, E. de Meulemeester (I.) proposed to treat yeast with gum arabic; H. van Laer (IX.) used 2 per cent. of common salt, and H. Buchner and M. Gruber (I.) used ether. Yeast extracts of this kind are met with in commerce under various names: siris, wuck, ovos, &c. (*see* p. 168, vol. ii.).

§ 318.—General Properties of Expressed Yeast Juice.

The expressed yeast juice prepared by the method of Buchner and Hahn is a clear, though opalescent, liquid of a yellow to brownish yellow colour, and with a strong smell and taste of yeast. When freshly prepared, it has a faintly acid reaction, though Ahrens (I.) states that that prepared by him is slightly alkaline, but quickly turns acid. The fresh juice prepared by Wroblewski (III.) was also faintly alkaline.

According to this worker (IV.), the juice is optically inactive, which is rather surprising in view of the protein content. E. Buchner's (X.) investigations on this point have not yet led to any decisive results.

When the yeast juice is treated with strong alkali, mineral acids or acetic acid, a voluminous precipitate is formed. On being warmed, coagulation of protein occurs at 35°–40° C., becoming much stronger at higher temperatures, so that the whole mass seemed curdled. This is probably the first time that a preparation so rich in protein has been obtained from the cells of budding fungi. By means of partial coagulation, Wroblewski (III.) was able to differentiate several coagulable proteids. He points out that the temperature of coagulation (41° C.) of the one proteid coincides with that at which the fermentative action of the juice ceases; and that furthermore the proteid coagulating at 41° C. appears to be digested before any of the others.

According to E. Buchner (X.), the chemical analysis of different samples of juice gave the following results: sp. gr. 1.027–1.057; content of dry matter 8.5–14.5 per cent., coagulable protein 5–6 per cent., total nitrogen 0.82–1.45 per cent., organic phosphorus about 0.228 per cent., organic sulphur about 0.065 per cent., ash 1.3–1.8 per cent. (*see also* p. 192, vol. ii.). The ash constituents are: potassium, sodium, calcium, magnesium, phosphoric acid, sulphuric acid, chlorine and silica (the latter from the kieselguhr). Ahrens (I.) found in the ash-free substance, 45.4 per cent. of carbon, 7.5 per cent. of hydrogen and 10.64 per cent. of nitrogen (*see also* p. 204, vol. ii.). Wroblewski (I. and III.) has also identified the following substances in expressed yeast juice: albumens, globulins, mucinous substances, proteoses, peptones, nucleoalbumens, compound carbohydrates and a special crystalline

body that leaves behind, on combustion, a quantity of phosphoriferous ash. Other substances present are : tyrosin, leucin, glutamic acid, nitrogenous bases, xanthin bodies, a substance capable of changing sulphur to sulphuretted hydrogen and iodine to hydriodic acid; lecithin, glycerin, calcium phosphate, magnesium phosphate, peculiar volatile bodies and various others.

As was shown by Buchner and Rapp (V.), expressed yeast juice can be desiccated without injuring any of its properties. The best method is to concentrate it to the thickness of syrup in a Soxhlet vacuum apparatus, after which it can be brought to complete dryness by exposing it, in thin layers, to the air, at a temperature of 22° C., or at 34°–35° C. In the case of Munich yeast juice, the fermentative power was unimpaired by this treatment, but, according to E. Buchner (X.), a loss of 18–74 per cent. in this respect is sustained by Berlin yeast juice.

The most interesting feature of expressed yeast juice is the enzymes it contains, which include: a fermentative enzyme, a hydrolytic enzyme (decomposing maltose, saccharose and glycogen), a proteolytic enzyme, an oxidising enzyme, a reducing enzyme, one that decomposes fats, another that splits up hydrogen peroxide, and a lab enzyme (*see* chapters lxv. and lxvi.). The most important is the one under whose influence sugar is decomposed into alcohol and carbon dioxide. According to the recent labours of Buchner and Meisenheimer (II.), lactic acid plays an important part in the decomposition of sugar, and must be regarded as an intermediate product. Both these workers and also Mazé (III.) attribute the fission of sugar in alcoholic fermentation—with the intermediate production of lactic acid—to the action of two different enzymes ; and they give the name of yeast zymase to the one that decomposes the sugar into lactic acid, whilst they propose the name, lactacidase, for the enzyme that transforms the lactic acid into carbon dioxide and alcohol. The quantitative determination of the absolute amount of fermentative enzymes in expressed yeast juice has not yet been carried out. The only possible means, so far, of obtaining information in this direction is by comparing the fermentative value of two different juices under identical conditions. All samples of expressed yeast juice exhibit fermentative power, provided the yeast from which they were recovered possessed any fermentative enzyme at all or was able to store up such an enzyme even temporarily. The fermentative power and fermentative energy, however, differ considerably, and depend on the race of yeast, and on the conditions (*see* § 320) under which the yeast was treated for recovering the juice. The amount of carbon dioxide furnished by 20 c.c. of pressed yeast juice, in presence of 8 grms. of saccharose and 0.2 c.c. of toluene (as antiseptic), at the end of ninety-six hours at 22° C., is 0.7–1.87 grms. According to E. Buchner (X.), 10.5 c.c. of juice and 3.5 per cent. of a 60 per cent. solution of

saccharose furnish up to 30 c.c. of gas, after ninety minutes at 28° C ; that is to say, two and a half times the volume of the original liquid. Other workers observed greater fluctuations in the fermentative power. Thus, Wroblewski (III.) obtained 1.8–10 c.c. of carbon dioxide from 3.5 c.c. of juice, 14 c.c. of water and 3.5 c.c. of a 60 per cent. solution of saccharose. The results obtained by Macfadyen, Morris, and Rowland (I.) with top-fermentation yeast are so irregular that—as these workers admit —no clear information is obtainable therefrom. More favourable results were obtained by Harden and Young (I.) with similar fermentation yeast. Buchner and Antoni (I.) found that fermentation with expressed yeast juice goes on with equal power in an atmosphere of oxygen or hydrogen. Expressed juice has been prepared in Munich from bottom-fermentation yeast by Buchner and Rapp (I.–VIII.); in Berlin by Buchner, Albert, Spitta, Meisenheimer and Antoni from the bottom yeast of three different breweries; by Lange (II.) from pressed yeast; by R. Green (III.) from *Saccharomyces cerevisiæ* (Hansen); by Takahashi (I.) from Saké yeast; by Wroblewski (I., II., IV., V.) from commercial yeast and wine yeast, pure cultures of beer yeast and Okoci distillery yeast; by Macfadyen, Morris and Rowland (I.) and by Harden and Young (I.) from top-fermentation yeast.

Before proceeding to describe the other peculiarities of expressed yeast juice, it may be mentioned that, up to the present, all the observations on the extent to which the fermentative enzyme are influenced by various factors have been made with the juice alone and not with the pure enzyme itself. Consequently, the degree to which the results obtained has been affected, one way or the other, by the other constituents of the juice, must be left out of consideration.

Influences of this kind come into play during the storage of the expressed juice. Buchner and Rapp (I.) observed that the juice loses its efficiency in proportion to the length of time and increasing temperature of storage; and the same peculiarity was also noticed by R. Albert and by Macfadyen, Morris and Rowland (I.). Storage in ice affords the best means of preserving the fermentative power of the juice. The loss of power is ascribed by E. Buchner (III.) to extensive alterations, such as are set up by the autodigestion of the juice (*see* chapter lxvi.); but another cause might be traced to the gradual development of an acid reaction, Ahrens (I.) having found that, in the course of a night, the acidity increases from 0.305 per cent. to 0.81 per cent. (expressed as lactic acid).

Both the digestive action and the production of acid may be largely prevented by desiccation (*see* p. 463). As a matter of fact, the stability of the dried juice is considerable, for, according to Buchner and Rapp (VI. and VIII.) no appreciable diminution

of fermentative power can be detected at the end of a year; and these results were confirmed by R. Albert (I.). The retention of fermentative power by sterile permanent yeast will be mentioned on p. 476.

A similar autofermentation to that occurring in living yeast (*see* chapter lxv.), is also noticed in expressed yeast juice. It is attributed to the glycogen content of the yeast (*see* p. 171, vol. ii.). In conformity with Will's (XX.) discovery of the low percentage of glycogen in Munich pressed yeast, the autofermentation in Munich yeast juice is slight, amounting—according to Buchner and Rapp (VII.)—to not more than corresponds to 0.45 grm. of carbon dioxide per 100 c.c. of the juice. According to E. Buchner (VII.) it is greater in Berlin yeast juice, and corresponds to 0.40–1.10 grms. of carbon dioxide per 100 c.c. of juice. The maximum value—exceeding even the fermentation in presence of saccharose—was that obtained by Macfadyen, Morris and Rowland (I.) with juice from top-fermentation yeast, namely, 65–900 c.c. of gas per 100 c.c. of the juice; unfortunately, however, the cause of this unusually extensive autofermentation was not further investigated. The result is out of harmony with those of E. Buchner (VII.), and is probably attributable to the presence of living bacteria. In a subsequent investigation conducted by Harden and Young (I.), the auto-fermentation, though greater than in Buchner's experiments, did not exhibit such considerable differences as in those of Macfadyen, Morris and Rowland.

Divergent results have also been obtained by the various workers on filtering the juice through a bacterium filter. Whereas Buchner and Rapp (I.), and also Macfadyen, Morris and Rowland (I.), observed merely a diminution in the fermentative power of the juice when forced through a Chamberland filter—especially in comparing the first and subsequent fractions—Wroblewski (III.) found that this function of the juice was entirely destroyed by the treatment in question. Similar results were obtained by Stavenhagen (I.) after filtering the juice through a Kitasato filter. This effect is probably explained by the circumstance that bacteria filters do not permit the transfusion of proteids to more than a slight extent, if at all (*see* vol. i., pp. 99, 100).

Hence the filtration of expressed yeast juice through a very fine filter may result in a loss of proteids, and, therefore, of the fermentative enzyme. For this reason it is important to bear the initial fermentative power of the juice in mind during experiments of this kind; for, since a decrease of this property must be expected from filtration, the initial power must be very high if the juice is to exert any fermentative action at all afterwards. This is the sole explanation of the unfavourable result obtained by Stavenhagen. It should also be mentioned

that Buchner and Rapp (I.), by using a Chamberland filter, obtained a yeast juice that was perfectly devoid of cells, but exhibited active fermentative power despite the entire absence of germs.

§ 319.—Changes set up in Expressed Yeast Juice by External Physical or Chemical Influences, or by Living Organisms.

The influence of temperature on alcoholic fermentation with expressed yeast juice was investigated by E. Buchner (X.), who found that the fermentative power was maintained longest at 5°–7° C., but that fermentation commenced soonest at 28°–30° C., the absolute maximum effect being obtained with a temperature of 12°–14° C. In repeating these experiments, Macfadyen, Morris and Rowland (I.) observed that the fermentative efficiency is increased at higher temperatures; but they left the time factor out of consideration, confining their experiments to a period of only forty-eight hours.

Desiccated yeast juice, on the other hand, will stand higher temperatures without loss of fermentative power. Thus, according to Buchner and Rapp (VI.), yeast juice that has been dried very carefully may be heated to 85° C. for eight hours without suffering any considerable loss of power, and even at 97° C. the fermentative power is not entirely destroyed. Buchner (VIII.) states that the precipitate obtained with alcohol and ether (*see* p. 471) continues to excite fermentation after being heated to 105°–110° C. for four hours; and the same authority (V.) says that the permanent yeast (to be described later on) does not entirely lose its power when heated to 110° C. for six hours in the air, or to 100° C. for eight hours followed by heating for ten hours at 110° C. in a current of hydrogen, though it is destroyed by exposure to 140°–145° C. for an hour.

On the basis of existing knowledge it might be presumed, *a priori*, that the dialysis of the fermentative enzyme through animal membranes would be difficult, if feasible at all; and as a matter of fact, Buchner and Rapp (II. and IV.) have established that the enzyme cannot be extracted from the living yeast by lixiviation, nor can any considerable proportion be obtained by dialysis through parchment paper. Similar results were obtained by R. Albert (I.) with sterile permanent yeast, no fermentative enzyme being extractable with the aid of water or sugar solution unless the cell membranes had been previously destroyed. According to the newer researches of Harden and Young (III.) and Buchner and Antoni (II.) the juice is apparently inoperative at the end of forty-eight hours after having been dialysed through Martin's gelatin filter or in Gürber's apparatus at 0° C., though when united with the concentrated dialysate or with scalded press

juice, it becomes active once again (its power being increased threefold, or even more).

According to Buchner and Rapp (VI.), centrifugalising the expressed juice effects no change in its fermentative power, the various layers of the juice being of equal power before and after the treatment, provided the temperature has been kept within normal limits during the operation.

Alcoholic fermentation is an exothermic process, the decomposition of sugar into alcohol and carbon dioxide being accompanied by a greater disengagement than is observed during the action of other enzymes. This subject has been dealt with by Fitz (XII.), Berthelot (VII.), Bouffard (II.), Brown (VIII.) and Rubner (III. and IV.). Whereas Brown and Berthelot merely calculate that the heat liberated in the formation of the alcohol and carbon dioxide exceeds that requisite for the splitting up of the dextrose, and give the heat value as 67 and 33 calories respectively, Fitz, on the other hand, established by direct experiment that the temperature of an 18 per cent. solution of sugar increased by 18° C. during fermentation, but that 6° C. of this increase was due to a positive increment of heat external to the actual process of fermentation. The same thing was clearly shown in a primitive experiment by E. Buchner (X.), and Bouffard determined the heat of fermentation in a litre of grape must as 23.5 calories. Brown, in repeating this experiment with malt wort, found the heat of fermentation to be 119.2 cal. per gramme of maltose, or 21.4 cal. when referred to the gramme-molecule for comparison with Bouffard's figures. Finally, Rubner worked out accurate methods of determination, and recommended, in the first place, a differential method for estimating the heat of combustion of a nutrient medium before and after the growth of germs, and, secondly, a direct method of determining the heat liberated during the continuance of the vital activity. He determined the heat of combustion of bottom yeast as 4475 gramme-calories per grm. of dry substance, and that of top yeast as 4554 gramme-calories. Further experiments with alcoholic fermentation gave the mean value 149.5 gramme-calories (12 tests) as the heat of fermentation per grm. of saccharose.

In all cases the action of the enzyme is dependent, in a high degree, on the chemical reaction of the test liquid. According to Buchner and Rapp (I. and II.), the fermentative action of expressed yeast juice is accelerated by an addition of small quantities of alkalis, such as potassium carbonate, disodium phosphate and alkali arsenites. Wroblewski (III.) and also Harden and Young (III.) have expressed themselves in a similar sense, the first-named attributing considerable importance to phosphates, both alone and in presence of acids or alkalis. According to the first-named worker (I.), an addition of 0.05 per cent. of hydrochloric acid or acetic acid is injurious to the fermentative action (*see also*

p. 246, vol. ii.). Nitrous acid has a greater restrictive influence on cell-juice fermentation than any of its salts. E. Buchner (X.) found that acetic acid, tartaric acid, and especially lactic acid, had a less injurious effect, the initial diminution in the liberation of carbon dioxide disappearing when the experiment was prolonged for some time. In the case of added lactic acid (0.3 per cent.), the fermentative power was even higher than in the check experiments.

With regard to the influence of other salts on yeast juice fermentation (*see* p. 245, vol. ii.), E. Buchner and Rapp (III. and VIII.) ascertained, by means of quantitative experiments, that 1 per cent. solutions of sodium chloride and ammonium chloride retarded the fermentative power to only a small extent, whereas corresponding solutions of the sulphates of soda, ammonia and magnesia had a more serious effect, and calcium chloride was highly injurious, whereas barium chloride of equal strength was innocuous, and in fact rather favourable. According to Buchner and Antoni (II.), manganese sulphate, aluminium sulphate, ferrous sulphate and cobalt sulphate are either inoperative or only adverse in their effect. Turning to organic substances, urea and glycocoll increase the fermentative power, but, on the other hand, antipeptone, hemi-albuminose and protalbuminose are directly injurious. The phosphates, and especially the secondary phosphates, are particularly beneficial in yeast-juice fermentation, an addition of 1–4 per cent. having good results. Wroblewski (V.) finds that the optimum dose of secondary phosphates is 1.25 per cent. The beneficial effect of these salts is increased by the presence of acids and alkalis; and Wroblewski (V.) credits these salts with protective properties, exercised by neutralising the added acids and alkalis, so that the phosphates guard the living cell from the attacks of acids and bases. Additional matter on this point is furnished by the newer researches of Harden and Young (II.), according to whom the alkali phosphate added to the yeast juice is no longer precipitable by magnesia mixture after the fermentation is ended—a behaviour indicating the formation of an organic compound of phosphoric acid in the juice. From experiments made by Buchner and Antoni (II.), it appears that the addition of lecithin has considerable influence on zymase fermentation; and these authorities state that the active principle may consist of organic compounds of phosphoric acid.

Buchner and Rapp (VIII.) report that an extensive liberation of nitrogen follows the addition of nitrites to expressed yeast juice. The process is a purely chemical one and originates in the action of the amino acids and other amino compounds of the juice on the nitrites. The same observation was reported by Wroblewski (IV.), who also found that an addition of 0.25 per cent. of sodium nitrite increased the fermentative activity of yeast juice.

Although the yeast juice of itself, and particularly in view of the large proportion of added sugar, is bound to restrict the development of micro-organisms, it has been considered advisable to add some antiseptic in carrying on fermentations with the juice. According to Buchner (X.), corrosive sublimate renders yeast juice very turbid and destroys the fermentative power; and it is stated by Buchner and Antoni (I.) that the same effect is produced by even a 0.55 per cent. solution of ammonium fluoride or sodium fluoride. Sodium azoimide (dose 0.36–0.71 per cent.) diminishes the fermentative power, whereas the converse result is reported of a 0.5 per cent. solution of quinine sulphate by Palladin (I.), Gromow and Grigoriew (I.) and also by Buchner and Antoni (I.).

It is well known that hydrocyanic acid temporarily arrests the activity of most enzymes completely, the effect passing off when the volatile acid has been expelled by passing a current of air through the liquid. This behaviour has also been observed by Buchner and Rapp (I.) with the enzymes of alcoholic fermentation. In the earlier experiments of these workers (1–4), in yeast juice fermentations, extensive use was made of arsenites (*see* p. 244, vol. ii.) as antiseptics, but these were afterwards abandoned. In this connection, Abeles has pointed out that substances entering into direct combination with the proteids of yeast juice—an observation previously made by Biernacki (I.)—lose their toxic properties toward micro-organisms, and that 2 per cent. of sodium arsenite is incapable of restricting either the growth or the fermentative power of the cells, a portion of the latter being still active after the fixation of the arsenite. In consequence of this observation the use of arsenites was abandoned, more particularly because of their irregular action on fermentation under certain conditions. For instance, an addition of 2 per cent. of arsenite prevented fermentation by yeast juice when the yeast had been stored, or the juice had been dialysed or diluted, or finally when dried juice (prepared at 35° C.) was used. Buchner and Rapp (VII.) attribute this peculiar behaviour to the disappearance or diminution of the high molecular proteids, so that proteids afford a certain amount of protection against the injurious action of arsenite, the same effect being produced by sugar when added in considerable quantity with, or directly after, the arsenite. The addition of 5 per cent. of arsenite completely arrests the fermentative power of the juice.

The influence of formaldehyde was investigated by Wroblewski (IV.), who found that an addition of 0.05 per cent. reduced the fermentative action to a very low level, whereas Macfadyen, Morris and Rowland (I.) observed a favourable effect with an addition of 0.0005 per cent. Buchner and Antoni (I.) found that the fermentative power was reduced to one-fifth by 0.12 per cent., and to between one-third and three-

fifths by 0.24 per cent. in the case of active juice. Wroblewski (IV.) also investigated the influence of hydroxylamine hydrochloride, and found that an additional 0.65 per cent. extinguished the fermentative power of the juice.

Other antiseptics that have found extensive use in fermentation experiments are chloroform, thymol and toluene (*see* p. 247, vol. ii.). Now chloroform, though applicable for this purpose, causes a slight premature separation of proteids. Thymol is better, but is surpassed by toluene, which latter has also been largely employed by E. Fischer and P. Lindner (II.). Both of them possess sufficient antiseptic power. R. Albert (I.) claimed that higher fermentation values are obtainable in presence of thymol than with toluene, but the accuracy of the previous statement was afterwards confirmed by Buchner (VII.). In the experiments of Macfadyen, Morris and Rowland (I.) on the influence of antiseptics on yeast juice fermentation, the results were so contradictory in presence of sugar, that the authors admitted the desirability of further experimentation on this point, which task was afterwards undertaken by Harden and Young (I.).

Glycerin and saccharose in large quantities also restrict development, living organisms either dying off quickly or at least losing their reproductive power in strong solutions of glycerin. Buchner (X.), however, found that fermentation with yeast juice still continued vigorously, even when the total content of glycerin or saccharose attained 45 per cent.

The influence of various quantities of sugar on the progress of yeast-juice fermentation may also be dealt with in this place. Buchner and Rapp (VII.) found that the quantity of carbon dioxide liberated by yeast juice attained the maximum in presence of a large addition of sugar (30–40 per cent.). Conversely, a small quantity of sugar (5–15 per cent) must be selected when the fermentation is desired to terminate early. Macfadyen, Morris and Rowland (I.) obtained diametrically opposite results, the erroneous character of which, however, was pointed out by Harden and Young (I.).

Finally, alcohol must be mentioned as an antiseptic, and also as a precipitant. Experiments in this connection with yeast juice were carried out by Herzog (I.), Wroblewski (IV.) and Buchner and Antoni (I.). The first and two last-named of these workers found that the fermentative action of yeast juice on sugar is diminished as the amount of added alcohol is increased. The fermentative power of the juice, however, was not finally extinguished by 15 per cent. of alcohol; and it would seem as though the limit of the production of alcohol in cell-less fermentation were higher than with living yeast cells (*see also* p. 240, vol. ii.). According to the researches of Wroblewski the addition of 10 per cent. of alcohol restricts fermentation, whilst 20 per cent. arrests it completely.

Alcohol has long been in use as a precipitant for isolating enzymes. Since, however, prolonged contact with alcohol must be avoided with the more stable enzymes (*e.g.*, invertase), caution is all the more necessary in the case of the more sensitive enzyme of alcoholic fermentation. According to Albert and Buchner (III.), the whole of the fermentative enzyme can be recovered, without loss, when the expressed yeast juice is treated with at least twelve times its own volume of absolute alcohol—or preferably with a mixture of 800 c.c. of absolute alcohol and 400 c.c. of ether per 100 c.c. of yeast juice—the liquid being aspirated off as rapidly as possible, and the precipitate washed with ether and dried over sulphuric acid in a vacuum desiccator. The precipitated fermentative enzyme is completely soluble in water containing 2.5–20 per cent. of glycerin. In order to retain the fermentative power of the precipitate intact, the simplest method is to suspend it in the solvent. The portion dissolved by the aid of water and glycerin can be reprecipitated with ether-alcohol, without greatly impairing the efficiency of the preparation. The proteids are, of course, the chief constituent of the precipitates, so that only an insignificant proportion of the total weight of the precipitate consists of fermentative enzyme.

When methyl alcohol is used as precipitant (*see* p. 242, vol. ii.), the fermentative power of the precipitate is, strange to say, entirely destroyed. Ether by itself, as first pointed out by Will (XXXV.), causes the production of a jelly, which is rich in the fermentative enzyme. Favourable results have also been obtained with acetone for precipitating the proteids of yeast juice. At first the present writer made the mistake of using an insufficient quantity of the acetone, at least a tenfold volume being requisite for throwing down the whole of the fermentative enzyme. According to the later researches of Buchner and Antoni (II.) the precipitates obtained with smaller quantities of acetone are deficient in the phosphoric acid compounds essential to successful fermentation, whilst increasing the quantity of acetone causes an increased proportion of saline matter to be thrown down in the precipitate.

In addition to the foregoing reagents, Buchner (X.) employed ammonium sulphate and also cholesterin, according to the method of Brücke, as a precipitant of the fermentative enzyme of yeast juice. In the former case, however, no fermentation could be observed at all, and merely traces in the latter. Ahrens (I.) used zinc sulphate and alcohol, and Wroblewski (IV.) ammonium sulphate, for the same purpose, but neither of them tested the fermentative power of the precipitates. Wroblewski employed partial precipitation, and obtained five precipitates with an equal number of filtrates. In this partial precipitation the proteids—which, as already mentioned (p. 462) are coagulated at different temperatures—are thrown down in an incomplete manner. Finally, R. Green (III.) also confirmed the fact that the fermen-

tative enzyme is carried down with the precipitates produced in yeast juice.

Mention may be made in this place of the investigations carried out with diluted yeast juice. Wroblewski (V) ascertained that dilution is accompanied by an unexpected extensive weakening of the fermentative power, whilst Macfadyen, Morris and Rowland (I.) found that the addition of an equal volume of water noticeably retarded the autofermentation of the juice, and that double the quantity arrested the liberation of gas almost completely. Buchner (VII.), however, in repeating these experiments with greater accuracy, obtained entirely different results, no perceptible decrease of fermentative power being obtained by diluting the juice with a fourfold volume of 9 per cent. sugar solution; whilst it was only on dilution with 2–3 volumes of water that a gradual diminution was noticeable, and even this did not exceed 20–25 per cent. of the total fermentative power. Buchner (X.), moreover, was able to observe the occurrence of fermentation, in certain circumstances, even when the juice had been diluted twenty-fivefold. According to the recent investigations of Harden and Young (I.), the dilution of the juice obtained from top-fermentation yeast has only a very slight influence on its autofermentation.

The fermentative enzyme suffers injury through digestive enzymes. As already mentioned, the fermentative power of yeast juice diminishes rapidily during storage, a result attributed to the proteolytic enzymes of the juice (*see* chap. lxvi.). Buchner and Rapp (I.) have shown that when yeast juice is treated with trypsin, papayotin or pancreatin, it loses its fermentative power more rapidly than the check samples, either on account of the direct action of the digestive enzymes, or indirectly in consequence of the decomposition of the high molecular proteids that protect the fermentative enzyme.

One of the most essential conditions for the investigation of cell-less fermentation is, of course, to exclude all action on the part of living organisms. H. Lange (II.) tried to ascertain how far the presence of yeast cells in the crude juice affects the development of fermentation phenomena, and found that even when the proportion was ten times greater than the normal, it was incapable of setting up fermentation with anything approaching the same degree of vigour in concentrated solutions of sugar. Similar results were obtained, in this connection, by H. Will (XX.). Buchner and Rapp (I.) made intentional additions of yeast cells and stale juice contaminated with bacteria; but in no case did the fermentative effect surpass that of the fresh juice. It should also be mentioned that—according to the experiments of Geret (I.) and Rapp (II.)—sterile permanent yeast, and therefore also the fluid contents of yeast, possess certain bactericidal properties.

§ 320.—Buchner's Zymase or Alcoholase.

E. Buchner explained the fermentative effect of expressed yeast juice as being due to the action of an enzyme, which he proposed to call zymase. This was the name applied by Béchamp (VIII.) in 1872, for the enzyme we now term invertase; whilst other workers regard the word zymase as synonymous with yeast enzymes in general. In order to prevent the misunderstandings likely to arise from this multiplicity of meanings, we will in future refer to the enzyme of alcoholic fermentation as "alcoholase."

At present nothing definite can be stated with regard to the chemical nature of alcoholase. This enzyme forms a merely insignificant proportion of expressed yeast juice. According to Wroblewski (V.), it is colloidal. Certain other facts speak in favour of its proteid nature, whilst others again indicate a morphological connection. Since the enzyme has not yet been isolated in a pure state, we can only deduce its nature from experiments with yeast juice. The characteristic property is its ability to split up certain sugars—compare Buchner and Meisenheimer (II.). In the dried state it appears to be fairly stable. It is incapable of dialysing through the cell membrane. Under certain conditions it is not destroyed by heating. It is sensitive in variable degree toward chemical reagents, acids being injurious, whereas alkalis in small quantities are beneficial. It is sensitive toward alcohol, but less so toward alcohol-ether or acetone.

Besides expressed yeast juice we have another preparation of yeast suitable for the study of alcoholase and other yeast enzymes, namely, sterile permanent yeast. Ordinarily, the fermentation technologist applies the term permanent yeast to yeast prepared so as to enable it to be despatched to considerable distances; but the substance we are now considering must not be confounded with this. Owing to the difficulties in the way of investigators preparing yeast juice themselves, or obtaining it in large quantities for the purpose of further research into the nature of alcoholase, considerable value attaches to a preparation that can be made by any one without any special appliances, and that is also very stable. Furthermore, permanent yeast presents many advantages over yeast juice as a material for the investigation of fermentation phenomena, inasmuch as the whole of the alcoholase can be recovered from the prepared yeast by a skilled operator. The presence of the uninjured cells give rise to difficulty only in certain investigations, *e.g.*, the extraction of alcoholase; and in such cases the cells must first be opened by trituration with or without sand.

The basis of the methods of preparing permanent yeast consists in the elimination of water, which is effected either by

careful drying or by means of chemical agents, such as alcohol-ether—R. Albert (I.)—or acetone—Albert, Buchner, and Rapp (I.). In the last-named method the operation must not be regarded as one of plasmolysis, effected by the reagent, but as an extraction of moisture, the reagent penetrating the cell membrane and the strata of protoplasm, whereby all chemical reactions are arrested.

The acetonised permanent yeast, prepared from bottom-fermentation beer yeast, and known in commerce as zymin, is a white and practically sterile powder, as dry as dust, and containing 5.5 to 6.5 per cent. of water. The fermentative capacity calculated for 2 grms. of the preparation, disseminated in 10 c.c. of water, with 4 grms. of saccharose, and 0.2 c.c. of toluene as antiseptic, fermented for seventy-two hours at about 22° C., corresponds to 0.96–1.09 grm. of carbon dioxide. According to Albert, Buchner and Rapp (I.), the fermentative power amounts to 0.40–0.49 grm. of carbon dioxide in the first twenty-four hours, 0.36–4.45 grm. during the second similar period, 0.07–0.17 grm. in the third period and 0–0.02 grm. in the fourth. Gromow and Grigoriew (I.) found that the addition of fresh zymin to a sample that has already become enfeebled causes renewed liberation of carbon dioxide to a greater extent than would be the case if the two quantities of zymin had been employed together at the outset. Hence the work of the newly added zymin is facilitated by the fermentation products of the amount employed at first. The preparation of acetonised permanent yeast is protected by patents ; and the product itself is obtainable from Anton Schroder, of 45 Landwehrstrasse, Munich. It has found application for medicinal purposes, and has been used experimentally for baking by Komers and E. von Haunalter (I.) and for the detection of sugar in urine by Münzer (I.).

Sterile permanent yeast is suitable for further investigations on alcoholase. On this account mention may be made in this place of a series of questions, such as the amount of alcoholase present in yeast, and the formation, stability, accumulation and isolation of this enzyme.

Of these, the content and formation of alcoholase in yeast interest us more particularly. For investigations of this kind, permanent yeast has proved especially adapted, since it enables the total content of alcoholase to be ascertained, both previous to fermentation and at any other stage. Observation has long since demonstrated that the amount of alcoholase present in yeast is a factor varying with the physiological condition of the latter. On this point, Will (XXXV.) expresses himself as follows : "It is possible that, like the peptonising enzyme, zymase is present only under certain conditions ; and it is conceivable that yeast which has settled down after primary fermentation, filled with reserve substances and in a certain state of quiescence,

contains very little zymase, if any." According to LANGE (II.) the alcoholase content is dependent to some extent on the nitrogen content (*see* p. 259, vol. ii.), which latter is stated by HAYDUCK (V.) to be a measure of the fermentative power of the yeast. GREEN (III.) also found that the formation of alcoholase is an intermittent function; and this view is confirmed by the results of the different workers who obtained expressed yeast juice with little or no fermentative power. The behaviour of yeast that will no longer excite normal fermentation in beer wort after being used a certain number of times is also attributable to this cause. All these facts clearly indicate that the alcoholase content, and therefore the fermentative power, of yeast necessarily vary with the age of the yeast and divers other circumstances as well as with the character of the organism. This is a point that should be borne in mind in further investigations, the more readily so because the preparation of acetonised permanent yeast has afforded us a reliable means of arresting and determining at any stage all the reactions proceeding in the cell.

The alcoholase content of yeast during storage at low temperatures, under ice water, and in the regenerative process, has been traced in this way. The present is a most appropriate moment for devoting closer attention to the regenerative process of HAYDUCK (VI.). Previous to the time of that worker, brewers had frequently noticed that yeast after repeated use gradually ceased to give a satisfactory "break" (*see* p. 187, vol. ii.) and furnished a less compact sedimental yeast (*see* p. 231, vol. ii.). HAYDUCK (V.), uninfluenced by E. C. Hansen's recent discovery of the existence of a multiplicity of yeast species, many of them capable of causing haze in beer, attributed one cause of yeast degeneration (*see* p. 268, vol. ii.) to a surfeit of nitrogenous food (p. 215, vol. ii.), having traced, by analysis, the growing nitrogen content in the dry residue during the repeated employment of the yeast in brewing practice. In 1884 he attempted to lessen this injurious surplus by allowing the yeast to develop in a vigorously aerated wort at a higher temperature than that of the fermentation room, though not so high as to check reproduction. Then, stimulated by the favourable results obtained independently by a brewer, whose name has not transpired, HAYDUCK (VI.) replaced wort by a boiled solution of sugar (hopped in order to suppress bacteria) which, when vigorously aerated, gave a yeast crop comparatively poorer in nitrogen. In the present more complete state of knowledge respecting the nature of fermentation disturbances, there is no need to labour the point that this treatment might occasionally increase the quantity of any pre-existing disease yeasts in the sample, and certainly could not preclude that possibility. The method did not find any practical application. Nevertheless, it is worthy of mention, since, though as stated above it might occasionally lead to highly undesirable

results, and must therefore be regarded as unreliable and defective in principle, it revealed a noteworthy fact, namely, that the yeast treated in this way was rendered more efficient as a fermentative agent than it had been previously. This was confirmed by R. Albert (II.), by preparing and comparing the effect of expressed juice from samples of yeast before and after regenerative treatment. Additional confirmation was supplied by E. Buchner and A. Spitta (I.), on repeating the experiment, acetonised permanent yeast being found more advantageous than yeast juice. From these results it seems probable that the stock of alcoholase in the yeast cells is low during the period when the "head" on the fermenting wort is most abundant, the alcoholase being apparently destroyed as it is formed, and not accumulated. After the yeast has been stored at a temperature of 0° C. the fermentative power is increased by 21 per cent. in three and a half hours, and 17 per cent. in twenty hours. It has also been found that yeast stored under ice water for twenty-four hours does not show any increase or diminution in the quantity of alcoholase present.

According to E. Buchner (X.), regenerated yeast is not that which contains a large store of alcoholase, but such as is capable of producing this enzyme quickly. If the yeast possessed a high initial fermentative power, one can hardly expect any increase from regeneration and storage. The product furnished by regeneration with a 20 per cent. solution of sugar was not particularly good in comparison with that resulting from the use of an 8 per cent. solution. The addition of 1 per cent. of asparagin to Hayduck's solution containing no nitrogenous matter led to a slight diminution in the alcoholase content of the regenerated yeast, without any subsequent recovery during storage. According to the more recent researches of Lange (III.), however, the fermentative power of yeast juice can be increased as much as ninefold if the yeast, before trituration, be immersed in a solution of saccharose containing asparagin as the chief adjunct. From this it appears that asparagin favours the production of alcoholase in the living cell. Further experiments on this point are highly desirable.

Whereas, in the experiments with yeast juice, the stability of the alcoholase was found to be very low, the fermentative power remained practically unimpaired at the end of twelve months in the case of specially well-dried juice (*see* p. 465). According to Albert, Buchner and Rapp (I.) a similar result was obtained with acetonised permanent yeast, the fermentative power of which decreased only by 10–19 per cent. at the end of six months' storage in tightly stoppered bottles at room temperature. Possibly the tendency toward loss of germinating power could be still further prevented by diminishing the content of water.

Buchner (III.) attributes the diminution of fermenting

power to the action of endotryptase, which is formed in the yeast cells under certain conditions, but may also disappear again. A high temperature seems to assist the development and action of this enzyme. Up to the present it has not been found possible to separate alcoholase and endotryptase, both enzymes being apparently acted on equally by favourable or unfavourable influences, and on this account several points still remain unsolved. A. HARDEN (I.) found that serum strengthens the fermentative power of yeast juice, and observed at the same time a retarding influence on endotryptase (*see also* the remarks on quinine sulphate, p. 469).

The isolation of alcoholase is a highly important question. When it is remembered that none of the other enzymes has been completely isolated up to the present, a satisfactory solution of this problem can hardly be expected in view of the short time that has elapsed since the discovery of alcoholase. Nevertheless, a certain degree of progress has been made, especially when it is remembered that a highly labile substance is in question. In this connection AHRENS (I.) succeeded in increasing the fermentative power of yeast juice by freezing out the water. Other experiments in the same direction relate to precipitation with alcohol, and more particularly with alcohol-ether or acetone. AHRENS (I.) cooled the juice down to -2° C., and obtained by this means a loose mass of ice, which consisted chiefly of pure water and was separable from the liquid constituents. By repeating this treatment a product of increased fermentative power was obtained, and MEISENHEIMER (I.), working on the same lines, found that the increase amounted to about 48 per cent. in the lowermost (5th) stratum. The precipitation of yeast juice by alcohol-ether or acetone has been already mentioned on p. 471; but this treatment does not effect any concentration of the alcoholase, the whole of the proteids being thrown down at the same time. The question whether fractional precipitation would furnish the desired result was examined by ALBERT and BUCHNER (III.), who found that while the first precipitation with a little alcohol throws down the bulk of the proteids, the enzyme is also deposited, and the second precipitate, with a larger amount of alcohol, is devoid of fermentative power. Experiments of this kind suffer from the circumstance that the precipitates are only very gradually soluble in water; and whilst it is true that solution is facilitated by an addition of glycerin, the resulting advantage is slight, since all the admixtures present are thrown down during the reprecipitation, and consequently no concentration of the enzyme is secured. The same thing happens when acetone is used as precipitant. A more favourable prospect was afforded by the experiments of R. ALBERT (I.), performed with extracts from sterile permanent yeast. Since this material still contains the unbruised cells, and therefore the enzyme cannot

be extracted under such conditions, the cell walls and envelope of coagulated protoplasm must be broken down before proceeding to the operation of extracting the enzyme with glycerin and water. Albert (I.) therefore allows 50 grms. of the permanent yeast to dry along with 100 grms. of quartz sand, and then triturates the mass with 100 c.c. of water, the liquid portion being afterwards separated from the solids by means of hydraulic pressure or an aspirator. Precipitation with alcohol ether furnishes 2–3 grms. of a yellowish white powder, which differs from the yeast juice precipitates in being readily soluble, and of being precipitable again and again without appreciable loss of fermentative power. With regard to these experiments, all that need be mentioned is that the use of quartz sand in triturating permanent yeast is not free from objection, since dry grinding for ten minutes results in a noticeable diminution of fermentative power. Nevertheless it is probable that further progress toward the isolation of alcoholase may be accomplished in this manner, especially if loss of fermentative power be still further prevented by discontinuing the use of quartz sand, and if alcohol-ether be replaced by mixtures of acetone and ether or other innocuous precipitants, provided no very high degree of purity is expected in the resulting preparation.

The proof that the fermentative enzyme cannot be extracted from sterile permanent yeast, unless the cells have previously been ruptured by mechanical means, also demonstrates clearly that fermentation goes on inside the yeast cells, and not externally. This also follows from the circumstance that alcoholase cannot be dialysed, and that glycogen is not fermented by beer yeast until the cell membranes have been broken. Hence, alcoholase is an endoenzyme.

§ 321.—The Position of Alcoholase with Relation to the other Enzymes.

Before considering the relative position of alcoholase to the other enzymes, we will devote some attention to the discussions that have attended the discovery of this enzyme. So long as the separation of the fermentative enzyme from the living yeast cells had not become an accomplished fact, differences of opinion between scientists were readily conceivable; but even after the result in question had been achieved, an experimental solution afforded of the highly important problem, and all the errors of reinvestigation corrected, the doubting spirits began to advance other objections. At first it was sought to ascribe the fermentation to micro-organisms still present in the juice; but this objection fell to the ground when active antiseptics were used in all cell-juice fermentations, and after Lange had shown (II.) that the fermentative effect of the juice could not be produced by ten times the number of yeast cells found in the crude juice. Others

put forward the opinion that the liberation of carbon dioxide was due to the fission of the plasma, or other causes. Another view that soon obtained prominence and found many supporters was that the fermentation resulted from the insignificant quantity of living plasma still present in the juice—a conception by no means novel in connection with enzymes. Kupfer and Voit (I.), soon after the discovery of cell-less fermentation, expressed the opinion that the same was *probably* due to fragments of protoplasm ; and Abeles (I.) spoke positively in favour of this hypothesis after advancing several proofs in favour of the theory. The same view was shared by Macfadyen, Morris, and Rowland (I.), Beijerinck (XXVII.), Wehmer (XXXIII.), Behrens (XVII.), C. J. Lintner (VII.), Soxhlet (II.), Iwanowski and Obrastzow (I.), and H. Fischer (I. and II.). On the other hand, Duclaux (XXI.), R. Green (IV.), Rey-Pailhade (V.), Pfeffer (VII.), A. Richter (II.), and A. J. J. Vandevelde (II.) supported the purely enzymatic theory of fermentation.

At present we will only refer to the views of Abeles and of Macfadyen, Morris, and Rowland. Abeles (I.) says : "The fermentative power is dependent on the total dissolved, or more properly speaking suspended, organic mass contained in the yeast juice." Albert and Buchner (I.) showed, on the contrary, that a constituent precipitated from the yeast juice still possesses fermentative power when redissolved. If it be urged, on the other hand, in support of Abeles's plasmal theory that yeast reproduction occurs despite the plasmal poison, Abeles correctly points out that the toxic action on organised ferments depends less on the concentration of the poison than on the quantitative ratio between protoplasm and poison. The careful experiments of Buchner and Rapp (VI.) demonstrated that antiseptics which, like toluene and choloroform, do not enter into direct chemical combination with the proteids of yeast juice, will suppress the fermentative action of even large quantities of living yeast cells, and that the carbon dioxide liberated in these circumstances is formed entirely by the amount of stored-up alcoholase left out of consideration by Abeles. The last-named also stated that young cells in particular effect the fermentation of sugar solution (as observed by Wiesner thirty years before) after desiccation and even after exposure to 100 C. for several hours, the cells also retaining their reproductive capacity. Buchner in his heating experiments invariably found that the yeast cells were killed during the process. Macfadyen, Morris, and Rowland (I.), and also Wroblewski (V.), observed that twofold dilution of the yeast juice practically arrested the fermentative power ; and they consider that this behaviour is so greatly opposed to that of enzymes under the same conditions as to constitute a serious objection to the enzyme theory accepted by Buchner. However—as mentioned on p. 472—this result was not obtained in the experiments of Buchner (VII.) or in the

more recent ones of HARDEN and YOUNG (I.). Finally, several physiological reasons may be advanced against the plasmal theory. For instance, the acceptance of this hypothesis affords no explanation of the circumstance that juice incapable of producing fermentation should occasionally be furnished by exceedingly vigorous yeast, although the hypothetical particles of plasma are present in the juice, or of the fact that the fermentative enzyme can be concentrated as was shown by R. ALBERT (II.), BUCHNER and SPITTA (I.) and LANGE (III.). In such case we can hardly assume that an alteration of the whole protoplasm has occurred.

In view of all these facts, and also of the behaviour of yeast juice in presence of toluene and 40 per cent. sugar solution, after centrifugalising, treatment in the Chamberland filter, desiccation, storage, heating, precipitation, extraction of the precipitate, extraction of killed permanent yeast with glycerine and water, and the reprecipitation of such solution, Buchner rightly concludes that no living agent is present in yeast juice. Those holding an opposite opinion will be obliged to furnish a new definition of what is meant by "life"—a course leading merely to useless polemics. It is an indubitable fact that yeast juice does not represent the cell in its entirety, but the cell contents freed from membrane and other insoluble constituents, that is to say, a product forming only part of the erstwhile living cells. To assume that, under certain conditions, the parts that have been separated from the living cells and are totally incapable of growth will be able to continue living, is a novel and highly improbable idea. On the other hand, the hypothesis that these separated, soluble components of the cell have retained their activity and are capable of exerting it under certain conditions, accords with all existing experience and observation, and appears to be correct. Moreover, it has been demonstrated that the fermentative agent forms only an insignificant proportion of the soluble cell-substance, that it is soluble in water; and that it can be precipitated from triturated permanent yeast, redissolved and reprecipitated—properties that had never been expected of living protoplasm.

We may now proceed with the comparison between alcoholase and other enzymes. In the first place it must be remembered that the properties of all known enzymes vary more or less considerably, so that it is by no means surprising to find that the more labile alcoholase exhibits points of difference from other enzymes, which differences do not justify denial of its enzymatic nature. Alcoholase exhibits the same properties as other enzymes, including solubility in water, with or without glycerine; precipitability by alcohol, ether, or acetone; the faculty of being carried down with other precipitated substances, such as calcium phosphate, precipitated protein, &c., and finally, susceptibility toward chemical reagents and protoplasmal poisons. Differences are exhibited to some extent in respect of its

incapability of dialysing, except with difficulty or under certain conditions; in its greater susceptibility to high temperatures—though this is less noticeable in the dry state than when in solution—in which particular it is on a level with urase and the inverting enzyme of *Monilia candida;* and also with regard to the merely occasional appearance of the enzyme in the living cell—in which respect, nevertheless, analogies can be found in the vegetable kingdom. The only important difference, however, consists in the greater amount of heat disengaged by this enzyme, and the slowness of its action. Neumeister (I.) is firmly convinced on this account, that the question is one of collaboration between various substances outside the living cell, which substances have retained the powers they originally possessed in the protoplasm.

The next question that arises is the allocation of alcoholase to its proper sub-group among the enzymes, and also whether it may be present as zymogen in the yeast cell. Buchner (X.) proposes to class alcoholase as the representative of a new sub-group—fermentative enzymes—of the large class of enzymes, whilst Duclaux (XXII.) ranges it with the enzymes of nutrition, and Wroblewski (V.) places it in the third group of catalysers, which are closely allied to the morphotic constituents of protoplasm. E. Buchner does not assume the presence of zymogen in the yeast cell, but Wroblewski (V.), on the other hand, is partly in favour of the existence of such a body.

The method employed in the preparation of sterile permanent yeast may also be applied, with advantage, to other ferments. In this way E. Buchner and J. Meisenheimer (III. and I.) have investigated the enzymes of fermentation by fission fungi, namely, lactic and acetic fermentations, as well as by *Monilia candida* and a lactose yeast; and the same course was adopted by F. Rothenbach and L. Eberlein (I.) with *Bacterium Pasteurianum.* In all these cases, fermentation, or the production of the corresponding acid, was obtained by using sterile preparations of this kind. The same method can also be applied with higher plants, especially when labile enzymes are in question. An enzyme similar to alcoholase was found by Stoklasa, Jelinek, and Vitek (I.), and Stoklasa and Simacek (I.) in sugar beet, peas, potatoes, flowers, meat, and lung tissue; by Marpmann (VIII.) in honey; by Simacek (I.) in pancreas; and by Arnheim and Rosenbaum (I.) in pancreas, muscle, and liver. Living yeast is often preferably employed in dealing with questions of a general nature relating to enzymes; and it is probable that the new preparation, zymin (*see* p. 474), may be suitable for these investigations, on account of its excellent keeping properties, ease in weighing, and definite fermentative power. Indeed, experiments of this kind have already been conducted with zymin by Palladin (I.), Telesnin (I.), Gromow and Grigoriew (I.), Herzog (III.), and Euler (I.).

CHAPTER LXIV.

THE CHEMISTRY OF ALCOHOLIC FERMENTATION.

By Dr. ARMINIUS BAU,
Chemist to the Kaiserbrauerei, Bremen.

§ 322. The Chemistry and Chief Products of Alcoholic Fermentation.

As already remarked in the introduction to vol. i., it must have been noticed, at a very early date, in connection with the production of wine, mead, and other alcoholic beverages from must, diluted honey, and similar raw materials, that the original sweet flavour disappeared, a frothy head being formed and a gas disengaged; and that the effect of the fermented liquor on the human organism was quite different from that of the fresh grape juice or sweet solutions of other substances employed. In spite of this, however, the changes occurring during fermentation long remained in obscurity; and the first researches in this direction were devoted to the origin of the alcohol, rather than to the remarkable formation of the frothy head or the liberation of the pungent-smelling gas. When the Alexandrian school had improved the originally primitive apparatus of distillation, experiments were made in distilling wine, and a product was obtained exhibiting the intoxicating properties of that beverage in an intensified degree. The discovery and preparation of alcohol resulted from the invention and elaboration of methods of distillation. A description of these latter can be found in the commentary on the works of Democritus—who was presumably one of the earliest alchemists of whom we have any knowledge—published by Synesios, who studied in Alexandria at the beginning of the fifth century. It is known that wine was distilled as long ago as the eighth century of our era, spirit of wine having been referred to in the writings of the alchemist Geber. It is also probable that early attempts were made to concentrate and rectify the aqueous distillate from wine by redistillation; and the Spaniard, Raymundus Lullus (1235–1315), found that this result could be effected by treating the distillate with caustic potash, followed by redistillation. About the year 1413, Basilius Valentinus wrote a clearer description of the method for obtaining a more highly concentrated product; but

it was not until 1796 that the Russian chemist, Tobias Lowitz, succeeded in preparing anhydrous alcohol by combining the use of hygroscopic agents with fractional distillation.

On account of its origin, alcohol—which name is derived from the Arabic—was known as spiritus vini (spirit of wine) or spiritus vitis (grape spirit). The scientific name of this fermentation product is ethyl alcohol, or, according to the newer terminology, methyl carbinol. The chemical formula is CH_3–CH_2.OH, the methyl group CH_3 being saturated with the carbinol CH_2 (OH), or, as regarded from another point of view, the ethyl group C_2H_5 with the hydroxyl (OH). Ethyl alcohol is generally termed alcohol, for short, so that when this latter name is encountered in the literature, ethyl alcohol is always implied. In all other cases, the special nature of the alcohol is indicated by a prefix, *e.g.*, methyl, propyl, butyl, &c.

The manner in which alcohol is formed remained unknown until J. J. Becher, in 1669, expressed the opinion that saccharine liquids alone are capable of fermentation. The nature of the gas liberated during the process also remained unknown for a long time. It is true that B. van Helmont (1577–1644), who was a chemist as well as a physician, again pointed to the fact that a liberation of "gas" occurs during alcoholic fermentation. Nevertheless we are unable to gather from his work, *Ortus medicinæ vel opera et opuscula omnia* (first printed in 1648) whether he was really aware of the nature of the gas in question; and it was left for MacBride, in 1764, to correlate the gas of fermentation with the gas carbonum, or gas sylvestre as van Helmont called it, and to establish its identity with carbon dioxide (CO_2).

With regard to the quantitative yield of the main products of fermentation, Cavendish found the amount of carbon dioxide formed equal to 27 per cent. of the sugar decomposed. Lavoisier (II.) then attempted to determine the quantitative yield of both products, and obtained from 1 cwt. of sugar 35 lb. 5 oz. 4 dr. 19 grs. of carbon dioxide and 57 lb. 11 oz. 14 dr. 19 grs. of anhydrous alcohol, 4 lb. 1 oz. 4 dr. 3 gr. of sugar remaining undecomposed. Expressed in percentages these results gave 36.8 per cent. of carbon dioxide and 60.1 per cent. of alcohol. The fact that these values differ from those obtained at a later date is not surprising, because, on the one hand, analytical methods were not so well developed then as they are now, and, on the other hand, errors in the recovery of the decomposition products were induced by the enormous weight (1 cwt.) of sugar used. It may also be mentioned that Dubrunfaut (IV.), about fifty years later, employed 2559 kilos. of sugar in a single fermentation experiment. Under such conditions it required the genius of a Lavoisier, a Gay-Lussac, or a Dubrunfaut to obtain values in any way approaching the truth.

On the basis of his experiments, Gay-Lussac (II.) established the equation of fermentation as:

$$\underset{\text{Saccharose.}}{C_{12}H_{24}O_{12}} = \underset{\text{Alcohol.}}{4C_2H_6O} + \underset{\text{Carbon dioxide.}}{2CO_2}$$

(The formulæ have been rearranged in accordance with the atomic weights now accepted.) He, however, committed the error of giving an inaccurate composition of the sugar; but this was corrected by Dumas and Boullay (I.), who showed that Gay-Lussac's equation was true solely for glucose $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. As will be set forth more fully on p. 511, saccharose cannot be fermented until it has taken up a molecule of water. The products of the equation given above would necessarily amount to 48.89 per cent. of carbon dioxide and 51.11 per cent. of alcohol. Dubrunfaut (IV.) obtained in his experiment 45.17 per cent. of carbon dioxide and 46.15 per cent. of alcohol, and demonstrated that the theoretical yield of both products was unattainable.

Pasteur (XXXI.) prepared the way for more precise investigation by showing the constant appearance of by-products in alcoholic fermentation, whilst a portion of the sugar is consumed in building up the cell-substance. Elion (V.) proved that yeast assimilates sugar during fermentation, which sugar is not transformed into fermentation products. According to Wortmann (XX.) again, about 5 per cent. of the sugar present is consumed by the yeast, for the elaboration of its own substance and not for fermentation. This latter term, strictly speaking, is confined to the process of decomposition that attacks about 95 per cent. of the sugar; and therefore the full amount of the sugar cannot, in any case, undergo decomposition into alcohol and carbon dioxide.

It would occupy too much space and weary the reader to recount all the various attempts made to determine quantitatively the main products of fermentation, and we will, therefore, mention only those giving the highest results. Thus, Pasteur (XXXI.) obtained 46.4 per cent. of carbon dioxide, Jodlbauer (I.) 46.54 per cent., and Kosutány (I.) 47.5 per cent.; whilst with regard to alchohol Pasteur obtained 48.3 per cent., Jodlbauer 48.67 per cent., and Kosutány 47.5–48.08 per cent. Hence, in the most favourable instances, the proportion of the theoretical yield amounted to 95.2 per cent. in respect of both alcohol and carbon dioxide; so that, for the bulk of the sugar decomposed during alcoholic fermentation, the equation

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O$$

— which was not directly challenged by Pasteur (XXXI.) — still holds good.

We will also consider the case of cell-less fermentation (*see* p. 464, vol. ii.). Büchner and Rapp (IX.), working with 26 grms. of saccharose, obtained 12.2 grms. of carbon dioxide and 12.4 grms.

of alcohol, figures corresponding to 46.9 per cent. and 47.6 per cent. respectively. In a second experiment, however, these workers (2) obtained 50.4 per cent. of alcohol; but these figures should not be compared with those given above, the latter having been obtained with grape sugar (glucose, *see* p. 513), which furnished, theoretically, 48.89 per cent. of carbon dioxide and 51.11 per cent. of alcohol, whereas not more than 51.45 per cent. and 53.62 per cent. respectively can be formed from saccharose.

In order to obtain the above-mentioned yields highly favourable working conditions are essential; but it is immaterial whether the whole of the sugar is fermented or a portion remains behind, provided the weight of sugar actually fermented be ascertained and referred to the alcohol and carbon dioxide recovered. Jodlbauer (I). points out that, with increasing age, yeast undergoes some modification, inasmuch as the quantity of carbon dioxide produced from the fully fermented sugar diminishes progressively. This is true of pure yeast as well as of ordinary yeast. Jodlbauer obtained 49.02, 48.97, and 49.17 per cent. of carbon dioxide from saccharose, when using fresh yeast, but only 47.67, 47.44, and 46.98 per cent. with the same yeast grown old. If, on the other hand, instead of interrupting the fermentation directly after the sugar has completely disappeared, the carbon dioxide determination be carried further, a surplus of carbon dioxide, formed by the autofermentation (*see* § 334) of the yeast, is often obtained.

The ratio of alcohol to carbon dioxide produced is therefore approximately 1 : 1; and in zymase fermentation—according to Buchner and Hahn (I.)—it varies between 1 : 0.90 and 1 : 1.01. However, if the ratio be examined during the various stages of fermentation, it appears—according to Lindet and Marsais (I.)—that the proportion of carbon dioxide to alcohol is lower at the beginning of the process than it is toward the end. These workers obtained the following relative values of alcohol : carbon dioxide at the start :

1 : 0.93, 1 : 0.79, 1 : 0.89, 1 : 0.91, and 1 : 0.79,

and

1 : 1.01, 1 : 1.00, 1 : 1.09, 1 : 1.03, and 1 : 1.02

at the end. The results were unaffected by the fermentation temperature or by the presence or absence of acid in the wort. The amount of yeast produced per 1 grm. of alcohol was found by Lindet and Marsais to range from 0.048 grm. in the initial stage of fermentation to 0.0002 grm. at the end.

The simplest way of expressing the chemical reaction of fermentation is by the equation already given, viz.:

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O.$$

Of course the decomposition of the sugar is not effected in this elementary manner. As far back as 1858, Traube (I.) expressed

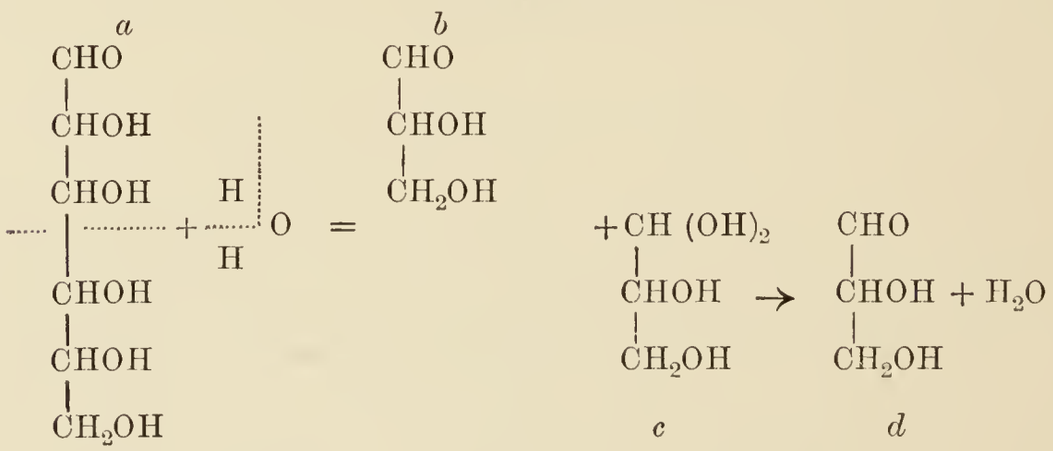
the opinion that the changes suffered by organic substances through the action of ferments—fermentation—are nearly always effected with the active collaboration of water. Similarly, Hoppe-Seyler (VII.) wrote: "All fermentative processes go on in an undisturbed manner only when the solutions are in a sufficiently diluted state; and the chemical collaboration of water seems to be essential in all cases." Hence, the equation of alcoholic fermentation should be expressed as:

$$C_6H_{12}O_6 + 2H_2O = 2H_2CO_3 + 2C_2H_6O,$$

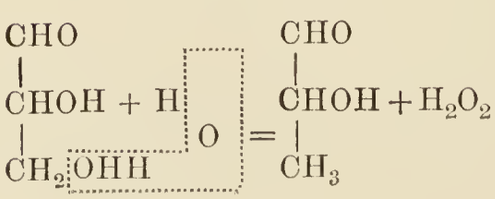
carbonic acid being formed in addition to alcohol.

A slight divergence may be permitted here. The substance generally called "carbonic acid" is really carbon dioxide or carbonic anhydride, CO_2, the acid properly so-called having the formula H_2CO_3. This latter readily decomposes into carbon dioxide and water, in accordance with the equation $H_2CO_3 = H_2O + C_2O$, so that it is merely a temporary intermediate product. According to A. Baeyer (I.) alcoholic fermentation proceeds in two stages. In the first of these, the hydroxyl groups of the sugar undergo displacement, accompanied by reduction of the one series of carbohydrate groups and an accumulation of oxygen in the other, the carbon chain then being subjected to fission where the oxygen has accumulated. This results in the formation of either the extreme anhydride of ethyl-carbonic acid or that of lactic acid—corresponding to alcoholic fermentation on the one hand and lactic fermentation on the other. Other workers, *e.g.*, Wagner (II.), Rayman and Kruis (I.), also look upon the alternate hydration and dehydration of the carbon atom as probable, without, however, going more fully into the elucidation of the fermentation process.

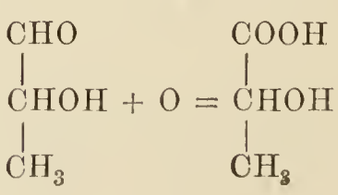
Formula I.



Formula II.



Formula III.



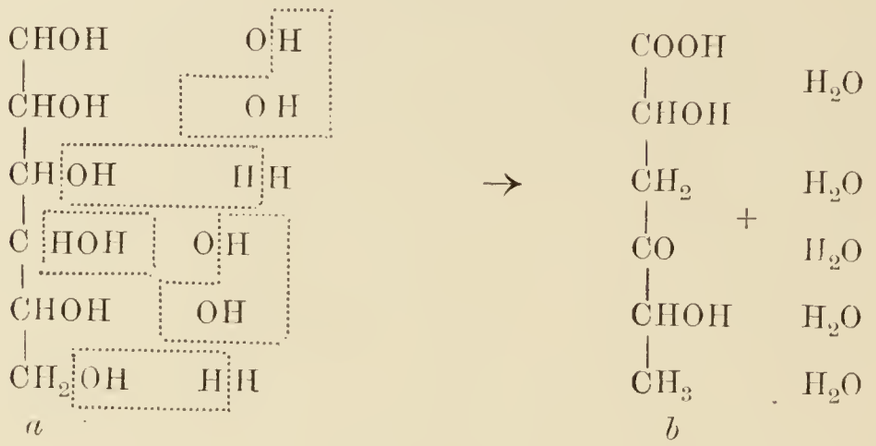
Nencki (V.) is also of opinion that the absorption of water into the sugar molecule is essential to alcoholic fermentation, and gives an exhaustive description of the transformation of the sugar into lactic acid. We must follow his train of thought, since Buchner also has brought out a similar hypothesis with regard to alcoholic fermentation. The sugar molecule *a* (*see* Formula I.) takes up one molecule of water and decomposes into dioxypropionic aldehyde *b*, and an intermediate product *c*, which parts with water and is also transformed into dioxypropionic aldehyde *d*. This latter reacts with water (Formula II.) and forms lactic aldehyde and hydrogen peroxide, which decomposes into water and nascent oxygen, the latter then oxidising the lactic aldehyde to lactic acid (Formula III.). Lactic acid, however, contains the elements of the chief products of fermentation, namely, alcohol and carbon dioxide:

$$\begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_3} \end{array} = \mathrm{CO_2} + \begin{array}{l} \mathrm{CH_2OH} \\ | \\ \mathrm{CH_3} \end{array}$$

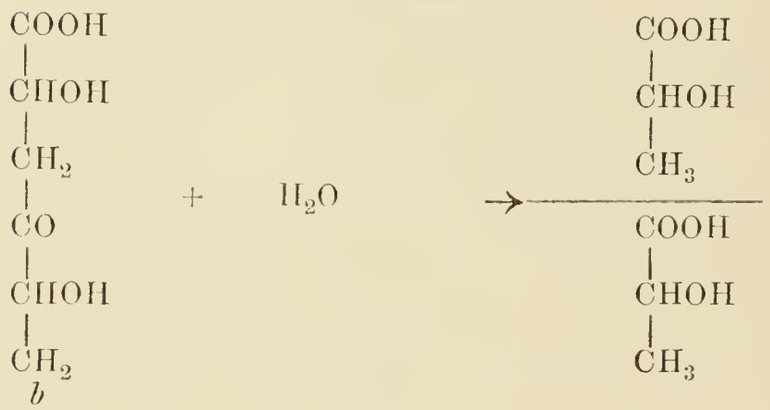
Buchner and Meisenheimer (IV.) detected acetic acid and lactic acid in their cell-less fermentation experiments (*see* p. 475, vol. ii.). The amount of this last-named acid was determined in the fresh yeast juice, the experiment being repeated after leaving the juice to stand four days with and without an addition of sugar. In two experiments without added sugar, the original lactic acid in the yeast juice was found to have disappeared, whilst with added sugar it remained, and in one case increased by 100 per cent. Of course no bacteria were present in the yeast juice. On adding 1.5 grm. of lactic acid to 500 c.c. of yeast juice and leaving for a day, the whole of the acid disappeared; but in other experiments no diminution of the added acid occurred. Subsequently, however, in three sets of experiments the formation of lactic acid was observed—whether an addition of this acid had been given or not—the amount varying, however, in inverse ratio to the quantity of acid added, owing to the adverse influence of the latter. The varying behaviour of the yeast juice samples is probably due to the character of the yeast, the tendency of the juice to produce lactic acid increasing with the age of the yeast from which it has been obtained. In cases, however, where the formation of lactic acid could be observed in the absence of added sugar, the result is obtained at the cost of the glycogen present in the yeast (*see* p. 172, vol. ii.). The chief result of this experiment is that lactic acid plays an important part in the decomposition of sugar, and probably occurs, as an intermediate product, in alcoholic fermentation. This phenomenon may be expressed graphically by the aid of Formula IV. The sugar molecule *a* (glucose) takes up four molecules of water—set down here as

hydroxyl groups and hydrogen atoms—and at once parts with five molecules of water, so that a temporary intermediate product, *b*, a dioxy-γ-ketone acid, is formed. This latter combines with one molecule of water (*see* Formula V.) and decomposes into two molecules of lactic acid. That, from the theoretical point of view, this acid may be regarded as the source of the carbon dioxide and alcohol has already been mentioned on p. 487, vol. ii.

FORMULA IV.



FORMULA V.

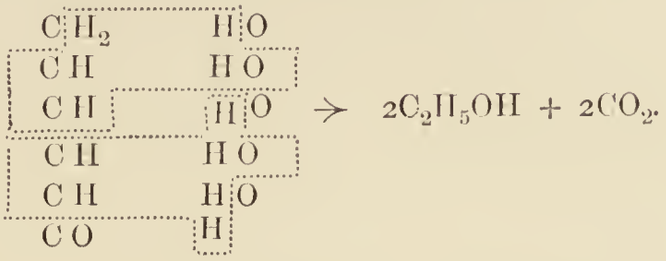


The researches of GRÜSS (I.) led him to conclude that the reducing agent he discovered in yeast (*see* chap. lxvi.) should be regarded as hydrogenase. This would first split up the sugar molecule into carbon dioxide and hydrogen, the latter then causing a separation of alcohol and water from a second and third molecule of sugar, as expressed in the following typical equations:

1. $$\left\{\begin{array}{rcl} CH_2OH + H_2O & \rightarrow & CO_2 + 5H \\ 4(CHOH + H_2O) & \rightarrow & 4(CO_2 + 4H) = 4CO_2 + 8H_2 \\ COH + H_2O & \rightarrow & CO_2 + 3H \end{array}\right.$$

2. $$\left\{\begin{array}{rcl} 2CH_2OH + H_2 & \rightarrow & C_2H_5OH + H_2O \\ 2(4CHOH) + 8H_2 & \rightarrow & 4C_2H_5OH + 4H_2O \\ 2COH + 3H_2 & \rightarrow & C_2H_5OH + H_2O \end{array}\right.$$

According to Grüss, there is a still simpler explanation of alcoholic fermentation expressed by the following formula:



This view is not open to discussion, since it coincides with the old equation $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$, which does not afford the slightest insight into the mechanism of fermentation. The other explanation put forward by Grüss presupposes the collaboration of three molecules of sugar, and is therefore more complex than the hypotheses previously mentioned. A valuable support would naturally be afforded to these latter if one could isolate the intermediate product between sugar and lactic acid, *i.e.*, Nencki's dioxypropionic aldehyde or Buchner's dioxy-γ-ketonic acid.

The remarkable property of yeast juice which brings about the disappearance of lactic acid at one time and its production at another, may be explained by the assumption of two different enzymes, one capable of decomposing sugar into lactic acid and the other transforming this acid into alcohol and carbon dioxide. With both enzymes in excess or continually replenished, only the final products of the reaction will be recoverable. This is the case in fermentation with living yeast, with which there is also the possibility of the intermediate products disappearing in consequence of the phenomena of nutrition. The first enzyme, namely, that decomposing the sugar into lactic acid, has been named zymase, or more precisely yeast zymase, by Buchner and Meisenheimer (II.), whilst they apply the name lactacidase to the second enzyme, by which the lactic acid is transformed into alcohol and carbon dioxide. These workers have abandoned the idea that a dioxy-γ-ketonic acid is formed as an intermediate product between sugar and lactic acid; though as stated by A. Wohl and Nef, methylglyoxal may possibly be formed as a product of this kind. This is in harmony with the circumstance that inactive acid alone is invariably formed in the fermentation of saccharose and glucose, as might be expected with methylglyoxal (CH_3-CO-CHO) as the regular transition product.

The question now arises whether similar transpositions can be effected by purely chemical means, without the intervention of enzymes; and a positive result would render Buchner's hypothesis more feasible. Duclaux's (II.) observation that alcohol and carbon dioxide are formed in a solution of glucose treated with caustic potash (*see* p. 459, vol. ii.), has been confirmed by Buchner and Meisenheimer (I.), who obtained 2 per cent. of alcohol from sugar treated in this way. When caustic potash is replaced by

baryta water or lime water, the carbohydrate yields 50 per cent. of lactic acid, without any production of alcohol, so that the decomposition of lactic acid into alcohol requires the action of the more powerful alkali. If potassium lactate be electrolysed by the KOLBE (I.) method, carbon dioxide and aldehyde are formed; and, according to DOSSIOS (I.), carbon dioxide and acetic acid are produced when lactic acid is warmed with diluted chromic acid. Now, aldehyde and acetic acid are the simplest of derivatives from alcohol, so that a purely chemical proof is afforded of the presence of the atomic aggregations, carbon dioxide and alcohol, in lactic acid. According to Duclaux, calcium lactate in aqueous solution is decomposed in sunlight and air directly to alcohol, calcium carbonate and calcium acetate; and Hanriot states that the same salt furnishes large quantities of alcohol and acetone when heated with caustic lime. Mazé found that *Allescheria* (*Eurotiopsis*) *Gayoni* (*see* pp. 348 and 368, vol. ii.), also produces alcohol when grown in a nutrient solution containing lactic acid.

§ 323. The Non-volatile By-products of Alcoholic Fermentation, Glycerine, Isobutyleneglycol, Succinic Acid, Oxalic Acid, Lactic Acid.

In considering the main products of alcoholic fermentation in the preceding paragraph, it was stated that only about 95 per cent. of the total weight of the sugar is converted into alcohol and carbon dioxide.

LAVOISIER (II.) had already observed the occurrence, in his fermentation experiments, of an organic acid, which he assumed to be acetic acid, corresponding to 2.63 per cent. of the fermented sugar. PASTEUR (XXXI.), however, was the first to emphasise the fact that alcoholic fermentation always results in by-products, the formation of which involves the consumption of about 5–6.5 per cent. of the sugar. In 1857 he named succinic acid—previously discovered by C. SCHMIDT (II.)—as one of these by-products, and in 1858 discovered glycerine, together with fat, cellulose and other (unnamed) substances. Following up the idea that these substances originated in the sugar, he established the following equation for the decomposition of this latter:

$$\underset{\text{Glucose.}}{49C_6H_{12}O_6} + 30H_2O = \underset{\text{Succinic acid.}}{12C_4H_6O_4} + \underset{\text{Glycerine.}}{72C_3H_8O_3} + 30CO_2.$$

From 100 parts, by weight, of sugar subjected to fermentation, Pasteur obtained 3.607–3.64 parts of glycerine, 0.673–0.76 parts of succinic acid, and 1.2–1.3 parts of other substances.

With regard to glycerine, it had already been found by BOUSSINGAULT (II.) that no definite relation exists between this and the sugar consumed; and also that yeast itself furnishes

glycerine when digested with water at 40°–41° C., 30 grs. of yeast yielding 0.19–0.335 grm., or 2.5–2.87 per cent. when referred to the weight of the sugar. The production of glycerine is increased when the fermentation is accelerated by working under reduced pressure, with an augmented quantity of yeast and at a higher temperature. Both Moritz (II.) and also Thylmann and Hilger (I.) found the production of glycerine diminished by retarded fermentation and lower temperature, as well as at temperatures exceeding 35° C. accompanied by the first-named condition, whereas, on the other hand, it is increased on the yeast being provided with added nutriment, on accelerated fermentation, and by using more highly concentrated solutions of sugar. In Moritz's experiments the ratio between the alcohol and glycerine produced ranged from 100 : 9.3 to 100 : 13.8, and in those of Thylmann and Hilger, 100 : 1.638 to 100 : 11.78, *i.e.*, between very wide limits.

Moritz (II.) reports that, according to Müller-Thurgau, the amount of glycerine produced is determined by the greater or smaller vital energy of the yeast, and is in direct relation therewith, the fluctuations in the alcohol-glycerine ratio being thus explained. The absence of a definite relation between the sugar consumed and the glycerine produced was also mentioned by Straub (I.), whose results agreed in other respects with those of Kulisch (IV.) and of Thylmann and Hilger. The amount of glycerine does not increase proportionally with the alcohol content; it is increased by supplying the yeast with an abundance of food, especially such as contain nitrogenous substances.

After Müller-Thurgau, in 1884, had expressed the opinion that glycerine is a metabolic product of yeast and not one of fermentation, the following result was obtained by Wortmann (XX.) from the examination of 41 samples of must, fermented with pure yeast: namely, that the normal ratio of alcohol to glycerine varies between 100 : 7 and 100 : 14. Pure yeasts furnis ha lower average yield of glycerine. The alcohol-glycerine ratio is not a criterion of the quality of the wine. The quantity of glycerine produced is not proportionate to the number of active yeast cells present, but is largely dependent on the specific glycerine-forming capacity of the race of yeast, as well as on the composition of the must. The production of glycerine is not influenced by the ash content of the must, or the quantity of yeast; and there is no mutual relation at all between the various fermentation and metabolic products. At a later date, Wortmann (VII.) expressed himself still more strongly on this point. The amount of any nutrient substance present in must or taken up by the yeast forms no measure of the quantity of any metabolic product obtained. The formation of alcohol and carbon dioxide proceeds quite independently of the formation of glycerine.

Laborde (VII.) also regards the amount of glycerine produced as a characteristic racial feature of the various yeasts, having

obtained 2.5–7.75 per cent. of glycerine (mean 3 per cent.) per 100 grms. of sugar, from one and the same wine must. The production of glycerine is in inverse ratio to the activity of the yeast, and increases more particularly when nitrogenous foodstuffs to which the yeast has not been habituated—such as Liebig's meat extract, yeast water, &c.—are added to the must. An increase is also observed at higher fermentation temperatures, with stronger sugar solutions, and when the medium is strongly acidified with tartaric acid. On the other hand, a diminution is observed when artificial nutrient solutions are used, as well as in sugar solutions to which alcohol has been added previous to fermentation. To some extent the production of glycerine by a given yeast will vary according to the kind of sugar present, 3.15 grms., for instance, being obtained from galactose and inverted milk sugar, as compared with 2.45 grms. from glucose, fructose, saccharose, and maltose under identical conditions. A lactose yeast produced 1.75 grms. of glycerine per 100 grms. of sugar in a solution of lactose, but 3.16 grms. in the case of inverted lactose.

At the commencement of fermentation the formation of glycerine is smaller than toward the end, Effront (XI.), for instance, obtaining at the end of

24	48	72	and 94 hours
0.15	0.35	0.40	and 0.91 per cent. of glycerine.

According to this same worker (XII.), yeast that has been habituated to preservatives (*see* vol. 1, p. 251) will also produce glycerine, the capacity, however, diminishing progressively as the habituation proceeds, so that, eventually, it is possible to obtain fermentations that run their course in accordance with the theoretical equation, no by-products being formed.

A very large number of experiments have been devoted to determining the ratio between alcohol and glycerine in wine; and a few of the figures may be reproduced here. For example, according to Bornträger (XIII.), the ratio in question is 100 : 6.0; Retkow (I.) gives it as 100 : 5.6–12.8 in white wine and 100 : 7.0–11.83 in red wine; and Windisch (II.) 100 : 6.1–10.2. The fluctuation of the values obtained by accurate experiment with reliable wines makes it impossible to place any legal restrictions on the amount of glycerine, and such a measure would only open the door to chicanery.

As regards the formation of glycerine in beer, Borgmann (I.) fermented samples of one and the same wort with two different pure cultures of yeast, and found the beers to contain 0.109 and 0.137 per cent., respectively, of glycerine, the ratio of alcohol to glycerine being therefore 100 : 2.63 and 100 : 3.24. In beers prepared without pure-culture yeasts, the ratio was 100 : 4.14 to 100 : 5.497. Amthor (IV.) also fermented beer worts with pure cultures of eight different races of yeast, and found the

percentage of glycerine remarkably low, being only 0.1113 per cent., as compared with an average of 0.144 in beer from Elsass, and 0.1266 per cent. in Bavarian beer. The minimum ratio of alcohol to glycerine was 100 : 1.65, the maximum being 100 : 4.3, and the mean 10 : 2.38.

Following the example of Pasteur (XXXI.), most workers regarded sugar as the source of glycerine during fermentation. Udransky (I.), however, who took yeast that was free from sugar and contained originally 0.053 per cent. of glycerine, and digested it with alcohol, found that, without any autodigestion having occurred in the yeast, the glycerine content increased by 116–285 per cent. of the original quantity—and even by 355.2 per cent. at the end of 13 months—though no sugar had been added. Lecithin is probably the antecedent from which the glycerine is formed, since, under certain conditions, that substance—which was also found in yeast by Hoppe-Seyler (VII.) (*see* p. 174, vol. ii.)—decomposes into fatty acids, cholin and glycerophosphoric acid, the latter being readily split up into phosphoric acid and glycerine. Duclaux (XXVI.) believes in the existence of special enzymes that furnish glycerine and succinic acid, pure zymase (alcoholase) probably decomposing sugar completely into carbon dioxide and alcohol. Buchner and Rapp (XI.) nevertheless consider that, from the chemical point of view, this decomposition of sugar is a far more complex process than the inversion of sugar, for instance, so that the constant appearance of by-products is not surprising, these being found in all complicated reactions. The problem has been solved by means of an experiment in cell-less fermentation, in which Buchner and Rapp (X.) found that 100 grms. of saccharose furnished 0.5 grm. of glycerine and 0.3 grm. of succinic acid, that is to say, smaller proportions than Pasteur (XXXI.) and others obtained in fermentations with yeast. After Laxa (II.) had discovered a fat-decomposing enzyme, lipase, in yeast (*see* p. 66, vol. ii.), Delbrück (XI. and XII.) expressed the opinion that glycerine is produced by the decomposition of fat (the glycerine ester of a fatty acid) by lipase. A similar hypothesis had previously been advanced by Rommier (II.). Distillery washes always contain fat, from the raw grain used, but in the preparation of wort, most of the fat is left behind, though it may be assumed that a little fat—and especially lecithin—is present in the wort, in an emulsified condition if not in solution. On the other hand, the glycerine content of beer (and also wine) is so high that it can hardly be derived from the fat present in the wort. Yeast, however, according to Nägeli and Loew (III.), always contains fat (*see* p. 173, vol. ii.), small globules of which can be often detected in the cells under the microscope (*see* p. 155, vol. ii.). Fat is therefore occasionally stored up by the yeast cells, and at other times decomposed again by lipase, the fission products (glycerine) finding their way into

the beer or other fermented liquid. It is probable that Buchner's expressed yeast juice contains lipase and fat (from the yeast). This supposition has much to recommend it, since, despite numerous researches, no regular connection has been discovered to exist between the amount of sugar fermented and of glycerine formed.

Isobutylene glycol was found by Henninger (I.) in a red Bordeaux wine, the amount being estimated at 0.05 per cent., or one-fiftieth of the quantity of glycerine present. It is doubtful, however, whether this substance is a fermentation product; and it most probably existed in the must already, or was formed during the process of recovery. According to Butlerow (I.), isobutylene is formed in the decomposition of amyl alcohol by heat. Sanson (I.) frequently detected isobutylene glycol, and according to Windisch (V.) it occurs also in cherry brandy.

Experiments in connection with glycerine have frequently been combined with the determination of succinic acid. Beissenhirtz (I.) is usually credited with the discovery of this substance in alcoholic fermentation, though he only found it in a case of acetous fermentation of a mixture of bread, carob beans, honey, vinegar, brandy, and water. In 1853, Schunck (II.), in the course of an alcoholic fermentation set up, as he believed, by the enzyme of madder—erythrozyme—(*see* p. 459, vol. ii.) observed the formation of carbon dioxide, a little hydrogen, and considerable quantities of alcohol, accompanied by a small amount of succinic acid. However, the proof that this acid is a constant by-product of alcoholic fermentation was due to C. Schmidt (II.) and Pasteur (XXXI.), the latter, as already mentioned, obtaining 0.673–0.76 per cent. of this acid from sugar, and explaining its formation by the equation given on p. 490, vol. ii.

Broadly speaking, the hypotheses on the formation of succinic acid are the same as in respect of glycerine. According to Boussingault (II.), the yield of succinic acid increases with the temperature, quantity of yeast, and reduction of atmospheric pressure; Norin and Cloudon (I.), on the other hand, stating that it decreases when air is excluded, and increases when the fermentation is conducted with access of air. The amount produced during the various stages of fermentation varies. Kayser and Dienert (I.) found that the quantity increases at first, diminishing toward the close of fermentation, whilst Effront (XII.), on the contrary, observed a continuous increase in the amount of this acid, the maximum being reached in the final stages of the process, *e.g.*:

after 24	48	72	and 96 hours
0.025	0.045	0.068	and 0.092 per cent.

Thylmann and Hilger (I.), *inter alia*, ascribe the increase or decrease of succinic acid to the same causes that operate in the case of glycerine.

Exhaustive researches on succinic acid were undertaken by A. Rau (II.), who, for the most part, employed 15 per cent. solutions of saccharose, glucose, and maltose, with or without nutrient substances. Three different yeasts were employed, at temperatures of 15°, 25°, and 35° C., air being excluded in some cases; and the principle of intermittent fermentations also applied. The results showed that the total acidity is considerably increased at the higher temperatures, no alteration in this respect being obtained by the addition of nutrient substances. No great fluctuation was observed in the content of succinic acid, nor does the yield appear to be seriously influenced by the kind of sugar, or the presence or absence of air or of yeast foods. Pure yeast and pressed yeast, with vigorous fermentative power, gave a higher yield of succinic acid than ordinary beer yeast. The formation of the acid goes on, *pari passu*, with the production of alcohol and decomposition of the sugar. With intermittent fermentation, and at 35° C., however, the ratio of alcohol to succinic acid was 100 : 0.439; but in the three subsequent pauses the ratio was 100 : 0.875, 100 : 0.89, and 100 : 0.823 respectively, or practically identical. A comparison of the production of glycerine and succinic acid shows that low temperature, whilst unaffecting the formation of the acid, restricts that of glycerine. The presence or absence of nutrient substances has no influence on the production of the acid, whereas providing the yeast with abundant nutrition causes an increase in the yield of glycerine. (This excessive feeding of the yeast probably leads to an accumulation of fat, which is afterwards decomposed—*see* p. 494, vol. ii.). The production of succinic acid is independent of that of glycerine, and Pasteur's equation (*see* p. 490, vol. ii.) is inapplicable. This view of the formation of succinic acid is also shared by Straub (I.). Duclaux (XXVI.) attributes it (like glycerine) to the action of a separate enzyme, but adduces no proof in support of the hypothesis.

Succinic acid is produced in fermentation with expressed yeast juice. In one case, reported by Buchner and Rapp (X.), 1250 c.c. of the juice contained 0.2 grm. of the acid before fermentation, but after the fermentation of 100 grms. of saccharose, the amount was found to be 0.5 grm., an increase of 0.3 grm.

Opinions are still divided with regard to the source of succinic acid, most workers regarding sugar as the raw material—as in the case of glycerine—though it is worthy of note that the amount of this acid produced under different conditions of fermentation is invariably small. The amount of succinic acid present in beer is also small, *i.e.*, 0.0026–0. 0039 per cent. according to Straub (I.). Blumenthal (I.) states that micro-organisms produce this acid from both carbohydrates and protein, on which account Grüss (II.) believes that the source, in the latter case, is to be found in asparagin, the assumption being that this substance is first transformed by

yeast oxydase into aspartic acid, which is then decomposed into malic acid, according to the equation :

$$2\left|\begin{matrix}CH - CO(NH)\\ CH(NH_2) - COOH\end{matrix}\right. + 3O_2 = 2\left|\begin{matrix}CH - COOH\\ CH(OH) - COOH\end{matrix}\right. + 2N_2 + 2H_2O$$

Asparagin — Malic acid

Owing to the simultaneous presence of a reducing substance (*see* chap. lxvi.) in the yeast cell, the abstraction of the hydroxyl group from the malic acid might be effected by its agency, succinic acid, $COOH\text{-}CH_2\text{-}CH_2\text{-}COOH$, being thus formed. According to the Grüss hypothesis, free nitrogen must be formed ; but this has not yet been observed during alcoholic fermentation. With regard to the quantitative proportions in which this element could appear in comparison with carbon dioxide, 1 grm. of sugar could—taking Pasteur's figures as a basis—yield 464 mgrms. of carbon dioxide and 7.6 mgrms. of succinic acid, the formation of the latter being accompanied—according to the Grüss equation—by the liberation of 1.8 mgrm. of nitrogen. Converted into volume, these figures would be equal, at 15° C. and 760 mm. pressure, to 253 c.c. of carbon dioxide and 1.5 c.c. of nitrogen, or, in round numbers, 0.6 per cent. of the gas. Pasteur (XXXII.), who determined the carbon dioxide volumetrically, does not mention the presence of nitrogen ; but he himself described the experiment as a very delicate one, so that the smaller amount of nitrogen that might be produced with a diminished yield of succinic acid might well escape detection. In order to substantiate the Grüss hypothesis it would be necessary to test the fermentation gases for the presence of free nitrogen, and to show that this latter is formed in direct ratio to the amount of succinic acid produced. It would also have to be proved that yeast actually forms succinic acid from asparagin.

Oxalic acid is formed during fermentation by various organisms, *e.g.*, by *Saccharomyces Hansenii*, Zopf. (XV.)—compare p. 283, vol. ii.—but it has not been definitely found to result from alcoholic fermentation by yeast, though crystals of oxalate (*see* p. 118, vol. ii.) are often observed when yeast is examined under the microscope, their presence being ascribed by Prior (V.) to the formation of small quantities of oxalic acid during fermentation. Whether this originates in the sugar, however, or was already formed in the fermented solution, is quite undecided.

Lactic acid was discovered in certain fermentations by Dubrunfaut (IV.) in 1856, though it should be remembered that he did not work with pure yeast, and, since lactic bacteria are abundant, experiments of this kind, to be worthy of consideration, must be performed with yeast perfectly free from bacteria. Pasteur (XXXI.) was unable to detect any lactic acid in the fermentations with *his* pure yeast ; and only in one single instance did he men-

tion that a very small quantity of the sugar had been converted into lactic acid. Effront (XII.) ascribes the formation of lactic acid to a transformation of the proteid substances. In the fermentation of sugar by expressed yeast juice, Ahrens (I.) observed a non-volatile acid, which he stated to be lactic acid, but Buchner and Meisenheimer (IV.) were the first to prove in an indubitable manner that this acid is a constant by-product of alcoholic fermentation, and originates in the sugar (*see* p. 464, vol. ii.).

§ 324. Volatile Acids and Aldehydes as By-products of Alcoholic Fermentation. Influence of Oxygen on Fermentation.

The acids in § 323 are classified as non-volatile, or fixed acids, and are contrasted, in zymotechnology, with the volatile acids, all of which—so far as concerns those present in fermentation products—belong to the fatty series. (Prior VI.) fermented one and the same beer wort with seventeen different pure yeasts, and determined the acidity in the resulting beers. The quantity of the fixed organic acids formed per 100 c.c. of beer ranged from the equivalent of 2.1 to 5.4 c.c. of decinormal caustic soda, and that of the volatile acids between 2.1 and 5.8 c.c. of alkali. For every 100 c.c. of alkali required to neutralise the fixed organic acids, the quantity consumed in neutralising the volatile acids formed by the various yeasts was 62.4–180.9 c.c. Consequently the acidity due to the several races of yeasts fluctuates between wide limits. According to Alfred Rau (II.), the quantity of volatile acids formed increases considerably at higher fermentation temperatures (35° C.); and pressed yeast produces a larger amount than beer yeast. Straub (I.) found that admission of air increases the production of volatile acids; and according to Biourge (I.), the quantity formed is independent of the alcohol produced. The concentration of the fermenting liquid is without any appreciable influence, but, on the other hand, the yield is proportional to the duration of fermentation, especially if the completely fermented solution be stored for some time. Amthor (II.) found this to be especially the case with *Saccharomyces apiculatus* (*see* p. 434, vol. ii.).

Formic acid is the first member of the volatile series. It was observed here as long ago as 1891, by Kruis and Rayman (II.), in beers that had been left standing in contact with deposited yeast for some years (*see* p. 126, vol. ii.). They also found it in sterile beer worts after prolonged storage, and therefore attributed its formation to a chemical reaction occurring in the wort itself, and probably connected with the transformation of protein. Khoudababachian (I.) detected formic acid in fresh grape must, the quantity increasing during fermentation when the surrounding

conditions were unfavourable to the yeast. According to Liebermann (IV.) and Kiticsan (I.), traces also occur in normal wines, though, as observed by Duclaux (I.), it disappears readily in presence of yeast Thomas (II.) mentions the occurrence of formic acid in aqueous infusions of malt culms; nevertheless it is also formed in fermentation, especially when a large surface of the liquid is exposed and nitrogen compounds are present. Of these latter, urea, either alone or in association with ammonium bicarbonate, is best adapted to increase the output of formic acid, which can be still further augmented by the addition of calcium carbonate. During cell-less fermentation, Buchner and Meisenheimer (I.) found traces of a volatile acid very similar to formic acid. The frequent occurrence of the latter among the products of bacterial fermentation has already been mentioned on p. 181 of vol. i.

Acetic acid was recognised at an early date as a by-product of alcoholic fermentation, by Lavoisier (II.). Duclaux (XXVII.) and Béchamp (XIII.) also found acetic acid constantly, though only as traces; and these observations were confirmed by Schützenberger (II.). Owing to the wipespread occurrence of acetic bacteria, the acid can only be regarded as a by-product of alcoholic fermentation when yeast, free from bacteria, is employed; just as was remarked with regard to lactic acid. Kruis and Rayman (II.) failed to detect acetic acid at all, though they made it the object of special attention; whereas Thomas (II.) obtained it regularly, though merely as traces. Buchner and Meisenheimer (IV. and II.) found it invariably in fermentations with expressed yeast juice free from bacteria; and it is therefore certain that the acid is a normal by-product of alcoholic fermentation. The yeast juice, prior to fermentation, contained 0.004–0.010 per cent. of acetic acid, and afterwards 0.08–0.33 per cent., so that considerable quantities were produced during the operation. The specimens of yeast juice that fermented lactic acid furnished considerable quantities of acetic acid, the converse being the case with such of them as produced lactic acid in abundance. The name glucacetase has been applied to the yeast enzyme that is assumed to split up glucose into three molecules of acetic acid. The hypothesis advanced by Biourge (I.) to account for the formation of acetic acid is that the volatile acids must be regarded as the result of assimilation processes, and not of the decomposition of sugar, yeast itself yielding a considerable amount of acid on distillation. According to Prior (V.), however, it is more feasible to suppose—and this is indicated by the experiments of Giltay and Aberson (II.)—that the oxygen consumed in fermentation oxidises a portion of the alcohol in the interior of the yeast cell. The influence of oxygen on fermentation—a point to which we shall revert shortly—also plays a part in this case. It cannot, however, be credited as the sole cause of the production of acetic acid during fermentation, the experiments

of Buchner and Meisenheimer (IV.) having proved this acid to be a normal by-product of that phenomenon. It is true that the amount so produced is small, though, according to Thylmann and Hilger (I.), it is greater when the solution contains a higher proportion (30–40 per cent.) of sugar. When it is formed to any extent otherwise, its production should perhaps be attributed to the oxidation of the alcohol, probably with the assistance of bacteria. Acetic acid, though only in small quantities, is also found in fusel oils.

The third member of the fatty-acid series is propionic acid, which was found by Winkler (II.) and Béchamp (XIV.) in diseased wines. According to Ordonneau (I.), it is also occasionally present, as an ester, in cognac. Strecker (I.) and Kramer (I.) attribute its origin to lactic acid, in the case of certain fermentations—a point worthy of note, seeing that this acid is a normal by-product of alcoholic fermentation.

Butyric acid, which is readily produced by certain bacteria, was detected—either in the free state, or more frequently combined as an ester—in cognac by Duclaux (XXVIII.) and Ordonneau (I.), and in potato fusel oil and cherry brandy by K. Windisch (III. and IV.).

Valeric acid was found by Kruis and Rayman (II.) in a sterilised wort that had been kept for several years. They ascribed its origin—as well as that of the other higher fatty acids still to be mentioned—to the decomposition of complex nitrogenous foodstuffs, *e.g.*, protein. Duclaux (XXVIII.) has also found it occasionally in diseased wines.

Among the higher fatty acids, caproic acid, caprylic acid, pelargonic acid and capric acid occur, in combination with alcohols as esters, in the fusel oils (*see* next paragraph) and in cognac, whilst œnanthylic acid appears only in wine, or cognac. On this point *see* Pelouze and Liebig (I.), Delffs (I.), Fehling (I.), A. Fischer (I.), Grimm (I.), Duclaux (XXVIII.), Ordonneau (I.), K. Windisch (III. and IV.), Kruis and Rayman (II.), and Schüpphaus (I.).

Taking the total fatty acids as representing 100, Windisch (III.) gives the proportions of the individual fatty acids in the esters as follow: in potato fusel oil 3.5 of acetic acid, 0.5 of butyric acid, 14 of caproic acid, 34 of caprylic acid, 12 of pelargonic acid, and 36 of capric acid; in corn fusel oil, acetic acid 2.7, butyric acid 0.4, caproic acid 13.2, caprylic acid 26.7, pelargonic acid 12.9, and capric acid 44.1. Hilger (I.) also found stearic acid, palmitic acid and lauric acid in a sample of corn spirit. These acids originate undoubtedly from decomposed fat; and it is highly probable that the other higher fatty acids, from butyric acid to capric acid, are produced from the fat (*see* p. 494, vol. ii.) contained in the mash and in the yeast cells, as the result of decomposition by yeast lipase; compare Bau (XXII.).

The aldehydes that are always formed during fermentation should be regarded as intermediate products between the fatty acids and the alcohols. Ordinary aldehyde (acetaldehyde) was observed by Béchamp (XIII.) and Röser (II.). The cause of its formation will be dealt with shortly. As was shown by Durin (II.), it can be easily recovered by strongly cooling the fermentation gases in suitable vessels. In works where yeast is produced by the aeration process, a considerable amount of aldehyde is formed under the influence of atmospheric oxygen, especially in mashes of maize, rice, and malt, the resulting spirit having an evil smell and taste in consequence of its high content of aldehyde—*see* Mærcker (II.). Jaksch (III.) also states that aldehyde is formed during alcoholic fermentation. Kayser (XIII.) regards aldehyde as a product of the activity of the *Saccharomycetes;* and, according to Kruis and Rayman (II.), considerable quantities of this substance are formed when a film is produced and there is a plentiful accession of air. Hence aldehyde is the result of the oxidation of nascent alcohol. According to Ilges, it is not formed during fermentation, but only in the distilling apparatus, by contact of the spirit vapour with air—compare Mærcker (IV.). During the oxidation of alcohol, the formation of aldehyde is accompanied by the production of acetal, the diethyl ether of aldehyde, $CH_3.CH(OC_2H_5)_2$; and, according to Geuther (I.) and Windisch (IV.), it occurs in fairly considerable quantities in fermentation products, whilst Ordonneau (I.) found it in cognac. Its formation can be easily explained, since, according to Durin (II.), alcohol and aldehyde come into contact in the nascent state during the formation of the latter substance, so that the two may unite to acetal, with elimination of one molecule of water.

The influence of oxygen on alcholic fermentation by yeast will now be considered, though its action on cell reproduction and respiration has already been dealt with exhaustively on p. 231 *et seq.*, vol. i. It may be mentioned at once that the favourable influence of aeration on the fermentation of must, worts, and mashes has long been recognised in practice, and was fully established by a series of fermentation technologists between the years 1867 and 1874. Blankenhorn did this in an imperfect manner, then Moritz (partly in collaboration with Haas); also by Molnar in the case of wine musts, and by Adolf Mayer (VII.) with nutrient solutions. More accurate researches were undertaken, with wort by R. Pedersen (I.) in 1878. It was ascertained that aeration increases both the working action on the extract and also the (absolute) reproductive power of the yeast, though the amount of extract consumed per unit weight of the yeast crop is smaller in aerated worts than in others. The difference, however, is not large—as was proved arithmetically by D. Iwanowski (I.) in 1893. In repeating Pedersen's experiment, E. C. Hansen (p. 233, vol. ii.) found that the amount of extract consumed per

cell of the yeast is smaller in cultures that have been aerated. The results of this test, however, cannot be accepted unconditionally, the passage of air being accompanied by subsidiary effects, inasmuch as it sets up vibrations in the wort liquid and thus stimulates reproduction. According to Rapp (I.), the fermentative activity on the other hand is diminished by powerful vibration, so that the aforesaid stimulative effect is counteracted to an extent that has not been precisely determined. Moreover, the air in its passage carries off volatile metabolic products, including those of an injurious character, thus freeing the nutrient medium from poisonous constituents, to an extent varying according to circumstances. This favourable influence is absent in all the parallel experiments in which a similar "rousing" with inert gas has not been performed. If hydrogen (which is very difficult to obtain in a perfectly pure state) be used for this purpose, it is stated by Korff (I.) that the resulting acids differ considerably from those formed when in aerated cultures, a circumstance that will readily be understood in view of the chemical action exerted by atmospheric oxygen.

The difficulty and complication of the task of investigating the influence of oxygen on alcoholic fermentation by means of yeast are increased by the powerful stimulus imparted by this gas to the reproduction of the cells. An attempt to counteract this disturbing factor was made by A. J. Brown (IV.). Starting from his own observation that no appreciable reproduction occurs in wort pitched with a larger number of cells than can be grown therein from a minimum sowing, he made large sowings in a mixture of yeast water and glucose, passing either air, hydrogen, or carbon dioxide through the liquid. By operating in this way it was found that, whilst the number of cells remained practically unaltered throughout the experiment, the amount of sugar fermented was larger in the case of the aerated cultures. The conclusion deduced therefrom, that a given number of cells of approximately the same total weight will ferment more sugar in presence of air than without, was opposed by Duclaux (XVIII.) on arithmetical grounds, which, however, were rejected by Brown (VII.).

Brown's experiments need to be repeated, as was pointed out by H. van Laer (XI.) and Iwanowski (I.), since the assumption on which they are based conflicts with general experience. The absence of any reproduction in the excessive sowing was probably due to some unfavourable constitution of the nutrient medium employed, a factor whose influence has been shown by the experiments of N. von Chudiakow (I.), who supports the view that oxygen has a restrictive influence on alcoholic fermentation. Another circumstance left entirely out of consideration is the fact that a number of cells perish during fermentation and undergo dissolution, so that when the same quantity of yeast is found at the beginning and end of the process, it cannot be assumed, with

certainty, that no reproduction has occurred. An unduly large sowing of yeast also modifies the composition of the nutrient medium, owing to the diffusion of soluble matters from the yeast, more particularly from the dead cells. It is still an open question, however, whether this change persists when air, carbon dioxide, or hydrogen that is not perfectly pure, is blown through the solution.

Although the results of the majority of these experiments tend to indicate that the amount of work performed per unit of yeast is smaller in presence of oxygen, and that oxygen therefore restricts fermentation, they cannot, however, be regarded as decisive. For this to be the case it is an essential condition to show that each cell has been continuously exposed to the influence of the oxygen throughout the entire experiment. Now, in the case of liquid cultures, the yeast cells frequently agglomerate to small lumps, the interior of which cannot be reliably demonstrated to be accessible to oxygen; and the metabolism proceeding inside these lumps differs from that in the outer cells that are exposed to the ascending bubbles of air. This objection applies with still greater force to all cultures on solid media, including streak cultures on sugar-gelatin, the cells below the surface being practically shut off from the oxygen in contact with those on the surface. The only way to afford decisive proof is by experiments in cell-less fermentation with zymase, and therefore the question whether the fermentation set up by yeast is influenced in one way or another by oxygen must be regarded as still unsettled.

Practical experience in the alcohol industry is not opposed to the foregoing particulars. The purpose of the aeration regarded as necessary or useful in the case of fermenting mashes, especially molasses, distillery wash and pressed yeast factories, is mainly to increase the yeast crop and not to augment the fermentative power of the individual cells. Any prolongation of the rousing process beyond the attainment of this object is for the purpose of utilising the favourable supplementary effects of this treatment as indicated on p. 501, vol. ii. Due precaution must be observed in this, since otherwise considerable loss may arise from another supplementary effect, namely, the volatilisation of the alcohol. These circumstances are merely referred to now, to prevent the impression that the fact that aeration accelerates and increases the fermentation of a liquid medium affords proof that oxygen stimulates fermentation. The amount of alcohol carried away by the liberated carbon dioxide was investigated by Eck (I.) as long ago as 1875, in the case of distillery washes; and the loss determined by Riss (I.) in experiments made with a saccharine medium containing mineral salts, amounted to 1.12 per cent., referred to the quantity of alcohol present in the fermented liquid.

Finally, brief consideration may be devoted to Pasteur's conception of alcoholic fermentation as life without air (vol i. p. 20).

The experimental basis on which this theory was constructed, so far as yeast is concerned, was shown to be untenable by Nägeli (II.). The assumption that the plentiful admission of oxygen to cultures of yeast causes this latter to develop like an aerobic thread fungus and not set up alcoholic fermentation has not been proved, such proof entailing the determination of the ratio between the carbon dioxide liberated, the alcohol produced, and the resulting yeast crop. The solution of this task was first undertaken by Giltay and Aberson (II.); but their experiments are open to objection, though not in the direction mentioned by Duclaux (XVIII.) in a criticism refuted by Giltay (I.). This worker and Aberson found that over 60 per cent. of the sugar consumed was converted into alcohol and carbon dioxide, even in strongly aerated yeast cultures in which energetic respiration occurred at the same time. A similar result was obtained by Buchner and Rapp (V.); but neither set of experiments was conclusive, the conclusions being only a matter of probability, and therefore tending to refute the accuracy of Pasteur's hypothesis.

The chemical action of oxygen in alcoholic fermentation is a far simpler question than this physiological influence. As already stated on p. 501, vol. ii., alcohol readily undergoes oxidation to aldehyde and acetic acid. According to Durin (II.), the carbon dioxide liberated during fermentation constitutes a compact froth, and the alcohol distributed all over the surface of the minute bubbles of gas is readily transformed into aldehyde. Röser (II.) also found a larger quantity of aldehyde in fermentation conducted with admission of air than without aeration. In any event, however, some action is exerted by yeast oxydases in connection with the carrying of oxygen, especially when a film is produced by the yeast. If a fermented liquid be left, together with the whole of the yeast, in contact with air for some time, Kruis and Rayman (II.) state that nearly all the alcohol formed during fermentation is oxidised to carbon dioxide and water. The part played by atmospheric oxygen during the storage of wines was referred to by Pasteur (XXXIII.), who found that young wine will retain its original character for a long time when stored out of contact with air. Which of the substances, however, combine with the oxygen, and what products result, still remain undetermined. According to Wortmann (VII.) there is no doubt that considerable changes are produced by atmospheric oxygen during the storage of wine (*see* p. 509, vol. ii.); but the view that has prevailed since Pasteur's time, namely, that the matter is one of oxidation, is not applicable in its entirety since physiological processes probably form a contributory factor. The changes in question are chiefly ascribed by Wortmann to the collaboration of organisms. According to Rapp (I.), the formation of esters is also increased by the access of air—a circumstance that can be easily explained, since the acids resulting from the oxidation of alcohols combine more

readily with alcohol, in the nascent state, to form esters, than is the case with acids already formed.

§ 325. Alcohols and Esters (Bouquet Principles) as Volatile By-products of Alcoholic Fermentation. Other By-products.

Whereas, in the preceding paragraphs, we have dealt with the volatile products belonging to the aliphatic series and containing relatively large amounts of oxygen and little hydrogen—such as the volatile acids and the aldehydes, with the general formulæ, $C_nH_{2n}O_2$ and $C_nH_{2n}O$—we will now proceed to treat of bodies with the formula $C_nH_{2n}+2O$, the first of which series is methyl alcohol, $CH_3.OH$. This is often formed during bacterial fermentation, but may also occur through the decomposition of intermediate products (glucosides) in alcoholic fermentation by yeast. This circumstance will be referred to later on (*see* p. 510, vol. ii.).

The second member of the series, namely, ethyl alcohol, one of the chief products of fermentation, has been dealt with in § 322.

The higher alcohols are abundantly represented in cognac, and in the fusel oils discovered by Scheele (II.) in 1785. They occur in the free state, and also as esters in combination with fatty acids.

Primary propyl alcohol, $CH_3.CH_2.CH_2.OH$, was obtained by Chancel (I.) from the fusel oil of grape-husk spirit, and also by Fittig (I.). According to K. Windisch (III. and IX.), Kruis and Rayman (II.) and Ordonneau (I.) it occurs constantly in crude potato and corn spirit, cherry brandy and cognac.

Secondary, or isopropyl alcohol, $CH_3.CH(OH).CH_3$, is said to have been obtained by Berthelot (VIII.), and was found in crude potato spirit by Rabuteau (II.).

Normal butyl alcohol, $CH_3.(CH_2)_2.CH_2.(OH)$, is formed, according to Fitz (XIII.) and Emmerling (VII.), in the fermentation of glycerine (a constant by-product of alcoholic fermentation) by fission fungi. It was isolated from crude potato spirit by Rabuteau (II.), from crude corn spirit by Emmerling (VII.), and from cognac by Ordonneau (II.). According to this last worker, it is probably a normal product of fermentation by wine yeasts, whereas beer yeast, which furnishes other secondary products, produces isobutyl alcohol, $(CH_3)_2.CH.CH_2.CH_2(OH)$. This last substance had already been obtained by Wurtz (I.) from the crude spirits furnished by beetroot, potatoes, and grain; it was also found by Pierre and Puchot (I.), K. Windisch (III. and IV.), Rabuteau (II.) and by Kruis and Rayman (II.).

Primary pentyl or amyl alcohol, $CH_3.(CH_2)_3.CH_2(OH)$, seems, according to Wyschnegradsky (I.), to occur along with the isomeric amyl alcohols in fusel oil.

Fermentation amyl alcohol, or isoamyl alcohol constitutes the bulk of fusel oil. It occurs as ordinary, optically inactive alcohol, $(CH_3)_2.CH.CH_2.CH_2(OH)$, but, according to Marckwald (I.), is invariably accompanied by the active alcohol

$$CH_3.CH_2\diagup\!\!\diagdown^{CH_3}CH.CH_2(OH)$$

which rotates the plane of polarised light towards the left. Le Bel states that it is converted into the dextro-rotatory modification under the influence of mould fungi. The name, amyl alcohol, was bestowed by Cahours (I.) because the alcohol is formed from materials containing starch. The amyl alcohols are found in all technical fusel oils, and to a smaller extent in cognac as well; compare also Pedler (I.), Krutsch (I. and II.), Balard (I.), and Halenke and Kurtz (I.).

Various hypotheses have been advanced to account for the formation of the fusel oils. According to Breefeld (XVIII.), by-products are formed as soon as the materials requisite for the continued growth of the yeast are exhausted, the yeast dying and decomposition setting in as fermentation progresses. If the higher alcohols originate in normal fermentation, the amount so formed must, *pace* Lindet (IV.), bear a constant relation to the quantity of ethyl alcohol during the various stages. This, however, is not the case, the amount of fusel oils formed during the first fourteen hours being 0.36 part per 100 of alcohol, but 14.07 parts in twenty-four hours. This increase is apparently due to micro-organisms which do not come into action to their full extent until fermentation has terminated. The higher alcohols are products of a secondary fermentation, and are also formed, to a smaller extent, when the fermentation is accelerated by increasing the quantity of pitching yeast, or by adding sterile beer wort.

Perdrix (I.) established the fact that bacteria are able to produce fusel oils, this worker having isolated from Seine water a bacterium that furnished amyl alcohol. Péreire and Guignard (I.) found a similar bacillus in calcareous waters, and thought of utilising it technically; and Pringsheim (I.) described an amylic bacillus, isolated from American potatoes. Perdrix's bacillus, however, produces such a small quantity of this alcohol that it cannot be regarded as the sole agent in the formation of fusel oils. The experiments of Lindet (IV.), which showed that the amount of higher alcohols formed remained nearly constant, despite modifications in the conditions, might be explained by bacterial activity, although, as a matter of fact, it is not quite clear why the action of the bacteria was not influenced by the modifications in question. Stronger proof of bacterial agency in this connection is afforded by the experiments of Gayon and Dupetit (I.). The quantity of the fusel oil formed can be considerably lessened by the addition of bactericidal substances or by powerful aeration, which

suppresses anaerobic bacteria. It should be borne in mind that in nearly every case, except the so-called "Amylo process" (*see* pp. 94 *et seq.*, vol. ii.), the grape, potato, and grain mashes employed for fermentation are only imperfectly sterilised (addition of malt!), if at all, so that bacteria *may* gain access from the start.

In the case of brewing, where the worts are mostly sterilised, few reports are met with, in the literature, concerning the formation of higher alcohols during fermentation. Chapman (I.) distilled six samples of English beers and found, per 100 parts of crude alcohol, 0.051–0.250 part of fusel oil, as amyl alcohol, 0.021–0.062 part of esters, chiefly ethyl acetate, and traces of furfural. As mentioned already on p. 504, vol. ii., Ordonneau (I.) ascribes the formation of the higher alcohols to the vital activity of the yeast itself. Rayman and Kruis (I.) attempted to decide the question by fermenting sterile worts with pure cultures of four different yeasts and one of a species of *Mycoderma*. All the experiments in which *Sacch. cerevisiæ*, L., furnished a low yield of fusel oil, were performed with cells that had been cultivated for a long time in the laboratory under unfavourable conditions. It seems that the formation of amyl alcohol results from the yeast having reached a certain state of exhaustion. According to Mærcker (V.), on the other hand, it should be pointed out that grain mashes, which are particularly well adapted for the energetic nutrition and reproduction of yeast, greatly favour the production of fusel oil.

A high fermentation temperature is said by Kruis and Rayman (II.) to increase the yield of fusel oils. A yeast, otherwise incapable of producing fusel oil in malt worts, furnished a large quantity of amyl alcohol in an imperfectly sterilised yeast mash that had turned sour spontaneously with formation of lactic acid; but no higher alcohols were formed by yeast and aeration in a yeast that had been completely sterilised after turning sour. It was also found that in all cases where fusel oil was produced, the amount of acetaldehyde formed was merely small, and *vice versâ*. Hence, anaerobiotic conditions seem to contribute essentially to the formation of the amyl alcohols.

According to Gentil (I.), the above experiments do not sufficiently prove that fusel oils are produced by yeast, owing to the conditions adopted, namely, the selection of a yeast previously inhabiting a medium containing amyl alcohol, and the weakening of the yeast by an abnormally high temperature and shortened fermentation. Gentil himself failed to obtain amyl alcohol in fermenting a solution of saccharose containing malt peptone as yeast food.

In subsequent experiments, Kruis and Rayman (III.) found that, contrary to the results previously obtained, the formation of amyl alcohol is unaffected either by unfavourable composition of the nutrient medium or by the age and physiological condition

of the yeast. The alcohol is formed only when certain carbohydrates are present; and the assumption is put forward that amyl alcohol is formed, not from the hexoses (§ 326), but from other sugars that result from the polymerisation of the polysaccharides always present in the cereals employed as the raw material. In ten experiments with glucose, fructose, saccharose, and beet juice, ethyl alcohol alone was produced, though, on the other hand, amyl alcohol in considerable quantity resulted from the use of barley worts and inverted brewers' grains, which substances contain fat (*see below* on this page).

Emmerling (XI.), on the other hand, believes, from his tentative experiments, that fusel oils are not produced in more than minimum quantities, if at all, in fermentations from which bacteria have been rigorously excluded. He concludes that the amyl alcohols originate in carbohydrates, and are produced by bacteria that are of widespread occurrence and are almost invariably found on the skin of potatoes.

Great influence on the formation of fusel oils is exerted by the nature of the substance to be fermented, the presence of fat being a contributory factor according to Bornträger (II.). Fermentations with materials that have been freed from fat yield very little fusel oil.

Sugar and carbohydrates are usually regarded as the sources of the higher alcohols; but, according to Bau (XXII.), there is another possibility that should not be left out of consideration, namely, the formation of these substances from the fat that is ready formed in the mash and is generated from sugar by yeast, to be stored up and afterwards decomposed again. During fermentation the process of hydration is accompanied by those of oxidation and reduction. Moreover, in industrial mashes, one has to reckon with the presence of bacteria, a number of which are endowed with powerful reducing properties. According to Durin (III.), aldehydes are formed, not only by the oxidation of alcohol, but also by reducing actions occurring during fermentation, the nascent aldehyde being then capable of easy reduction to alcohol.

Lactic acid (*see* p. 481, vol. ii.) may be regarded as the originating material for the formation of propyl alcohol, this alcohol being found, according to Bouchardat (I.), among the products of lactic fermentation. Fitz (XIV.) states that it is also formed during the fermentation of glycerine by fission fungi.

According to Bau (XXII.), the higher alcohols originate from the fatty acids derived from fats; and indeed, butyric acid, caproic acid, caprylic acid, and capric acid are frequently met with in fats. When these acids are liberated by lipase (*see* p. 494, vol. ii.), they may, in the nascent state, be reduced to alcohols, especially in symbiotic fermentations by yeast and bacteria; indeed, numerous organisms are known that even eliminate free hydrogen.

There is one difficulty in the way of explaining the formation of the amyl alcohols by this hypothesis. They must be assumed to originate in valerianic acid, which, according to KRUIS and RAYMAN (II.) is formed, with other higher fatty acids, from nitrogenous compounds of complex structure. Even though, *pace* BRIEGER (I.), valerianic acid be actually capable of formation from protein, it is hardly feasible to suppose that this alone forms the source of the large quantities of amyl alcohol found in the fusel oils. CAHOURS and DEMARÇAIS (I.), it is true, state that fats yield valerianic acid by chemical means on distillation with superheated steam. Nevertheless, according to the highly important researches of EHRLICH (I.), both the amyl alcohols are formed from leucin and isoleucin (the fission products of protein), under the influence of the normal vital activity of yeast. D-leucin forms the source of the levo-rotatory *d*-amyl alcohol, whilst *r*-leucin is split up so as to form isoamyl alcohol and *d*-leucin. About 87 per cent. of the *l*-leucin is transformed by yeast into amyl alcohol. According to EFFRONT (XIV.), the autodigestion of yeast (*see* chap. lxvi.) forms another source of amyl alcohol, which, however, does not begin to appear until the process has reached an advanced stage. As the yeast cells die off, the formation of amyl alcohol ceases—a proof that this alcohol is produced, not by the vital activity of the yeast cells *per se*, but by the action of an enzyme excreted by the living cells. Hence, to a certain extent, Effront's opinion is at variance with the results of Ehrlich's experiments and consequently further investigation is required concerning the formation of fusel oil.

Methylpropylcarbinol, $C_3H_7CH(OH)CH_3$, was discovered by RABUTEAU (I.), and hexyl alcohol, namely, primary isohexyl, or caproyl alcohol, $(CH_3)_2C_4H_7(OH)$, was detected, by FAGET (I.), in the fusel oil of grape-husk spirit. According to K. WINDISCH (III.), this alcohol occurs, in small quantity, in crude grain spirit; and it has also been found by KRUIS and RAYMAN (II.) in crude potato spirit.

The presence of small quantities of heptyl alcohol or œnanthyl alcohol, $C_7H_{15}.OH$, was confirmed by K. WINDISCH (III.) in crude grain spirit, and FAGET (II.) obtained it from grape-husk spirit. Probably the alcohol recovered in the latter case was a primary isoheptyl alcohol, $(CH_3)_2C_5H_9(OH)$.

Finally, both normal and secondary nonyl alcohol, $C_9H_{19}.OH$ were discovered in crude potato spirit by HILGER (I.).

According to ROMMIER (II.) the fatty acids and alcohols produced during fermentation frequently combine while in the nascent state to form esters, which are also formed during the prolonged storage of fermented worts (wine) or distilled spirits (cognac). These bodies constitute a large proportion of the "bouquet" principles. The esters identified include the acetic acid compounds of ethyl and amyl alcohol, and the corresponding

compounds of butyric acid and the other higher fatty acids. The distillation of wine yeasts furnishes, according to PELOUZE and LIEBIG (I.), œnanthic ether, which later investigations have shown to consist chiefly of ethyl caprinate. In addition to traces of other esters, it is also said, by DELFFS (I.), to contain ethyl pelargonate, and—by A. FISCHER (I.)—caprinates and caprylates. According to Liebig, 40,000 parts of wine furnish 1 part of œnanthic ether, to which the characteristic odour of wine is principally due.

WORTMANN (XVI.) and MASTBAUM (I.) assert that the bouquet principles may be divided into four classes: (1) those originating in the raw materials; (2) those produced during the saccharification of the mash and during fermentation; (3) those formed during storage; and (4) those generated by distillation. The discussion of the first group does not come within the scope of the present chapter, although the substances concerned possess special importance in connection with the bouquet of wine and the character of many beers (hops). The odorous substances formed during fermentation are the above-mentioned esters of the alcohols and fatty acids, all of which are volatile. Some of these substances, the cause of the bouquet of wine, seem to be still unidentified, chemically. Some yeasts, notably LINDNER'S (XLII.) fruity-ether yeasts, produce large quantities of esters, chiefly acetic ether. In addition to these volatile bouquet principles—with which should be classed the fruity-smelling neutral ethyl succinate—certain non-volatile esters occur in wine, and probably also in beer. According to K. WINDISCH (V.) these esters contribute largely to the flavour of wine. They include acid ethyl succinate, and the fermentation esters of tartaric acid and malic acid, both of which acids exist, ready formed, in the grape. In addition to the primary bouquet principles—in the sense adopted by KOSUTANY (II.) and WORTMANN (XVI.)—introduced by the grapes themselves, various odorous substances are produced by the different yeasts. On this point compare KOSUTANY (II), MACH and PORTELE (III.), and PICHI (II.).

The bouquet principles developed during the storage and ripening of wine appear to result principally from the influence of oxygen (*see* p. 503, vol. ii.). CHOUARD (I.) found that a bouquet principle formed during primary fermentation; disappeared afterwards, probably as a result of some reducing process in fermentation; the bouquet may, however, reappear during storage, in consequence of oxidation. According to WORTMANN (VII.), the processes involved in this case are physiological, and not merely chemical reactions.

Changes, apart from continued fermentation, also occur during the storage of beer; these, according to NATHAN (II,), relating chiefly to the elimination of immature bouquet principles which impart an unripe flavour to the beer.

The distilled spirits, cognac and brandy, also undergo changes in storage, which changes are influenced by esterification, as well as by other factors, such as storing the products in casks that are (in contrast to those used for beer) neither lined with pitch nor varnished.

Besides these true by-products of alcoholic fermentation, we have to consider substances that exist ready formed in the raw materials, and are decomposed under the influence of fermentation. These substances are principally glucosides which, on being decomposed by enzymes, yield up their components to the fermented liquid. This explains the occurrence of hydrocyanic acid, benzoic acid, benzaldehyde, and benzaldehyde-cyanhydrin, for instance, in cherry brandy—according to K. Windisch (IV.)—and also of methyl alcohol in fermented fruit juices. In these latter, prepared from plums, cherries, and apples, Wolff (I.) found, almost invariably, methyl alcohol in the proportion of 1 per cent. of the ethyl alcohol present. In the case of wines prepared from grapes with the stalks unremoved, the resulting alcohol contained about 0.15–0.4 per cent. of methyl alcohol, whereas wines from grapes freed from stalks furnished 0.03 per cent. at most. The alcohol obtained by fermenting sugar with wine yeast was, on the other hand, entirely free from methyl alcohol in every case.

The nitrogenous constituents of the yeast (*see* p. 218, vol. ii.) and of the raw material undergo changes during fermentation. Thus fermentation products have been found to contain the following volatile and non-volatile compounds: ammonia, by Kruis and Rayman (II.) and by K. Windisch (IV.); trimethylamine, and other amines, by Ordonneau (I.) and Ludwig (I.); pyridin, collidin, &c., by Kramer and Pinner (I.) and Ordonneau (I.); *b*-glycosin, by Morin (I.) and Tanret (V.); derivatives of pyrazin and other bases, by Schrötter (I.), Oser (I.), Guérin (I.), Stöhr (I.), E. Bamberger and Einhorn (I.); leucin and tyrosin.

The distillates of fermented mashes and liquors also contain other chemical compounds, which were formerly believed to originate, at least in part, during alcoholic fermentation. Foremost among these is furfural, which was found by Kruis and Rayman, more particularly associated with the formation of large quantities of acetaldehyde. Furfural was discovered in 1882 by K. Förster (I.), in crude spirit, and also in the distillates from wine and beer. He ascribed its formation to the effect of the heat (boiling temperature) on the pentosans (*see* pp. 205 and 247, vol. ii.) contained in the raw materials. Kruis and Rayman regard furfural as a product of the metabolism of yeast, a view opposed by Chapman (I.). According to Lindet (VI.), it is formed only during the fermentation of worts from raw materials (cereal grains) that have been dissociated with acids, or when the fermented mash has been distilled by direct fire heat. No furfural

is obtained when the starch has been saccharified by diastase, and distillation has been effected by steam; so that it is not a product of fermentation. According to W. Windisch (IV.), it is produced by boiling acid solutions of carbohydrates, especially the widespread pentoses and pentosans, and is therefore formed in the distillation of the invariably slightly acid mashes, wines, and beers. This explains why C. Heim (I.) found Munich beer to be destitute of furfural. Brand (II.) and Heim (I.) failed to obtain confirmation of W. Windisch's (II.) hypothesis that the pasteurisation flavour of beer is due to furfural. It is, however, certain that when beer containing no furfural is boiled for a sufficient time, furfural makes its appearance; and the same is naturally the case with distillery washes and wine. The test recommended by Lenz (I.), namely, that the occurrence of the furfural affords decisive proof that a sample of cognac is a pure wine distillate, is unreliable.

K. Windisch (III. and IV.) states that fermentation products have also been found to contain terpene and terpene hydrate, as well as oils of high boiling-point, derived from the raw materials. Under certain conditions, sulphur compounds may also occur in the products of industrial fermentations, K. Windisch (V.), for instance, having found sulphuretted hydrogen in wine, whilst Barbet (I.) and Elwart (I.) observed sulphurous esters in spirits produced from molasses and sulphured saccharine juices. The formation of these compounds may be readily explained by the reducing action of yeast enzymes in presence of free sulphur or sulphur dioxide.

§ 326. Sugars Susceptible of Direct Fermentation.

As already mentioned on p. 484, vol. ii., saccharose will not ferment until it has taken up a molecule of water, in which operation it is transformed by the enzyme, invertase (*see* § 327), into two hexoses, glucose and fructose, according to the equation:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

For a long time this observation was unique of its kind, all attempts made to convert maltose—which has the same empirical composition as saccharose—into two hexoses by a similar enzymatic decomposition, having failed; so that it was held that maltose undergoes direct fermentation (compare Morris (III.), Hansen (LXIV.), Dastre (I.), Dünnenberger (I.), Mehring (I.), and Donath (II.)). E. Fischer (VII.), however, established beyond dispute that maltose is split up into two molecules of glucose by a special enzyme, maltase (*see* § 328), as a preliminary to fermentation. His discovery (VIII.), in collaboration with P. Lindner, that saccharose is also hydrolysed by *Monilia candida* (*see* p. 444, vol. ii.), which does not contain ordinary yeast invertase—which

discovery was followed by that of other instances—raised to the status of fact the axiom that all the di- and poly-saccharides must, in order to be capable of alcoholic fermentation by yeast, be first split up into simpler sugars by special yeast enzymes (which will be described later on). This circumstance forms the basis of differentiation between the directly fermentable sugars and the di- and poly-saccharides. The former are compounds, the carbon atoms of which are arranged in a simple chain, whilst the others are ether-like substances, in which separate carbon chains are connected together by one or more oxygen atoms. Those desirous of going more thoroughly into the study of the sugar group may be referred to the works of Tollens (II.) and E. O. von Lippmann (II.).

For the fermentable simple and compound sugars, E. Fischer (IX.) established the axiom that only such as contain a number of carbon atoms divisible by 3 are susceptible of true alcoholic fermentation by yeast.

The first members of this group would be the trioses ($C_3H_6O_3$), which do not occur in nature, but have been prepared artificially and play an important part in the synthesis of sugars. According to E. Fischer (X.), alditriose or *i*-glycerose, is capable of alcoholic fermentation; but this is contested by Wohl (I.) and Emmerling (VII.). This sugar is readily condensed to a compound with the formula $C_6H_{12}O_6$, which sugar is the cause of fermentation phenomena in glycerose syrup. The same applies to ketotriose (dioxyacetone), which is represented by the constitutional formula $OH.CH_2.CO.CH_2.OH$, and was obtained by Bertrand (VII.) in the fermentation of glycerine with *Bact. xylinum*. According to Emmerling (VIII.), it is unfermentable, the slight fermentation phenomena that make their appearance after prolonged warming being attributable to the condensation of the triose into a hexose.

Mention may also be made here of *d*-manno-nonose, a sugar that is also unknown as a natural product. It has the formula $C_9H_{18}O_9$ and, according to E. Fischer (XI.) is readily and completely fermented. Another sugar to be borne in mind is *d*-glycoheptose, which Lindner (XLII.) succeeded in fermenting by means of a yeast (No. 691 of the collection at the Berlin Brewing Institute) from the mucinous secretion of oak-trees. Since this sugar contains 7 atoms of carbon—in accordance with the formula $CH_2OH.(CHOH)_5.COH$—this observation urgently needs confirmation, since if it be correct it disposes of Fischer's axiom respecting the triplicity of the carbon atoms of fermentable sugars.

At one time it was also thought that the pentoses, *i.e.*, sugars with the formula $C_5H_{10}O_5$, including xylose and arabinose, as well as rhamnose, a methylpentose $C_6H_{14}O_6$, were susceptible of true alcoholic fermentation by yeast. A prolonged controversy was maintained on this point, on account of two circumstances: first, the mixed fermentations caused by the use of impure sowings

of yeast, and secondly (where pure yeast was used), the neglect to consider the fact that yeast is also able to assimilate such sugars and utilise them in the construction of new cells which are unable to ferment them. Lactose forms a well-known example of this kind. It seems by no means impossible that, given a sufficiently large sowing of yeast and small amount of sugar, the whole or a portion of the sugar present may be eliminated without any true fermentating taking place. Of course, where impure yeast or unsterilised nutrient solutions are used, the action of bacteria may come into play, a number of which are known to be capable of producing alcohol from pentoses. The proof that alcoholic fermentation cannot be set up in the pentoses by yeasts has been given by a number of workers, including Tollens and Glaubitz (I.), Scheibler, Cross and Bevan (I), Stone and Tollens (I.), Smith, E. O. von Lipmann (III.), E. Fischer (XVI.), and P Lindner (XXXV.). According to Buchner and Rapp (III.), expressed yeast juice is also inactive toward pentoses.

The real directly fermentable sugars are the hexoses, which have the general formula $C_6H_{12}O_6$; and indeed, only such members of this group as belong to the *d*-series, the *l*-compounds being unfermentable. Here also, as in the other kinds of sugars mentioned, a distinction is drawn between the aldoses and ketoses.

The most widely occurring and best known of the aldo-hexose sugars is *d*-glucose, also known as dextrose, grape sugar, starch sugar, and diabetic sugar. It is fermented by all organisms capable of inciting alcoholic fermentation, and therefore by all culture yeasts—of which, according to Lange (IV.), about 700 races are already known—and all wine yeasts. (*See also* pp. 207 *et seq.*, and p. 397, vol. ii.)

The sugar, *d*-mannose, also known as isomannose, seminose and carubinose, is only occasionally met with in nature, for example—according to Tsukamto (I.)—in the Japanese *Amorphophallus Konjaku*, and according to Prinsen-Geerligs (V.), Pellett (I.) and others, in various kinds of colonial molasses, in orange rind; and also, according to Grüss (III.), temporarily in germinating dates. On the other hand, it forms a regular constituent of the mannanes, which are of widespread occurrence throughout the vegetable kingdom and represent, to some extent, condensation products of mannose, either by itself or in association with other sugars. In the latter case the products are classed as conjugate mannanes. This sugar is fermented by all Lindner's yeasts (XXXV.) which also ferment *d*-glucose, except *Saccharomyces membranæfaciens*, *S. farinosus*, *S. Bailii*, a *S. apiculatus* from Leipzig mead and one from raspberry juice, *S. exiguus*, *Endoblasterma amycoides I.*, *E. liquefaciens*, a film yeast from marshmallow sap, a fruit-ether yeast from gall fermentation, and *Schizosaccharomyces Pombe*.

Whether *d*-galactose—also formerly termed lactose (not to be confounded with the di-saccharide lactose, or milk sugar, of the existing nomenclature) and lactoglycose—actually occurs in a free state in nature has not yet been definitely ascertained. In combination with *d*-glucose it forms milk sugar and melibiose. In the vegetable kingdom, *d*-galactose is found as a constituent of several glucosides, and especially of the widespread galactans, which may be divided into simple and conjugate galactans. True galactans are met with in barley, malt, and numerous seeds, and, according to Prinsen-Geerligs (V.) and E. O. von Lipmann (III.), also in the products and waste products of the cane-sugar and beet-sugar industries. According to Payen (III.) and Bauer (II.), gelose, the chief constituent of agar-agar, consists mainly of galactan. Conjugate galactans occur in vegetable mucilages: in yeast gum according to Schützenberger (III.), as galactoarabans in various seeds, according to E. Schulze (V.), whilst Lintner (VIII.) states that galactoxylan is a constituent of wheat, barley and malt. Conjugate galactans, differing with the origin of the material, are found in gum arabic. These bodies are also met with in the animal kingdom: associated with milk sugar in milk, according to Béchamp (XV.); whilst according to Thudichum (I.) they form a constituent of protagon. Lindner (XLII.) states that *d*-galactose is fermented by all yeasts that dissociate *d*-glucose, except the following species: *Sacch. membranæfaciens*, *S. farinosus*, *S. Bailii*, *S. apiculatus*, *Schizos. Pombe*, *Schizos. mellacei*, as well as a few yeasts from gall fermentation and cucumber pickle. On the other hand, strangely enough, it is fermented by two film-producing budding fungi (Nos. 127 and 374 of the Berlin collection) which leave glucose, mannose, and fructose intact. This report urgently needs confirmation, since the older statements on the fermentability of *d*-galactose are more divergent than in the case of any other sugar. It would occupy too much space to detail the communications on this point, and the reader is therefore referred to Bau's work (XXIII.), in which the older literature was critically reviewed. According to E. Fischer, galactose is fermented by the culture yeasts, by *S. pastorianus I. II.* and *III.*, *S. ellipsoideus I.* and *II.*, and *S. Marxianus*, as well as by milk-sugar yeast, whereas no fermentation is set up by *S. membranæfaciens* and *S. productivus*. Kozai (II.) reports that saké yeast will also ferment galactose.

Whilst the above three sugars of the hexose group are aldoses, the following representative of the ketohexoses must be added: *d*-fructose (levulose or fruit sugar), which is very widespread in nature and almost invariably accompanies *d*-glucose. A mixture of these two sugars in equal parts constitutes invert sugar, which term is also applied to their mixtures in any proportion. Fruit sugar is a constituent of several polysaccharides, including saccharose, melitriose, lupeose, stachyose, &c., of inulin and allied sub-

stances, and of yeast lævulan (*see* p. 175, vol. ii.). It is fermented by all organisms capable of fermenting *d*-glucose, with the single exception of a film yeast, LINDNER'S No. 178 (XLII.).

Only the above four hexoses (*d*-glucose, *d*-mannose, *d*-galactose and *d*-fructose) are fermented by yeasts, all the others being unfermentable.

CHAPTER LXV.

ENZYMES DECOMPOSING DISACCHARIDES AND POLYSACCHARIDES.

By Dr. A. BAU.

§ 327. Invertase.

The best known and most frequently investigated yeast enzyme is invertase, which was originally termed invertin, and has also been called saccharase, sucrase and euinvertase.

In its occurrence it is one of the most widespread of the enzymes. In the animal organism it is found in numerous organs, especially in the mucous membrane of the small intestine (particularly in warm-blooded animals): and it has also been found, by Erlenmeyer and A. von Planta (I.), Axenfeld (I.) and others, in insects. In the vegetable kingdom it occurs in the majority of plant organs: leaves, flowers and fruit (also in hop cones), &c., though in far smaller amount than in the lower fungi, mould fungi, yeasts and bacteria.

Invertases of different origin are not always of identical composition; and differences of action on saccharose are also observed in the invertases according to the method of preparation employed —compare Fernbach (VI.). For this reason, Bau (XII.) proposed the name "euinvertase" for the invertase occurring in the true yeast, until further investigation had shown whether the saccharose-dissociating enzymes obtained from such highly divergent materials were really identical. A special example of the variation in the individual invertases is afforded by *Monilia candida* (p. 444, vol. ii.) which, though decomposing and fermenting saccharose, does not, in the opinion of Fischer and P. Lindner (II.), contain true invertase. Hahn (III.) regards the enzyme of this fungus as an endoenzyme which is possibly combined with the protoplasm; and perhaps it is merely an enzymogen. Buchner and Meisenheimer (1.), who investigated the expressed juice of *Monilia candida*, found that the enzyme will not diffuse through parchment paper, differing remarkably, in this respect, from yeast invertase.

Invertase occurs extensively in yeasts, being found in all

culture yeasts used in brewing, distillery work and pressed yeast making, and therefore in all top- and bottom-fermentation yeasts of the *Sacch. cerevisiæ* type, both of the Frohberg and Saaz races comprised in the types OF, OS, UF, and US (*see* p. 540, vol. ii.). All the true wine yeasts also contain invertase; but, on the other hand, it is absent from *Schizos. octosporus* (*see* pp. 274–281, vol. ii.). Invertase is, however, present in a number of other budding fungi, though not in any of the examined races of *Sacch. apiculatus* (*see* p. 431, vol. ii.) and most *Torulaceæ* (*see* p. 398, vol. ii.). Of the latter, however, HARTMANN'S (I.) *Torula colliculosa* ferments saccharose without difficulty, whereas other species are incapable of decomposing this sugar. In this connection, further investigation is urgently required, since, in most of the work already done, attention was mainly directed to ascertaining whether the organisms employed were able to ferment saccharose, and not to determining the presence of invertase.

In the preparation of invertase in the purest, *i.e.*, most active, condition possible, it is the almost universal practice to employ *Sacch. cerevisiæ*, of either top- or bottom-fermentation type. In the older methods the first stage was to kill the yeast by means of alcohol or ether, in order to extract the invertase afterwards with water or glycerine—compare BERTHELOT (III.), LIEBIG (III.), HOPPE-SEYLER (IX.), GUNNING (I.), and DONATH (III.). The resulting solution is precipitated, fractionally, with strong or absolute alcohol, under which treatment the earlier fractions have less enzymatic power than the later ones. The precipitates are washed with absolute alcohol, and dried in the desiccator. In other methods, according to BARTH (II.) and AMTHOR (V.), the yeast is first carefully warmed, to expel the bulk of its moisture, and is then dried more energetically, and the resulting powder is extracted. An exceedingly powerful solution of invertase is obtained by allowing yeast to ferment spontaneously—O'SULLIVAN and TOMPSON (I.)—or by recovering the expressed juice by the Buchner method. According to ISSAEW (I.), plasmolysing pressed yeast with saccharose will furnish a very active invertase, from which the dissolved saccharose can be eliminated by fermentation. The relatively purest invertase, however, is obtained by killing the yeast and extracting it with glycerine or water, whether the killing be effected by treatment with alcohol or ether, or, preferably, by heating the carefully dried yeast to 100° C. and over. According to OSBORNE (I.), the yeast, after being killed with alcohol, should be digested with chloroform water at a moderate heat for some time, the filtrate being poured out into 96 per cent. alcohol. The deposited flakes are washed with alcohol, dried, and dissolved in 25 parts of water, the earthy phosphates still present being thrown down by a careful addition of ammonia, and the filtrate dialysed and then evaporated *in vacuo*. WROBLEWSKI (IV.) also employed dialysis for purifying the enzyme; but this worker

precipitated the invertase beforehand by saturating its solution with ammonium sulphate.

The chief property of invertase is its power of hydrolysing saccharose, which it splits up into one molecule each of *d*-glucose and *d*-fructose according to the equation

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

Whether the enzyme is also capable of hydrolysing other sugars is doubtful: *see* § 332 on this point.

As already mentioned on p. 511, vol. ii., yeast cannot ferment saccharose directly, the sugar needing to be first hydrolysed by invertase. It is uncertain whether this decomposition is effected inside or outside of the yeast cell. According to O'Sullivan (II.), the healthy yeast cell is incapable of diffusing invertase; and consequently the hydrolysis of the saccharose within the cell must precede fermentation. This opinion is shared by Hiepe (I.), who considers that hydrolysis is intimately connected with cell protoplasm, and that the operation is one in which physiological laws play as important a part as chemical laws; furthermore, that the admission of the saccharose into the cell, and the outward passage of the products of inversion are physiological processes. According to Fernbach (VI.), the rate at which yeast cells permit the escape of the enzyme is in inverse ratio to their age. In this connection Pottevin and Napias (I.) examined five yeasts in a peptonised solution of saccharose, and found that four of the races ceded invertase to the medium in the early stages of fermentation, whilst the fifth did not. These four yeasts yielded powerful solutions of invertase when macerated with chloroform water; but the fifth only parted with a little invertase after digestion for a fortnight. Hence the individual races of yeast appear to differ in respect of the cession of the enzyme to the circumambient medium. In general, however, it may be assumed that fresh yeast cells belonging to the groups *Sacch. cerevisiæ* and *Sacch. ellipsoideus I.* will allow invertase to diffuse through their cell membrane. Bau (XXIV.) and Donath (IV.) nevertheless found invertase in all fermented beverages.

On the constant occurrence of this enzyme in beer, Bau (XXV.) established a method of detecting whether beer has been pasteurised. One 20 c.c. sample of beer is boiled, and a similar quantity is left unboiled, each being treated with 20 c.c. of a 20 per cent. solution of saccharose, then kept for twenty-four hours at room temperature, treated with 0.5 c.c. of lead acetate, made up to 50 c.c. with distilled water, filtered and polarised. Should an appreciable difference be observed in the deviation of the angle of polarisation in the polarimeter, the beer has not been pasteurised; but if the two results be identical, or approximately so (slight differences in the reading being due to experimental error), the beer will certainly have been pasteurised, and probably at a temperature

exceeding 57°C. In the absence of a polarimeter, the test may be performed as follows: 5 c.c. of Fehling solution are boiled with 1 c.c. of test liquid (40 c.c. of beer and 40 c.c. of saccharose solution, after digestion for twenty-four hours). If the liquid remain blue, with a slight red precipitate, no invertase is present; in the opposite event the Fehling solution will be reduced completely.

Enzymatic action is also greatly influenced by temperature in the case of invertase; and whilst this action begins at about zero C., the optimum temperature is considerably higher. In the case of invertase from top-fermentation pressed yeast, A. Mayer (XI.) found this optimum temperature at 31° to over 36° C., and 44°–48° C. in that from bottom-fermentation yeast. On the other hand, according to Kjeldahl (I.), the optimum temperature for the activity of invertase from bottom yeast is 52.5° C., and that for the enzyme from top yeast, 56° C. A. Mayer (XI.) considers that these divergencies are explained, on the one hand by the invertase preparations being injured in the course of production, *e.g.*, by treatment with alcohol, and, on the other hand, by the fact that adherent impurities have a stimulating or restrictive influence on the enzymatic action according to their character. After very careful investigation O'Sullivan and Tompson (I.) determined the optimum temperature at 55–60° C. A considerable difference also exists in the reports as to the temperature at which this enzyme is destroyed, the explanation being the same as just given. For instance, alcohol lowers the destruction temperature, whereas high concentration and the presence of glycerine has the opposite effect. Prolonged exposure to a constant temperature also has an injurious effect, A. Mayer (XII.), for instance, finding the enzyme to be destroyed at 51° C. in some cases, whereas in others it remained active, though weak, at 65° and even at 66° C. According to O'Sullivan and Tompson (I.), the destruction temperature of invertase is 75° C.; and the same result was obtained by Bau (XXVI.), who did not prepare the enzyme in a pure state, but examined it direct in the cell by the Bokorny (IV.) method. This method obviates the injury always suffered by the enzyme in the course of isolation; but, on the other hand, allowance must be made for the fact that, when the experiment is repeated, the yeast may not be in the same physiological condition in all the tests. It is true that the conditions of nutrition of the yeast do not modify the properties of the enzyme; but its quantity and activity may be influenced by the accumulation or diminution of other substances present in the yeast cells. From additional experiments made, it may be assumed that yeast invertase, provided it has remained uninjured, will develop its maximum activity at 52°–56° C., and that it is certainly destroyed in aqueous solutions, and also in the yeast cell, by a temperature of 75° C. In an absolutely dry state it will stand far higher

temperatures. Both pure invertase and dry yeast will stand heating, without loss of enzymatic power, to temperatures assessed by A. Mayer (XIII.) at 97° C., by Bau (XII.) at 100° C. by Buchner (III.) at 145° C., and by Salkowski (X.) at as high as 160° C. According to Bau (XXVI.), yeast that has been dried at the ordinary temperature or heated to 105° C. retains invertase even at the end of five and three-quarter years.

In investigating the influence of chemical reagents on invertase, Bokerny (IV.) followed the principle of allowing these reagents to act on the yeast itself, in order to obviate any injury that the invertase may suffer in preparation. He reports that invertase remains unimpaired when the yeast is stored in absolute alcohol for three days at ordinary temperature, or for twenty days in 50–75 per cent. alcohol. The enzyme is also uninjured when the yeast is kept for two days in solutions containing 0.25–0.60 per cent. of oxalic acid, 0.1–0.5 per cent. of hydrofluoric acid, 2 per cent. of acetic acid, 2 per cent. of lactic acid or 5 per cent. of formaldehyde. The enzymatic power is also not destroyed by small quantities of mineral acids, alkalis, arsenites, hydrocyanic acid, chloroform, phenols, toluene and thymene, both of which latter were employed by Emil Fischer and P. Lindner (II.) in their investigations on enzymes. Similarly, Bau (XXVI.) examined yeast by digestion at 12°–17° C. for twenty-nine hours, and found that the invertase was destroyed by treating the yeast with 1 per cent. and 0.5 per cent. sodium hydroxide, and 0.1 per cent. silver nitrate, a weakening effect being produced in the case of 0.1 per cent. mercury chloride, whereas solutions of lower concentration remained inert. No injury was suffered by the invertase on treatment with organic acids, including tartaric acid of 4 per cent. strength.

With regard to the influence of light on invertase, the reports of workers differ. A. Mayer (XIV.) and Emmerling (XII.) failed to discover any such influence; but according to Downes and Blunt (I. and II.) and also Duclaux (XXIX.) the enzyme is sensitive towards light, especially in presence of air. Very dilute acids stimulate the activity of invertase; but the quantity used must be smaller in the case of mineral acids than of organic acids. For instance, according to Fernbach (VII.), 0.0025 per cent. of sulphuric acid in the solution produces optimum activity, whereas the same result requires the presence of 1 per cent. of acetic acid. Moreover, the reports of various workers differ on this point, *e.g.*, those of Kjeldahl (I.), Dumas (VII.), Nasse (I), Loew (X.), O'Sullivan and Tompson (I.) and Fernbach (VI.); presumably because they worked with invertase of divergent origin and method of preparation, and containing different extraneous substances. According to Nasse (II.), carbon dioxide accelerates hydrolysis by this enzyme, whereas carbon monoxide and oxygen have the opposite effect. All alkalis and alkaline salts are said by

Duclaux (XXIX.), O'Sullivan and Tompson (I.), and Fernbach (VI.) to have strongly adverse influence, even in small quantities. According to Nasse (II.) and Duclaux (XXIX.), small quantities of alkali chlorides and calcium chloride have a beneficial effect, whilst salts of the heavy metals are injurious. Alcohol, even as little as 5–10 per cent., is stated by A. Mayer (XV.), J. Moritz (III.), and O'Sullivan and Tompson (I.) to have a restrictive influence on hydrolysis; and, according to Griffith (I.), small quantities of salicylic acid have a similar effect.

In contrast to other enzymes, invertase seems to be completely inalterable. A. Mayer (XVI.) found that it is not attacked by putrefactive bacteria, although his experiment was not entirely free from objection, it being stated by Fermi and Montesano (I.) that certain bacteria themselves produce invertase, so that there is no proof whether the invertase found in the products of putrefaction really originated in the yeast or were excreted by the bacteria. Bau (XXVI.) investigated the mutual interaction of yeast enzymes, of which yeast-endotryptase (*see* chap. lxvi.), or yeast-peptase alone come under consideration. Yeast that had been liquefied at 45° C., or expressed yeast juice that had been kept for one to three weeks at 17°–20° C., or heated at 30° C. or 40° C. for an hour, still contained unimpaired invertase. The activity of this enzyme also remained intact when the yeast was digested for twenty-four hours at 37° C. with a solution containing the extremely large quantity of 1 per cent. of pepsin (Merck) and 0.1 per cent. of hydrochloric acid. It is true that, in these experiments, nothing was done to ascertain the quantities of invertase before and after the treatment with yeast endotryptase and pepsin respectively.

Attempts have already been made at the quantitative determination of invertase; but the method proposed by Fernbach (VIII.), like all quantitative methods for the determination of enzymes, is attended by the drawback that only the effect of the enzyme can be measured and not the amount of enzyme actually present. According to Fernbach, a number of samples (each measuring exactly 4 c.c.) of a 50 per cent solution of saccharose are treated with 1, 2, 3, &c., c.c. of the invertase solution under examination, each of the mixtures being then treated with 1 c.c. of decinormal acetic acid and made up to 10 c.c. The test-glasses are then warmed to 56° C. for an hour on the water-bath, cooled quickly and treated with a few drops of caustic soda to destroy the enzymatic action, the amount of invert sugar formed being determined by means of Fehling's solution. Fernbach estimates the unit of invertase as that capable of hydrolysing 0.2 grm. of saccharose in one hour at 56° C. and in presence of 1 per cent. of acetic acid.

According to Moritz and Morris (I.), the hydrolysis of saccharose by invertase is utilised in certain English breweries

by digesting beer yeast with the saccharose solution at 56° C. and running the inverted mixture into the hop back.

Invertase is also utilised practically in chemical analysis, for the determination of saccharose in cases where no reliable results can be obtained either by direct polarisation or by the Clerget-Herzfeld inversion method. According to the Convention for the Uniform Examination of Foodstuffs and Delicacies, VEREINBARUNGEN (I.), 100 c.c. of the solution under examination, *e.g.*, a 10 per cent solution of honey, are treated with 50 c.c. of a solution of invertase, prepared by the conventional method, the mixture being allowed to stand for two hours at 50°–55° C., and the invert sugar then determined either in the polarimeter or gravimetrically.

§ 328.—Maltase.

Whereas at one time it was thought that maltose was capable of direct fermentation, we have already seen, on p. 511, vol. ii., that this sugar also must be subjected to hydrolytic fission before it can be attacked by alcoholase.

Maltose, which was first discovered by Dubrunfaut, is also known as malt sugar, and, in the anhydrous condition, has the same empirical composition as saccharose, namely, $C_{12}H_{22}O_{11}$. Unlike the latter, however, it is not composed of two "simple" sugars, but consists of two molecules of *d*-glucose, condensed to maltose by the elimination of water. It occurs in nature, usually in small quantities in the leaves of various plants and, according to PURIEWITSCH (VIII.), is formed during the germination of seeds. It has also been found in germinated barley, and occasionally in green and cured malt, by numerous workers, including O'SULLIVAN, BROWN, and MORRIS (III.), JALOWETZ (II.), and others, whereas other observers, such as DÜLL (III.), LINTNER (IX.), and KRÖBLER (I.) deny or regard as doubtful its presence in malt. These divergent results are explained to some extent by the circumstance that some workers extract the malt with water, in order to examine the sugar content, during which treatment the diastase is afforded an opportunity of acting on the starch, whilst others have attempted to destroy the diastase previous to extraction for the practical purpose of the fermentation industry; however, it is immaterial whether maltose is already contained in malt or not, since the mashing process in brewing and distilling is designed for securing a more or less extensive conversion into maltose of the starch contained in the cereal grains.

In distillery work and in the manufacture of pressed yeast, attention is concentrated on attaining the utmost possible saccharification of the starch by diastase, whereas in brewing it is found desirable to regulate the process of saccharification, according to the type of beer required, in such a manner, by the employment of more or less highly cured malt, that the wort will

contain a larger or smaller quantity of maltose, according as the beer is to be lightly fermented and full flavoured, or highly fermented and vinous. In this connection it is customary to speak of the "attenuation" (degree of fermentation) of the beer, which, however, does not depend solely on the mashing process, but also on the kind of pitching yeast (*see* p. 268, vol. ii.) employed.

Though maltose can be hydrolysed by acids, the transformation—which results in the production of two molecules of *d*-glucose—is far less easily effected than is the case with saccharose. On the other hand, maltose is readily decomposed by the yeast enzyme maltase. A similar enzyme was discovered in maize by GEDULD (II.), who termed it "glucase"; but later workers, including LINTNER and KRÖBER (I.) have shown them to be different.

Maltase was discovered in yeast by EMIL FISCHER (VII.), after LINTNER (X.) had indicated the possibility of yeast possessing an enzyme capable of decomposing maltose. The original name for the enzyme was glucase or glycase, it being also called yeast glucase for closer identification; but at that time the nomenclature of the enzymes was in a state of confusion, some of them being named after the products to which they give rise, and others after the sugars they decompose (compare W. WINDISCH (V.)) and it was only later that the term maltase found general acceptance for the enzyme that decomposes maltose. In order to obviate any uncertainty, E. O. von LIPPMANN (IV.) proposed a new terminology, according to which the enzymes were to receive double names, the first portion indicating the sugar decomposed, and the second the product, or main product of the hydrolysis. Under this proposal the enzyme decomposing maltose would be termed maltoglycase or maltoglucase; but this name has not come into favour.

Maltase occurs in all races of culture yeasts of the *Sacch. cerevisiæ* group belonging to the UF, US, OF, and OS types, as well as all wine yeasts (compare pp. 278–280, vol. ii., and p. 283 *et seq.* vol. ii.). Special interest attaches to HARTMANN's (I.) *Torula colliculosa* (*see* p. 397, vol. ii.). As already mentioned, maltase also occurs in maize; likewise in mould fungi (*see* p. 362, vol. ii.), turnips, peas and potatoes, as well as in cereals (compare BEIJERINCK (XIII.), and STOKLASA and CZERNY (I.)). The low enzymatic influence exerted on maltose by barley leads to the supposition that, as in the case of wheat, rye, and rice, maltase is not inherent in this cereal, its presence being due to adherent mould fungi and yeasts (*see* p. 533, vol. ii.). Researches on this point would add to our knowledge on the occurrence of the maltases.

The preparation of "pure" maltase is attended with difficulties. On the one hand, according to E. FISCHER (VII.), and LINTNER and KRÖBER (I.), this enzyme is only sparingly soluble in water, and, on the other, it is very susceptible to alcohol, which

is generally used as a precipitant for enzymes. Moreover, it cannot be separated from invertase, and therefore, according to Emmerling, the best raw material for maltase is *Schizosaccharomyces octosporus*, which does not contain invertase.

The optimum temperature for this enzyme is given by Lintner and Kröber (I.) as 40° C., whereas that of Geduld's glucase (II.) varies between 57° and 60° C. This difference indicates that the various enzymes decomposing maltose are not identical; and it is therefore advisable to speak of the yeast enzyme as yeast-maltase, and not simply maltase.

The destruction temperature was determined by Lintner and Kröber (I.) as 55° C., which was also confirmed by Bau (XXVI.). Dry maltase has greater power of resisting high temperatures; for, though Bokorny (IV.) found that the maltase in a pressed yeast was destroyed in the drying process, E. Fischer (VII.), as well as Lintner and Kröber (I.), had previously ascertained that the enzyme would stand careful drying. According to Bau (XXVI.), the enzyme remains unimpaired when top- or bottom-fermentation yeast is dried at the ordinary temperature, or at 35°–37° C., the dried yeast being then heated for several hours at 105° C. (though in this case the maltase is slightly weakened), or stored for over five years. Bau regards maltase, however, as far more sensitive than invertase to desiccation; so that there is no considerable discrepancy between his statements and those of Bokorny.

The influence of chemical reagents was investigated more particularly by Bokorny (IV.) in the same manner as for invertase (*see* p. 520, vol ii.), the results showing maltase in pressed yeast to be more sensitive than that in brewery yeast. The enzyme remained unaffected by the action of 0.5 per cent. solutions of lactic acid and oxalic acid, caustic soda, 0.1 per cent. sulphuric acid and phenol, and by chloroform water (on this point *see* later). It was more or less enfeebled by 0.5 per cent. sulphuric acid, 1 per cent. acetic acid, 0.1 per cent. formaldehyde and thymol, 0.001 per cent. oil of turpentine, and 5 per cent. alcohol; whilst the following agents had a destructive effect: 0.1 per cent. hydrochloric acid, 1 per cent. oxalic acid, caustic soda, or phenol, 0.02 per cent. sublimate, 0.01 per cent. silver nitrate, and 10 per cent. alcohol. By subjecting bottom-fermentation yeast UF to similar treatment, Bau (XXVI.) observed a destructive effect on the maltase by 1 per cent. acetic acid, 0.5 and 1 per cent. oxalic acid, 1 per cent. lactic acid, 4 per cent. tartaric acid, 0.5 and 1 per cent. sulphuric acid, 0.91 per cent. hydrochloric acid, 1 per cent. caustic soda, 0.1–0.01 per cent. silver nitrate, and 0.1 per cent. sublimate. The enzyme was also injured by 0.2 per cent. oxalic acid, 1 per cent. sodium carbonate, 0.5 per cent. caustic soda, 0.02 per cent. sublimate, and 95 per cent. (vol.) alcohol. With reference to Bokorny's report (above) that maltase is uninjured

by chloroform water, it may be mentioned that, according to Morris (III.), fresh, intact yeast, unlike dried yeast, will not decompose maltose. It transpired, however, that Morris (IV.) had employed chloroform water to prevent fermentation during the digestion of the yeast with maltose, which reagent, according to Emil Fischer (XII.), and also Lintner and Kröber (I.), seriously injures or destroys maltase. Emmerling (XII.) states that maltase is unaltered by light.

All these experiments show that maltase is a far more sensitive enzyme than invertase. It is apparently unaffected by yeast tryptase, Bau (XXVI.) having found that low-fermentation yeast UF liquefied in five hours at 45° C., exerted a fairly powerful decomposing action on maltose, whilst, on the other hand, no maltase could be detected in expressed yeast juice that had been kept for eight days at about 20° C., or in another sample of the same juice three weeks old. The conditions causing the disappearance of this enzyme were not investigated: and a profitable field is therefore still open for the fermentation physiologist to extend our knowledge on yeast maltase.

Special interest also attaches to maltase, inasmuch as it exhibits not merely hydrolytic properties, but also acts as a synthetic agent. C. Hill (I.) found that, in presence of larger quantities of maltose, the decomposition of this sugar remains incomplete as soon as the solution has become enriched in glucose. Hill prepared his maltase solution by drying low-fermentation beer yeast on earthenware plates and heating the pulverised mass gradually to 100° C., the powder being digested with a tenfold quantity of a weak solution of sodium carbonate for three days, in presence of toluene. The filtrate completely decomposed a 2 per cent. solution of maltose, but not stronger solutions. The presence of glucose also hindered the complete hydrolysis of the maltose. When Hill allowed the maltase solution to act on a 40 per cent. solution of glucose, reversion was observed, 15 per cent. of the sugar being converted into a disaccharide, which Hill regarded as maltose. By means of experiments extending over several months, O. Emmerling (XIII.), however, showed that the reversion sugar is not maltose but isomaltose, namely, Fischer's (XIII.) unfermentable isomaltose, and not that of C. J. Lintner (XLVII.). These two isomaltoses are fundamentally different kinds of sugar, which merely have the same empirical composition, are derived from *d*-glucose and furnish identical phenylosazones melting at 151°–153° C. Unfortunately, we cannot here go into the much-discussed question of the existence of Lintner's isomaltose; but that of Fischer's isomaltose is regarded by Fischer (XIV.) and Ost (II.) as definitely established. Emmerling's claim that Fischer's isomaltose is formed by the action of yeast maltase was disputed by Hill (II.); but, after Emmerling (XII.) had succeeded in reconstructing amygdalin from

hydrocyanic acid, oil of bitter almonds, and *d*-glucose by the aid of yeast maltase, Hill (III.) became convinced of the existence of a second sugar, which he named revertose, associated with (Fischer's) isomaltose. On purification, this revertose, or revertobiose, forms strongly hygroscopic, crystalline incrustations, with a specific rotatory power of about $a_D = +91.5°$, and a reducing power equal to only about 47.5 per cent. that of maltose. Revertose needs closer investigation. Perhaps its origin is due to the invertase present in yeast extract. Attention may again be directed here to the circumstance that enzymes, in addition to the possession of hydrolytic or degradation properties, are also able to effect the synthesis of bodies of higher molecular weight, yeast maltase not being alone in the exhibition of this power.

§ 329.—Melibiase.

A polysaccharide known by the names melitose, gossypose, melitriose, and raffinose, with which we shall become more fully acquainted in § 332, can be hydrolysed, by the moderate influence of dilute acids, to two sugars, one of them being the well-known *d*-fructose, whilst the other is a disaccharide with the formula $C_{12}H_{22}O_{11}$, and to which the name melibiose was given by Scheibler and Mittelmeier (II.). Raffinose can also be split up into these two sugars by certain yeasts, Berthelot (X.), for instance, having found that the action of yeast on melitose produces a non-fermentable sugar, which he named eucalyn. According to Bau (XIII.), however, his subsequent reports about this compound are so contradictory that they cannot be utilised in connection with melibiose. In a further communication Berthelot (IV.) states that raffinose (melitriose) is completely fermented by good yeast, but to the extent of only about one-third by enfeebled bakers' yeast. Rischbiet and Tollens (I.) say that melitriose ferments readily and completely; but Loiseau (II.), on the other hand, states that this sugar, whilst completely fermented by low-fermentation beer yeast, is only consumed to the extent of one-third by high-fermentation yeast. Scheibler and Mittelmeier (III.) found that commercial yeast only fermented melitriose imperfectly, and that an amorphous sugar, namely, melibiose, was left in the fermentation residue. In contrast to these experiments, Bau (XIII.) demonstrated that pure cultures of low-fermentation yeasts ferment melitriose completely, whereas those of high-fermentation yeasts effect the separation of a sugar (melibiose) which remains unaltered. This worker (XXVII. and XVI.) then prepared large quantities of crystallised melibiose, both by the physiological and chemical methods, for particulars of which the reader is referred to the original treatise. As was found by Tollens and his colleague (VI.), and also by Scheibler and Mittelmeier (II.), this

disaccharide is split up by the energetic action of acids into two simple sugars, *d*-glucose and *d*-galactose. Hence it contains the same components as lactose (§ 330), but in a different state of chemical combination. According to Bau (XIII. and XV.), mineral acids and oxalic acid alone are suitable for the acid hydrolysis.

Whereas, like lactose, melibiose is only hydrolysed with difficulty by acids, it is readily split up by a yeast enzyme. After Bau (XIII.) expressed the opinion, in 1894, that melibiose is not fermentable direct, but must first be decomposed into its components, *d*-glucose and *d*-galactose, E. Fischer and P. Lindner (II.), as well as Bau (XII.) himself, working independently in the following year, detected in low-fermentation yeast an enzyme capable of effecting this transformation, and to which Bau gave the name melibiase.

Since the occurrence of melibiase in certain *Saccharomycetes* can be utilised as an important chemical means for the differentiation of groups of yeasts, we will now proceed to observe the general characteristics of this enzyme, a knowledge of which facilitates recognition of the value of diagnosing races of yeast on the basis of the action of this enzyme.

Melibiase—which, according to E. O. von Lippmann's (IV.) proposal, should be termed melibio-glucase—is said by Fischer and Lindner (II.), and also by Bau (XII., XXVI., XXVII.), to be somewhat sparingly soluble in water. The optimum temperature at which it decomposes melibiose into one molecule of *d*-glucose and one molecule of *d*-galactose, according to the equation $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$, is 50° C., though a considerable proportion of the disaccharide is split up at much lower temperatures. The destruction temperature is 70° C., though, on the other hand, as ascertained by Fischer and Lindner (II.), melibiase will stand desiccation. According to Bau (XXVI.), low-fermentation yeast that has been dried at 30°–37° C. may be heated to 100° C. for eight hours, or to 110° C. for five hours, without injury to the enzyme; and yeast dried in the above manner will retain its enzymatic activity for 5¾ years. In this respect, melibiase has the same power of resistance as invertase.

As regards the action of chemical agents, melibiase in yeast is destroyed by the influence of oxalic acid 1 per cent., sulphuric acid 1 and 0.5 per cent., hydrochloric acid 0.91 per cent., sodium hydroxide 1 per cent., silver nitrate 0.1 per cent., mercury chloride 0.1 and 0.02 per cent.; a more or less enfeebling effect being produced by acetic acid 1 per cent., oxalic acid 5 per cent., sulphuric acid 0.2 per cent., sodium carbonate 1 per cent., sodium hydroxide 0.5 per cent., silver nitrate 0.02 per cent., and alcohol 95 per cent. (vol.). Slight injury is caused by oxalic acid 0.2 per cent., and tartaric acid 4 per cent.

With regard to the influence of other enzymes: melibiase is almost as indifferent as invertase. In the method of experiment selected by Bau (XXVI.)—*see* pp. 521 and 525, vol. ii.—the only difference between this and the extremely resistant invertase was that, after the yeast had been treated with pepsin, melibiase could be detected in the filtered-off yeast cells, but not in the filtered solution. Melibiase seems therefore to be rather more sensitive than invertase, though it is far more resistant than maltase. It is also possible that melibiase is insoluble in faintly acid solid solutions, since the filtrate from the pepsin treatment contains free hydrochloric acid; whilst Hill (I.) for instance, found that faintly alkaline water is requisite for the extraction of maltase. Consequently, it must be left for further investigation to determine whether the sparing solubility of melibiase in water can be increased by the careful addition of alkali carbonates.

Melibiase occurs in all low-fermentation types of yeast, both Frohberg and Saaz, an exception being afforded, according to Lindner, by the low-fermentation beer yeasts, No. 2, No. 18, and No. 389 of the Berlin collection, these yeasts leaving melibiose "practically unfermented." According to Bau (XXVI.), however, the low-fermentation yeast No. 2 Victoria ferments melibiose, though slowly and sluggishly. Like certain other races, No. 18 is no longer grown in the Berlin collection, and should therefore be struck out of the scientific literature, the rediscovery, and especially the identification, of such yeasts being a matter of very low probability. Yeast No. 389, Gräfenthal, does not ferment melibiose, and in this respect forms a remarkable exception among the low-fermentation yeasts, all the others (according to the researches of Bau) containing melibiase.

As a rule the top-fermentation yeasts do not ferment melibiose, Lindner's report that pressed yeasts No. 430, No. 487, and No. 574 decompose this sugar being based on error. On the other hand, melibiose is fermented by the top-fermentation beer yeast, Liegnitz *a* No. 405, and by the pressed yeast, Winterhude, Race III. No. 139. The yeasts, No. 600 and No. 603, from Danish "Jopen" beer ferment melibiose; but contrary to Lindner's report, Broyhan yeast No. 330 (?) does not. These two classes of beer contain numerous organisms that cannot be classed along with culture yeasts; and it is therefore not surprising to find that they contain fungi capable of attacking melibiose. The only true top-fermentation yeasts that split up this sugar are the beer yeast Liegnitz *a* No. 405, and the pressed yeast Winterhude, Race III. No. 139.

In spite of the low fermentation temperature, low-fermentation yeasts occasionally assume a high-fermentation character, a peculiarity first observed by E. C. Hansen (LXV.)—*see* pp. 264 *et seq.*, vol. ii. The same thing was also noticed by Bau (VI.), at intervals, in Holland; and similar communications have been

made by other workers, an exhaustive report having been furnished by W. Henneberg (IV.). The low-fermentation yeast investigated by the latter and found to assume top-fermentation characteristics in a remarkable and constant manner, fermented melitriose completely; and as it also fermented melibiose, it consequently retained the characteristic property of low-fermentation yeast as well. The question therefore arises whether such top yeasts as ferment melibiose were originally low-fermentation yeasts that have acquired top-fermentation characteristics spontaneously and have retained them owing to the conditions of cultivation.

The extensive group of wine yeasts, together with the lactic acid yeasts, do not ferment melibiose. Lindner found that this sugar was attacked by Dürkheim No. 54 yeast and Küster Tokay yeast No. 534. However, since these yeasts are no longer cultivated and no further tests with them are possible, they should be struck out of the scientific literature. With the foregoing exceptions, all the races examined by Schukow (I.), Bau (XXVI. and XXVII.) and Lindner (XXXV.) were found to contain no melibiase, though Kalanthariantz (I.) found wine yeasts that were capable of splitting up melibiose. In one of these races, from Bari in Apulia, a decided hydrolysis of melibiose was observed on digesting the solution of the sugar with the yeast at 40° C., though no such action took place at 25°–30° C. Assmannshausen yeast also hydrolysed melibiose powerfully at 25° C.; but in view of Lindner's (XXXV.) statement that this yeast has no action on melibiose, the report of Kalanthariantz needs confirmation.

Of the wild yeasts that have been accurately defined in a botanical sense, *Sacch. Pastorianus I.* and *III.* ferment melibiose.

A special position is occupied by Logos yeast (*see* p. 276, vol. ii.), which, according to Bau (XV.) and Schukow (I.), does not ferment melibiose, though Lindner (XXXV.) obtained a different result. According to the results obtained by Bau (XXVI. and XXVII.), there are two races of this yeast, one of them fermenting melibiose, whilst the other does not. Similiar race divisions occur in the case of *Schizos. octosporus*, and *Monilia variabilis*, and especially *Torula colliculosa* (*see* p. 398, vol. ii.).

Summarising these investigations, it appears that, with the exception of Gräfenthal No. 389, all the culture low-fermentation yeasts ferment melibiose, as do also two high-fermentation culture yeasts, namely Liegnitz *a* No. 405 beer yeast and Winterhude pressed yeast Race III. No. 139, *Sacch. Pastorianus I.* and *III.*, two yeasts from Danzig Jopen beer, No. 600 and No. 603, a number of unnamed wild yeasts and one race of Logos yeast.

According to H. Gillot (V.), melibiose is also left unattacked by top-fermentation yeasts when readily assimilable sugars, such as grape sugar, are presented to the yeast at the same time.

Bau (VII.) based a method for detecting the adulteration of

pressed yeast by low-fermentation yeast on the exclusive faculty of the latter for fermenting melibiose.

In the case also of Buchner's permanent yeast, it is possible to detect whether the same is composed of top or bottom yeast, or of a mixture of both, the presence of melibiase being sufficient to demonstrate that of bottom yeast. The test is easily performed by the aid of melibiose, which sugar cannot be split up into its components, *d*-glucose and *d*-galactose, by any yeast enzyme other than melibiase. These components can be readily identified by means of phenylhydrazine, the experiment being carried out in the following manner: A 1 per cent. solution of melibiose, entirely free from any other kind of sugar, is treated with 2 per cent. (or a little more) of the yeast under examination, in presence of 1 per cent. of toluene, and kept for 1–3 days at about 25° C. The extract is filtered, and the filtrate is boiled with a small quantity of good bone black, then refiltered until clear, and the liquid tested with phenylhydrazine, 2 grms. of which, and 2 grms. of 50 per cent. acetic acid, are added for each gramme of melibiose employed, the mixture being heated for an hour on the boiling water bath. The mixture is poured out into cold water—about 3 vols. to each unit of melibiose solution originally taken—and filtered, the residue being washed once with water and then rinsed into a beaker, in which it is boiled up with water. If the resulting osazone dissolve completely in boiling water, no bottom yeast is present (at least in detectable quantity); but if the osazone remain undissolved, the presence of bottom yeast is demonstrated, since glucosazone and galactosazone are only sparingly soluble in boiling water. In addition to the ratio of solubility in hot water, the microscopical examination affords further indications, inasmuch as melibiosazone crystallises in fine needles, invariably arranged in stellar groups, whereas the two hexosazones chiefly form coarse, long, and thick needles. This difference will be sufficient for the experienced analyst, whilst those who wish to determine the character of the osazone by the melting-point and ultimate analysis must adopt a complicated method—on which point *see* Bau (XV.), who succeeded in detecting with certainty the presence of 10 per cent. of bottom yeast in top-fermentation permanent yeast by this method.

§ 330.—Lactase.

Milk sugar, or lactose, is one of the oldest known sugars, having been described by Fabricio Bartoletti as long ago as 1615. It occurs in the milk of mammals, cows' milk containing 3.6–5 per cent. (mean about 4.5 per cent.). goats' milk 3.26–6.65 per cent., ewes' milk 3.43–6.62 per cent., mares' milk 4.72–7.32 per cent., and the milk of the she-ass 5.29–7.63 per cent.

When lactose is boiled with dilute mineral acids, it is split up into equal molecules of *d*-glucose and *d*-galactose, the hydrolysis,

however, being only effected slowly and with difficulty. According to Ost (II.) the reaction is not complete unless one part of milk sugar be boiled with four parts of 2 per cent. sulphuric acid for six hours, or with ten parts of the same acid for four hours. Urech (I.) states that a solution of lactose containing 11.38 per cent. of hydrochloric acid will remain unaltered in the cold, even after the lapse of twenty-eight days; and that it is only when the proportion of acid reaches 32 per cent. that the sugar is almost completely decomposed within twelve hours at 23° C. Milk-sugar solution containing 4 per cent. of oxalic acid will remain unaltered after boiling for eight days; and according to Jones (I.) citric acid also is incapable of hydrolysing lactose. It therefore appears impossible for the lactose in a liquid containing that sugar to be decomposed merely by the organic acids formed by acid bacteria.

Like all di- and poly-saccharides, milk sugar is capable of being fermented directly, but requires to be previously split up into its components by a special enzyme, lactase.

As already mentioned on p. 163, vol. i., Beijerinck discovered this enzyme—to which the name, lacto-glucase, has been applied by E. O. von Lippman (IV.)—in *Sacch. Kefyr* and *Sacch. tyrocola*. According to E. Fischer (XV.), lactase cannot be extracted direct from lactose yeast with water, the cells having first to be triturated with ground glass, in order to bring the enzyme into solution. Lactose yeast that has been killed by means of chloroform also exerts a powerful hydrolytic action on lactose solutions. On the other hand, the enzyme can be readily extracted from Kefyr granules by water, on which account E. Fischer proposed to name it kefyr lactase in contradistinction to yeast lactase. This kefyr lactase has greater powers of resistance than maltase to the action of concentrated alcohol.

Buchner and Meisenheimer (I.) recovered from Armenian mazun yeast an expressed juice containing lactase. This yeast lactase will not diffuse through parchment paper, and is therefore —like the enzyme from *Monilia candida* (*see* p. 446, vol. i.)—an endoenzyme.

Among the fungi, *Mucor javanicus*—according to Wehmer (XIII.); *M. Cambodja*—according to Chrzaszcz (I.); and *Allescheria* (*Eurotiopsis*) *Gayoni*—according to Laborde (VIII.)—appear to contain this enzyme. Duclaux (XXX.) found that the matured mycelium of *Aspergillus niger* and *Penicillium glaucum* secretes lactase; but the point needs further investigation (*see* p. 363, vol. ii.).

According to E. Fischer (XV.), emulsin—which in any event is a mixture of enzymes—will also decompose lactose; and Beijerinck (XX.) and Bernheim (II.) report the capacity of a barley enzyme for hydrolysing milk sugar.

Lactase is widely encountered in the animal kingdom, and has been found by Kobert (IV.) in the juices of numerous lower

animals. It occurs in the mucous membrane of the stomach and intestines of infants and young animals, according to the statements of PAUTZ and VOGEL (IV.), WEINLAND (I.), FISCHER and NIEBEL (I.), and other workers.

The expressed juices recovered by STOKLASA and CZERNY (II.) from muscle, liver, lungs, and other organs, decompose and ferment milk sugar; and according to SIMACEK (II.), lactase is also present in pancreatic juice.

According to Weinland, the optimum temperature for lactase is 39° C.; but comprehensive research is needed on this point, as well as on the destruction temperature, and the favourable or adverse influence of chemicals and other substances on the enzyme, these properties of lactase being still undetermined.

Like maltase (*see* p. 525, vol. ii.), the remarkable property of reversion is also exhibited by lactase, especially that from kefyr. If 200 c.c. of extract kefyr granules be treated with 100 grms. of *d*-glucose and 100 grms. of *d*-galactose, in presence of 10 c.c. of toluene, and the mixture be kept in a tightly closed flask for fifteen days at 35° C., it is stated by E. FISCHER and ARMSTRONG (I.), that the mixture will furnish a new disaccharide, which they term isolactose. This sugar is split up again into *d*-glucose and *d*-galactose by a dilute solution of kefyr lactase, though not by emulsin. A highly interesting observation by E. Fischer and Armstrong is that isolactose is fermented by bottom yeast, but not by top yeast, the sugar thus behaving like melibiose with regard to these two races of yeast.

§ 331.—Trehalase.

The sugar, trehalose, consists of two molecules of *d*-glucose, which, however, are combined in a different manner from the two groups of grape sugar composing maltose, isomaltose, turanose, and probably other disaccharides, such as HILL'S (I.) revertose. They are hydrolysed with great difficulty by acids, the decomposition of over 99 per cent. of trehalose necessitating boiling for six hours with 5 per cent. sulphuric acid.

This sugar was formerly regarded as unfermentable, until Böning showed that the same "begins to ferment at the end of twelve hours in presence of best quality yeast that has been sufficiently washed"; compare TOLLENS (VII.).

BOURQUELOT (XIII.) afterwards discovered in *Aspergillus niger* (*see* p. 363, vol. ii.), an enzyme decomposing trehalose. For the preparation of this, trehalase—or trehalo-glucase, according to the nomenclature proposed by E. O. VON LIPPMANN (IV.)—he cultivated the mould fungus on Raulin's nutrient solution, triturated the culture with sand, extracted the water by means of 95 per cent. alcohol, and dried the residue *in vacuo*, the mass being then extracted with water and the filtrate precipitated with alcohol. The resulting mould-fungus trehalase, which also occurs

in species of *Penicillium* (*see* p. 363, vol. ii.), is destroyed by a temperature of 63° C. It is, however, doubtful whether the yeast enzyme that decomposes trehalose is identical with this mould-trehalase.

Bourquelot also found trehalase in barley and green fodder, and pointed out that the enzyme content, also detected in these raw materials by E. Fischer (XII.), originates in the mould fungi invariably present (*see* pp. 523, 524, vol. ii.). The French worker even went further, and expressed the opinion that trehalose is probably only fermented by yeasts when the latter have been grown in unsterilised malt worts, and have thus introduced trehalase derived from the raw material.

According to E. Fischer (XII.), however, pure yeast will hydrolyse trehalose, though invertase and filtered yeast extract do not. The behaviour of various yeast enzymes towards trehalose was then investigated by A. Kalanthariantz (I.), who found that certain wine yeasts hydrolysed 10–21.5 per cent. of trehalose at 22°–28° C., bottom-fermentation beer yeasts attacking 10–37.5 per cent. (at 24° C.), top yeasts, including Weissbier and Lichtenhain yeasts, hydrolysing 5–10 per cent., a number of other species 7.5–25 per cent., Kissly-Schtschi yeast 0 and 20 per cent., Logos 0 on one occasion and 25 per cent. on another, Pombe 0 on two occasions and 5 per cent. once. These results show considerable irregularity in the progress of hydrolysis, especially with the last-named yeasts. According to Delbrück (XIII.), trehalase can be detected in numerous wine, beer, and pressed yeasts.

The researches of Bau (XVIII.) failed to yield any definite result as to the presence, in bottom yeasts, of an enzyme capable of decomposing trehalose. These experiments were conducted at a fermentation temperature of 20°–25° C., and extended over four months. In the case of most of the organisms examined, the fermentation—if occurring at all—started slowly and pursued a sluggish course. The trehalose was gradually fermented by the yeasts US, UF, OS, Logos, *Sacch. ellipsoideus II.*, and *Sacch. Pastorianus I., II.* and *III.*, as well as by *Monilia candida*, only an inappreciable alteration of the sugar being produced by a lactose yeast, and very little, if any, by *Schizos. Pombe* and *Sacch. apiculatus*.

According to Kayser (IV.) the pineapple yeast (*see* p. 396, vol. ii.) ferments trehalose, the same effect being produced, according to Went (V.), by *Monilia sitophila;* by *Allescheria* (*Eurotiopsis*) *Gayoni*, according to Laborde (VI.); and by the so-called *Amylomyces* α and *Amylomyces* γ (*see* p. 89, vol. ii.), according to Rommel and Sitnikoff (I.).

Lindner (XXXIV.) states that trehalose is fermented by a yeast from Kissly-Schtschi, by *Monilia candida* and *M. variabilis*, by *Mucor Rouxii*, *Amylomyces*, by Danzig Jopen yeast No. 602, by a race (No. 402) of *Sacch. anomalus*, by nearly all the wine yeasts tried,

Sacch. Pastorianus I., II. and *III.*, *Sacch. ellipsoideus I.* and *II.*, *Sacch. cratericus*, by two yeasts from Breslau "Kretschmer" beer, and by culture bottom yeasts of the Frohberg type, though no definite result was obtained with Saaz yeast. In the continued experiments with races of the UF type, twenty-four yeasts fermented trehalose, whilst nine others gave negative or indefinite results. A majority of the culture top yeasts fermented this sugar only, rather more than 16 per cent. of them having no effect on trehalose; and similar behaviour was exhibited by most of the wild yeasts, only five out of thirty-seven races giving no result. With regard to Bau's statement that Logos yeast ferments trehalose, Lindner obtained a different result: and whilst Kalanthariantz found that three lactose yeasts split up trehalose, Lindner (XXXV.) could not obtain any fermentation of this sugar with the same yeasts.

Bau (XVIII.) has pointed out a general resemblance between the fermentation of trehalose and the course of fermentation of saccharose solution by *Monilia candida*, except that the operation proceeds more sluggishly.

Whereas Bourquelot's experiments indubitably prove that certain mould fungi contain an enzyme (trehalase) capable of splitting up trehalose, there does not seem to be any justification for assuming that this is also the case with the true yeasts—so far as present experience extends—the contrary being indicated in a striking degree by the irregularity of the decomposition and extreme sluggishness of the fermentation. The case is probably analogous with the behaviour of *Monilia candida* in presence of saccharose.

§ 332.—Raffinase.

Mudie (I.) discovered, in eucalyptus manna, a sugar, which was afterwards investigated by Berthelot (IX.), who called it melitose. This sugar has been already mentioned on p. 526, vol. ii. When Dubrunfaut, in 1850, observed that certain sugars deposited from beet molasses gave a polarimeter reading of over 100° (in the Soleil-Ventzke-Scheibler apparatus), Scheibler at first attributed this circumstance to the presence of an admixture of dextrin. This hypothesis, however, proved untenable, and the name, "plus sugar," was applied to the constituent causing this higher polarisation in beet sugar. In 1876, Loiseau succeeded in isolating this sugar in a pure state, and named it raffinose, because it was first recovered from sugar-refinery residues. The subsequent exhaustive researches of Tollens and his collaborators, and the simultaneously conducted investigations of Scheibler and Mittelmeier, revealed the identical nature of the sugars, melitose, raffinose, plus sugar and gossypose. The literature of these highly interesting labours—the study of which, in the original, is recommended to all young workers desiring to acquire a know-

ledge of the characteristics of the various sugars—will be found by referring to Bau (XVII.) and E. O. von Lippmann (V.).

Raffinose is a trisaccharide, *i.e.*, it consists of three simple sugars of the C_6 group, namely *d*-fructose, *d*-glucose and *d*-galactose. The two latter are in a more intimate state of mutual combination—they form melibiose (*see* p. 526, vol. ii.)—than that uniting them with the *d*-fructose. This latter is readily detachable, dilute acids (even weak acetic acid) sufficing to set up hydrolysis resulting in the separation of the trisaccharide into *d*-fructose and melibiose, according to the equation :

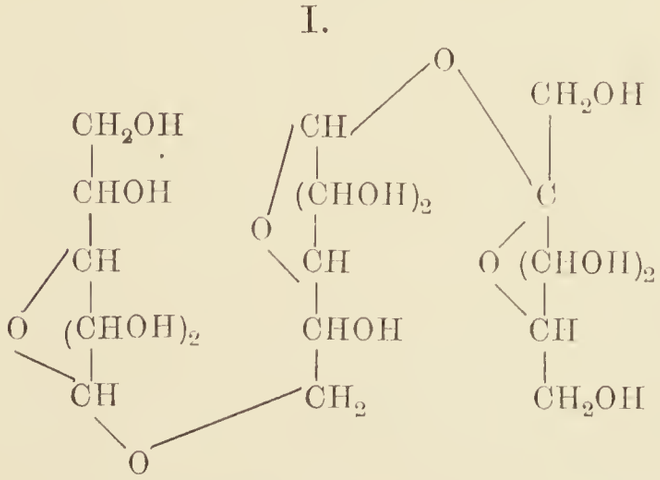
$$C_{18}H_{32}O_{16} + H_2O = C_6H_{12}O_6 + C_{12}H_{22}O_{11}.$$

The quantitative determination of raffinose in presence of saccharose is effected by several methods, which can be found in handbooks on the testing of sugar ; and Bau (XIII. and XXVII.) employed a method, based on fermentation physiology, whereby raffinose can also be detected in mixtures with other sugars. By the fermentation method he succeeded in determining the raffinose contained in beet-sugar molasses ; but the process requires so much time that it is only suitable for scientific investigations, and not for the practical conditions of the sugar industry.

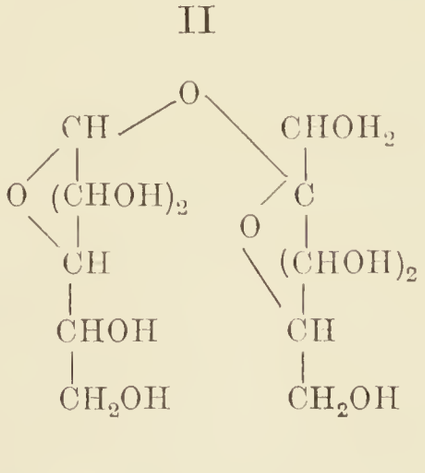
Raffinose is fermented by the great majority of yeasts that are able to ferment saccharose (*see* § 327). The fermentation of this trisaccharide is either complete—as with bottom yeasts—or else only about two-thirds of it are fermented, as is the case with most top-yeasts and a few other kinds, a fission-product, melibiose, being left behind (*see* p. 528, vol. ii.).

It was assumed that raffinose needed to be split up by a special yeast enzyme before it could be fermented ; but this opinion was opposed by Bau (XXX.), who asserted that *d*-fructose combines with melibiose to form raffinose, in exactly the same manner as it does with *d*-galactose to form saccharose. The method of combination is shown below, Formula I. representing raffinose, and II. saccharose :

I.



II



The fructose group on the right in these formulæ is combined in an identical manner with the melibiose group in the one case

and with the glucose radical in the other, so that the oxygen combination, indicated by thick type, can easily be resolved by invertase in both instances.

Lindner (XXXV.), however, objected that certain yeasts are able to ferment raffinose, but not saccharose, and that therefore the existence of a raffinase cannot be disputed. As an instance, mention may be made of *Schizosaccharomyces octosporus* which, according to E. Fischer and Lindner (IV.), contains raffinase but not invertase. On the other hand, Lindner's film yeasts No. 170 and 178 will ferment saccharose, leaving raffinose intact. In the case of animal invertase, Pautz and Vogel (I.), as also E. Fischer and Niebel (I.), found that the enzyme from the intestinal mucous membrane of dogs and horses will decompose saccharose, leaving raffinose unaltered.

From these statements, the existence of raffinase may be assumed to be demonstrated; but all that we know about it, apart from the fact that it splits up raffinose into *d*-fructose and melibiose, is that—as observed by Bau—it is decomposed at a temperature of 75° C., and is contained in Merck's invertase. According to Gillot (V.), raffinase occurs in mould fungi, as well as in yeasts.

In the case of polysaccharides, according to Bourquelot (XIV.), the enzymes act successively, so that in our case, the raffinose must first be hydrolysed by raffinase, before the melibiase can act on the liberated melibiose. No reversion takes place, and melibiase cannot attack raffinose itself.

In addition to raffinase—which merits exhaustive investigation—there are certainly other enzymes that are able to split up the polysaccharides; but as even less is known about these enzymes than about raffinase, we cannot devote a separate paragraph to them, and therefore they are briefly reviewed below.

Thus, it is doubtful whether the manna-trisaccharide, which Tanret (VI.) found in the manna of the ash-tree, is partially or completely hydrolysed by yeast, or not attacked at all.

The reports on the fermentation of melecitose by yeasts also require confirmation. According to Bourquelot and Hérissey (III. and V.), this sugar is decomposed by an enzyme of *Aspergillus niger*, into glucose and turanose. This latter sugar, a biose, composed of two *d*-glucose groups, may also be formed by the hydrolysis of melecitose by acids.

Bourquelot (XV.) states that only one-third of gentianose is fermented by yeast, the latter separating *d*-fructose and fermenting that sugar, but leaving the gentiobiose behind. However, under the influence of an enzyme of *Aspergillus niger*, and also of emulsin, gentiobiose can be split up, by further hydrolytic action, into two molecules of *d*-glucose.

A tetrasaccharide, stachyose, which was specially investigated by Tanret (VI.) and by A. von Planta and E. Schulze (I.), is

also only partially fermented by yeast, *d*-fructose being liberated and fermented, leaving a trisaccharide behind. Whether the latter—known as mannotriose—can be in turn attacked by yeast, has not yet been accurately determined. Emulsin, diastase, and *Aspergillus niger* act on stachyose in the same manner as yeast enzyme. Our knowledge of intimate properties of these enzymes that decompose the last-named polysaccharides is practically nil; and their investigation would therefore be extremely valuable, though complicated by the difficulty in the way of procuring the higher polysaccharides.

§ 333.—Fermentation of Dextrin by Yeast (Amylase).

As long ago as 1833, Biot and Persoz (I.) expressed the opinion that dextrin can be fermented by yeasts; though one of them, Persoz (I.), in collaboration with Payen, came to the conclusion, in the following year, that dextrin was unfermentable. This view was shared by G. Varry (I.), whereas Barfoed (I.) arrived at the opposite result.

In the course of their researches on the degradation of starch by diastase, Brown and Morris (IV.) found that dextrins are not directly fermentable by yeasts, but require first to be hydrolysed. Under certain conditions a few bottom yeasts, which act as the cause of secondary fermentation in English top-fermentation breweries, and are referred to by the authors in question as *Sacch. ellipticus* and *Sacch. Pastorianus*, are capable of hydrolysing dextrin, and thus afford an impetus to the direct fermentation of that substance. According to E. R. Moritz (I.), maltodextrins are not fermented during primary fermentation, but constitute the material for secondary fermentation.

According to the conception of the English chemists, the starch molecule is degraded, under the influence of diastase, in such a manner that a series of dextrins, the so-called amyloins, are formed, these constituting intermediate stages between soluble starch and maltose. These amyloins, which are also occasionally known as maltodextrins among German workers, are constituted according to the theory of Brown and Morris (V.)—in such a manner that m molecules of maltose, $C_{12}H_{22}O_{11}$, are combined with n molecules of dextrin, $C_{12}H_{20}O_{10}$. In the amyloins of low type, resulting from the more extensive degradation of the starch, the value m is far greater than the value n, the converse being the case with the amyloins that approximate more closely to starch in their composition. This clever hypothesis, however, failed to stand further theoretical and practical investigation; in which connection we need only mention the work of C. J. Lintner, who arrived at a different result in numerous published researches. In one of their experiments, Lintner and Düll (II.) found that the primary degradation-product of starch is an amylodextrin,

which is degraded into erythrodextrin by the action of diastase. Under the hydrolytic influence of malt enzyme, this product is transformed into achroodextrin, which furnishes maltose and Lintner's isomaltose—not to be confounded with Fischer's isomaltose, which is a well-defined, synthetic, non-fermentable sugar (*see* p. 525, vol. ii.). Varying results were obtained by other workers, both earlier and later; in fact all those who have taken up the problem of the degradation of starch by diastase—a question that has been under discussion for more than a century—have obtained different results, so that in the mouth of one author, the relatively established terms, amylodextrin and erythrodextrin, and more especially achroodextrin I., II., III., maltodextrin, &c., meant different substances to those implied by a second or third author, who believed himself to be working with the same compounds. An almost irremediable confusion exists in the nomenclature of these products, owing, no doubt, to the inadequate chemical and physical appliances at our disposal to enable the several transformation products to be isolated as well-defined individuals. Perhaps the exhaustive work of Moreau (I.) may finally clear the matter up. It would take up too much space to give even a brief historical review of the various hypotheses dealing with the intermediate products between starch and maltose; and we must therefore be content in this paragraph to class as "dextrin" all the intermediate bodies between starch and maltose that different authors have named amylo-, erythro- and achroodextrins, maltodextrins and amyloins, moreover, without drawing any distinction between the dextrins produced by diastase and those resulting from the action of acids on starch.

According to the views of English chemists, the amyloins effect the secondary fermentation of beer in the storage cask; which view is also expressed by E. R. Moritz (I.). If low-type amyloins be added to beer, the secondary fermentation assumes a more turbulent aspect and the beer matures earlier. In the opinion of Moritz, secondary fermentation is not effected by the true *Saccharomycetes*, but by the secondary-fermentation yeasts, with which Brown and Morris associate wild yeasts. This view was responsible for the sceptical attitude taken up in England with regard to the application of the Hansen method of pure-yeast culture to the production of top-fermentation beers. According to Moritz, the higher-grade amyloins, which dextrins approximate more closely in composition to starch, cause gradual secondary fermentation, so that the beer takes longer to mature.

Medicus and Immerheiser (I.) were led into quite a different sphere of labour on the fermentability of dextrins, by the examination of a suspected wine for the presence of added impure starch sugar. In their experiments on the fermentation of dextrin by yeasts they employed, in part, impure starch sugar, and in part prepared from this latter a so-called dextrin, which

they subjected to the action of commercial, but starch-free, pressed yeast. For example, 40 grms. of starch sugar were dissolved in 250 c.c. of water containing nutrient salts, the glucose being eliminated by fermentation. The resulting solution, corrected to the original volume, gave a polarimeter reading of $+7.65°$ in the first experiment, and $+6.90°$ in the second. After pitching the filtered solution with 5 grms. of pressed yeast, the polarimeter readings at the end of 6 days receded to $3.35°$ and $2.75°$ respectively; and after a further sojourn of 5 days at 30° C. the reading had fallen to 1.9 in both cases, an additional diminution ensuing after the addition of more yeast. In other experiments the same workers succeeded in eliminating the dextro-rotatory substances completely; but the process cannot be regarded as a true fermentation of dextrin in our sense of the term, neither carbon dioxide nor alcohol being produced.

E. von Raumer (I.) also, in the course of examining certain honeys suspected of containing added starch suger, endeavoured to ascertain how far dextrins can be fermented by yeasts. His results showed that pressed yeast, beer yeast and wine yeast behave differently, the first named attacking the dextrins powerfully, the latter not at all.

Fresenius (III.) was able to confirm the reports of Medicus and Immerheiser. According to his researches the so-called unfermentable dextro-rotatory substances are also fermentable by pressed yeast, though not affected by beer yeast. Fresenius, moreover, drew attention to the circumstance that film yeasts also eliminate dextrins.

Hence, it is important for the analyst who has to examine honey, or wine for added starch sugar, to know that he should never employ commercial pressed yeast. The "Convention," Vereinbarungen (II.), prescribes that 25 grms. of honey be dissolved in 200 c.c. of a nutrient solution and sterilised, and after cooling should be pitched with 5 c.c. of fluid, vigorous and, preferably, pure-culture wine yeast, *not pressed yeast*, fermentation being conducted at 20°–25° C. The liquid is then made up to 250 c.c. with water, after being clarified with alumina, or 3 c.c. of lead acetate and 2 c.c. of sodium sulphate solution, and is examimed in the polarimeter at 20° C. If the fermentation residue exhibits any considerable dextro-rotation, it should be examined for dextrin by precipitation with alcohol.

In the examination of wine for the presence of impure starch sugar, K. Windisch (VI.) recommends that 210 c.c. of the wine be concentrated to one-third by evaporation, and then diluted with water until the liquid contains not more than 15 per cent. of sugar. This solution is pitched with about 5 grms. of vigorous beer yeast that is free from optically active constituents, and fermentation is carried out to completion at a temperature of 20°–25° C.

The fermentation residue is tested for dextrin by a special method given by the same author (*l.c.*).

Lintner (XII.) opposed the views of Medicus, Fresenius and others by asserting that the earlier experiments were inconclusive, having been performed with impure dextrin or impure yeast. The dextrins in starch sugar are partly reversion products, such as the gallisin of Scheibler and Mittelmeier (IV.); and the pressed yeasts are not composed of uniform organisms, but contain many bacteria capable of attacking dextrin. Malt dextrin is unfermentable by pure yeast of *Sacch. cerevisiæ* type; and the numerous conflicting reports may also be explained by the circumstance that many of the workers employed dextrins containing sugars, and not the pure substance. E. von Raumer's observation of a difference in yeasts, inasmuch as pressed yeast attacks dextrin most powerfully, beer yeast acting in moderate degree, and wine yeast not at all, renders it fairly certain that his pressed yeast contained large numbers of bacteria, and the beer yeasts probably contained film yeasts, whilst the wine yeast was relatively pure. Shifferer (I.) also reports that malt dextrins resist the action of pure top yeast, though they are eliminated by treatment with impure commercial pressed yeast.

A new stage in our knowledge of dextrin fermentation was reached when the study of yeasts led to the establishment of certain definite types in the *Sacch. cerevisiæ* group. This was stimulated by the discovery of Saaz yeast (*see* p. 112, vol. ii.) and the differentiation between the Saaz and Frohberg types, the former of which gives a lower final attenuation than the latter. Since both top and bottom yeasts form well-defined races, we have the types: bottom Frohberg, bottom Saaz, top Frohberg, and top Saaz, distinguished by the signs: UF, US, OF, OS respectively. Yeasts of the OF and UF type produce the same final attenuation in beer wort, OS and US being also equal in this respect. It is true that insignificant fluctuations occur in one final attenuation, since the yeasts do not merely ferment the carbohydrates, but also assimilate and modify a number of other wort constituents, especially nitrogen compounds and salts, besides producing non-volatile products, especially glycerine and succinic acid (*see* p. 490) in varying quantities. This explains why the residual extract in one and the same wort that has been fermented by different yeasts, *e.g.*, of the UF type, is not identical in all cases, but frequently exhibits small differences.

In addition to the foregoing, H. van Laer (III.) established the Burton type of yeast, which ferments worts to a greater extent than the UF yeasts. He also differentiated intermediate types, giving an attenuation midway between the types already mentioned, and named them Saaz-Frohberg and Frohberg-Burton. These types, however, never found acceptance in fermentation physiology; and, in fact, van Laer (XII.) himself partly aban-

doned them on discovering Logos yeast. He then distinguished between the types S, F, and Logos, as well as the subsequently discredited intermediate types, Saaz-Frohberg and Frohberg-Logos.

A new type was discovered by LINDNER (XXX.) in *Schizos. Pombe* (*see* p. 293, vol. ii.), which is able to carry the fermentation of wort further than is the case with Logos yeast. This yeast has been exhaustively studied by W. WINDISCH (VI.) and F. ROTHENBACH (1.).

Consequently we have the following four types of yeast: S, F, Logos, and Pombe. The differences of attenuation produced by these yeasts are undoubtedly due to the fact that some of them are able to ferment dextrins, in addition to maltose (and hexoses). This faculty has been proved in the case of Logos yeast and *Schizos. Pombe*, though the cause of the difference between the types S and F has not yet been established on a scientific basis. It is true that numerous workers, especially PRIOR (VII.), have occupied themselves with this problem; but, up to the present, the researches and hypotheses of Prior, and also those of BAU (XXXII.), have not led to any final and recognised result.

The direct examination of the fermentability of dextrin by pure cultures of yeast was performed by LINDNER (XXXV.), who obtained the surprising result that many species ferment dextrin in a greater or smaller degree, even yeasts of the Saaz type being found by the same author (XLVII.) to be capable of attacking dextrin, namely, the top-fermentation OS yeasts, Nos. 150, 159, 160 and 403. On the other hand, Saaz yeast itself, and various other races belonging to the OS and US types, do not ferment dextrin. Certain other top-fermentation beer yeasts of the OF type, including Burton yeast, attack dextrin; and indeed, out of forty yeasts of this type examined, only eight left the dextrin intact.

Nearly all top yeasts from pressed-yeast factories ferment dextrin energetically, only one yeast from Bavaria, out of thirteen races examined, giving a doubtful result—compare LINDNER (XLVII.).

The true distillery yeasts, on the other hand, have, in general, little or no fermentative power on dextrin, 50 per cent. of the races examined leaving this carbohydrate intact.

Out of thirty-three low-fermentation beer yeasts, only one fermented dextrin energetically, another slightly; and in the case of fourteen races the result was doubtful—compare LINDNER (XLVII.), who also reports that a few races of wine yeast can ferment dextrin.

The film yeasts examined by Lindner have no action on dextrin; but, on the other hand, the species of the genus *Willia* (*see* p. 289, vol. ii.) seem to attack this carbohydrate slightly, only one species, *W. belgica*, giving decidedly negative results.

The wild yeasts with botanical names, *S. Pastorianus I.*, *II.*,

III., *S. ellipsoideus* and *S. cratericus*, ferment dextrin more or less strongly; of thirty-one other wild races examined, thirteen pure cultures were found to liberate carbon dioxide, faintly or decidedly, from dextrin solutions.

Of other organisms that ferment dextrin, mention may be made of: *S. exiguus*, *Monilia candida*, *Mon. variabilis*, *Sachsia suaveolens*, *Mucor Rouxii*, *Amylomyces* β and *Am.* γ, Logos yeast, *Schizos. Pombe*, *Schizos. mellacei*, and *Schizos. octosporus*. This carbohydrate is also attacked by a series of yeasts from Danzig Jopen beer.

According to P. Lindner's researches, no definite rule exists with reference to the fermentability of the dextrins by special yeasts; and it is urgently necessary that these investigations should be continued under the conditions laid down by C. J. Lintner, namely, the use of pure cultures and pure dextrins exclusively. The former condition was fulfilled by Lindner, but the solution of the second problem is complicated by the circumstance that, in spite of all that has been done, the dextrins resulting from the action of diastase on starch have not yet been isolated in the form of scientifically pure chemical entities.

However this may be, we may assume that the dextrins are not directly fermentable by yeasts, but that, like all polysaccharides, they must first be split up into directly fermentable sugars by the action of an enzyme. This enzyme, which has been named amylase, was found by Katz (II.) in various mould fungi, including species of *Penicillium* and *Aspergillus* (*see* p. 353, vol. ii.), as also in *Bact. megaterium*. Our knowledge of the amylase of yeast is at present very scanty, and much work is needed to amplify it.

Having now become acquainted with the present state of affairs with regard to the fermentation of dextrin, we are able to understand the patent taken out by Effront (XV.) for the cultivation of yeast possessing the power of fermenting that substance. According to the inventor, certain beer yeasts that are capable of slightly fermenting dextrin can have their powers in this respect considerably augmented by cultivation under favourable conditions. The yeasts are first grown in a medium that contains aldehyde, in addition to dextrose and mineral substances (potassium nitrate). After preparation in this manner, they exhibit a decided tendency to ferment dextrin; and this property can be further intensified by suitable treatment. The scientific explanation of the Effront process may be sought in two directions; either each beer yeast contains a dormant enzymogen, which can be stimulated to an increased production of amylase by suitable treatment, or else the method of cultivation (perhaps by spontaneous infection) favours certain yeasts that already produce amylase, so that they gain predominance in the cultures, under the conditions of environment selected by Effront, obeying the law of natural pure culture as established by Delbrück. No

accurate investigation has yet been instituted on either of these lines; and up to the present nothing is known as to the practical success of the Effront method.

§ 334.—The Autofermentation of Yeast.*

Except for an antecedent, though indefinite, announcement by Berthelot (III.), Pasteur may be regarded as having been the first to observe that, under certain conditions, the total carbon dioxide and alcohol produced from a solution of sugar by the action of yeast may exceed the theoretical yield calculated from the sugar initially present in the nutrient solution. Thus, for instance, 0 424 grm. of sugar and 10 grms. of yeast (calculated to dry substance), instead of furnishing about 110 c.c. of carbon dioxide, in accordance with the equation of decomposition, actually produced 300 c.c., together with 0.6 grm. of alcohol. Since only a portion and not the whole of this yield is covered by the quantity of sugar present in the nutrient solution, the remainder must have originated in the constituents of the yeast cells, so that the latter possess the faculty of fermenting, not merely the sugar in their vicinity, but also the material of their own corpus. As a matter of fact, under certain conditions (previous abundant nutrition, restriction of the access of air, &c.), considerable quantities of alcohol may be formed in a mass of yeast, without even a single trace of sugar having been added. The term applied to this phenomenon is "autofermentation."

Pasteur's explanation of the phenomenon was that the yeast cells contain a substance capable of being transformed into sugar and afterwards fermented; and, by boiling yeast with dilute sulphuric acid, he succeeded in extracting no less than 20 per cent. of fermentable sugar (calculated on the dry matter of the yeast). His assumption, however, that the cell wall is the source of this sugar was an error out of which his rival, Liebig (III.), made a good deal of capital. This latter worker showed, in the case of a number of samples of yeast, that the cellulose, forming the residue of the method of extraction employed at that time, was in considerably smaller amount than would be requisite to produce the total alcohol resulting from autofermentation. Thus, to mention only a single instance advanced by Liebig, 100.6 grms. of yeast, found by a preliminary experiment to contain 13.9 per cent. of so-called cellulose, furnished by autofermentation 13.9 per cent. of alcohol, whereas the theoretical yield from the amount of cellulose in question was only 11.3 per cent. at the most. Liebig regarded this observation as a proof of his own theory of fermentation; but it must be pointed out that, though the determination

* This paragraph has been chiefly drafted on the reports of Professor Lafar, to whom I am greatly indebted for his valuable assistance here, and in other portions of chapters lxiv. and lxv.—A. B.

corrected an erroneous (but by no means fundamental) error of his French opponent, it did not controvert the latter's explanation of the nature of autofermentation. The counter-hypothesis successfully brought forward against Liebig by Nägeli and Loew (II.), namely, that yeast contains a greater amount (up to 37 per cent.) of cellulose than reported by him is, however, untenable for the reason that it was based on the result of analytical determinations in which the so-called cellulose was not weighed separately, but was taken in conjunction with a mixture of other mucilaginous substances (*see* p. 175, vol. ii.), probably including glycogen, which at that time had not been identified.

After Salkowski (IV.) had observed that yeast digested with chloroform water does not undergo autofermentation, but yields a lævo-rotatory sugar, he arrived at the conviction that the source of this sugar was to be found in glycogen. The same result was attained by Cremer (I., V. and VII.), except that this worker showed the resulting sugar to consist of the dextro-rotatory *d*-glucose. The accuracy of this latter point was subsequently admitted by Salkowski (IV.).

Autofermentation, therefore, proceeds at the expense of yeast glycogen, the degradation of this carbohydrate being accompanied by other transformations that were formerly regarded—and also by Kutscher (II.)—as autofermentation. These phenomena relate to the transformation of the nitrogen compounds. According to M. Schenck (I.), however, a sharp distinction must be drawn between the autofermentation and the autodigestion of yeast. This latter form of decomposition will be dealt with in chapter lxvi.

Nevertheless, it will be advisable to mention here that aromatic perfumes are formed during autofermentation, probably as a direct accompaniment thereof. These fruity odours are emitted with special intensity when the yeast is stored in a pressed state, a fact well known in practice. Further investigations on the nature and causation of these aromatic substances are desirable.

Some particulars have already been given (p. 168 *et seq.*, vol. ii.) respecting glycogen and its hydrolysis by a yeast enzyme. This latter, to which the name glycogenase, has been given, seems to be incapable of diffusion through the cell membrane of the yeast, its action being confined to the interior of the cell. This may be concluded from the circumstance that the liberated enzyme in expressed yeast juice prepares added glycogen for fermentation. This glycogenase, which to some extent is still of a hypothetical character, is credited not merely with the faculty of hydrolysis but also, in a high degree, with the power of reconstructing glycogen under certain conditions. With regard to maltase and lactase it has already been mentioned, on pp. 525 and 532, vol. ii., that these two enzymes are capable of producing reversion effects in addition to their hydrolytic properties. The condition under which glycogen increases or disappears in the yeast cell have been exhaustively

investigated by HENNEBERG (II.); according to his view (VI.) it may be assumed that *Sacch. apiculatus* does not contain glycogen. Further investigation on the subject of glycogenase would constitute a valuable sphere of labour, since all that can be predicted with regard to its still hypothetical existence is the possession of the two properties mentioned.

A few remarks may be made here respecting the manner in which autofermentation may be influenced by various agencies. We have already seen on p. 172, vol. ii., that, according to Salkowski and Cremer, yeast is affected, by digestion with chloroform water, in such a manner that, whilst the glycogen is split up, the yeast does not undergo autofermentation. C. J. LINTNER (III.) states that the same effect can be produced by the addition of common salt, and he also investigated the action of other saline substances by adding, in all cases, 5 grms. of the salt under examination to each 10 grms. of aspirated bottom yeast rich in glycogen, containing about 25 per cent. of dry substance. Autofermentation was not set up in the samples treated with a chloride of sodium, magnesium, or aluminium, or with ammonium chloride, nitrate, or sulphate. A restrictive influence was produced by the sulphates of manganese and copper, as well as by potassium nitrate. On the other hand, a stimulating effect was brought about by the sulphates of sodium, zinc, magnesium or ferrous oxide, and by monopotassium phosphate. Hence, in certain circumstances, autofermentation may fail to occur in a yeast that is very rich in glycogen, when treated with restraining agents.

The autofermentation of yeast had a certain practical value for the analytic chemist, namely, when the sugar content of a sample submitted for examination is to be determined by the physiological method (*see* p. 427, vol. ii.). It is true that methods are available in which the influence of the yeast can be minimised, not only with regard to autofermentation, but also with reference to the other transformations (*see* pp. 482 and 510, vol. ii.) brought about by yeast, the sterilised solution being inoculated with an imponderable trace of yeast, as recommended by ELION (V.) and BAU (XXXI.). These methods, however, occupy a good deal of time, and though the time question may be neglected in researches of a purely scientific character, it prevents their application in practice, despite their accuracy. For a rapid examination, or commercial analysis, a larger sowing must be used, and in these circumstances the glycogen content and autofermentation of the yeast must be borne in mind, no matter whether the sugar be determined from the resulting carbon dioxide or the alcohol formed. These derivatives are neglected in the method of Elion and Bau, the only determination made being the loss of extract during fermentation, the result, supplemented by the cupric reduction and polarimeter tests, giving the actual content of sugar. In

commercial analyses a blank experiment has been recommended, in which the same quantity of yeast is employed with the liquid under examination in the one case, and with an equal volume of distilled water or nutrient solution free from sugar in the other. If the sugar content be determined from the carbon dioxide liberated, it has been thought that these precautions would eliminate the influence of the autofermentation of the yeast. The experiments of C. J. Lintner, however, have shown that the saline matters present act differently on autofermentation; and therefore, as the full composition of the liquid under examination is generally unknown, it is impracticable to carry out the blank experiment in such a manner that the yeast acts in a liquid that is identical with the one in question, except that sugar is absent. In the blank experiment one is working with unknown quantities, whose influence on autofermentation is still undetermined, so that new sources of error are introduced to an indefinite extent. It has been proposed to stop the fermentation experiment at the point at which a preliminary test has shown that the sugar of the counter experiment is completely fermented; but, as was seen on p. 173, vol. ii., the degradation of the glycogen begins at a time when fermentable sugars are still present in the nutrient medium. According to Jodlbauer, this danger does not exist when not more than two parts of moist yeast (containing about 25 per cent. of dry substance) are used to one part of sugar. The method recommended by him for the physiological determination of sugar is as follows: The nature of the sugar present in the samples is first ascertained by qualitative tests; and then the reducing power of the substance toward Fehling solution is determined (after hydrolysis in the case of saccharose or raffinose; this method being inapplicable in the case of trehalose), and from the results so obtained a calculation is made of the amount of substance that contains 2 grms. of the sugar. This amount—which in the case of solid substances is dissolved in 25 c.c. of water, is treated with 1 grm. of fresh beer yeast, that has been freed from water on an unglazed porcelain or earthenware plate, 1 c.c. of Hayduck's nutrient solution being added when the substance is low in nutritive substances. Fermentation is allowed to proceed at about 34° C., a weak current of hydrogen gas being drawn through the liquid, and the escaping carbon dioxide collected in an absorption apparatus. If the continued examination of a parallel experiment justifies the assumption that all the sugar in the main flask has just been consumed, then the main experiment is stopped, and the whole of the carbon dioxide remaining in the liquid and the free space of the flask is driven over into the absorption apparatus by careful boiling and the continued passage of a small current of hydrogen, the amount of the fermented sugar being determined from the increased weight of the absorption apparatus. In spite of all the excellent work performed by

Jodlbauer in this connection, it must be admitted that this method should only be resorted to when all other means have failed. The apparatus must be put together with great care in order to prevent, on the one hand, any escape of fermentation carbon dioxide throughout the whole of the experiment—lasting about twenty hours in presence of glucose, and twice as long in the case of saccharose—and, on the other, to exclude atmospheric carbon dioxide.

These particulars should also be borne in mind by those who wish to determine the amount of sugar in urine by the fermentation method. More precise information on the method employed for this purpose and the Einhorn saccharometer—which is similar to the fermentation flask noticed on p. 207 of vol. i.—may be found in the handbooks on Urine Analysis, especially that of Neubauer and Vogel, the last three editions of which have been supervised by H. Huppert (I.). All these handbooks advise a check experiment, to ascertain the amount of carbon dioxide liberated by the yeast on digestion with water only. Though the question of autofermentation is thus touched upon, the prescriptions given leave much to be desired on the score of accurracy, and in particular fail to bear in mind the possible occurrence of glycogen in the yeast. Excellent service in this connection has been rendered by E. Büchner and S. Mitscherlich (I.) by the elaboration of a method of preparing yeast free from glycogen. Utilising the observations of Henneberg (IV.), they treated yeast by spreading the pressed and screened material in a thin layer exposed to the air. On the yeast being kept in the ice-chest (at about 2° C.), no glycogen can be found after about a day; at about 20° C. it disappears in eight hours; and as quickly as 3–4 hours in the thermostat at 35°–45° C. As a rule the fermentative power of the yeast is unimpaired by this treatment. These workers state positively that yeast as free as possible from glycogen must be used for the detection of sugar in urine, since yeasts that are rich in glycogen may give rise to the erroneous impression that the urine under examination contains sugar. The permanent yeast sold under the name "zymin" is not suitable for this purpose, inasmuch as it contains glycogen (*see* p. 474, vol. ii.).

In conclusion it may be mentioned that the value of many of the scientific treatises dealing with the attenuation powers of yeast will assume a different proportion when examined with a view to ascertaining whether the possibility of the intervention of autofermentation has been duly considered by the authors. All experiments in which increased quantities of yeast have been employed in one and the same test require to be repeated and confirmed in this connection.

CHAPTER LXVI.

ENDOTRYPTASE AND PHILOTHION.

By Dr. M. HAHN (§ 335) and Dr. LAFAR (§ 336).

§ 335.—Endotryptase.

The existence of a proteolytic enzyme in yeast was indicated by the discoveries of several of the earliest workers, attention to the subject being drawn more particularly to the phenomena occurring in autofermentation (p. 543, vol. ii.). After it had been shown by Thénard (I.), Pasteur (XXXIV.), and Duclaux (XXXI.) that yeast loses weight during fermentation, and especially becomes poorer in nitrogen, Liebig (II.) discovered the presence of leucin in water surrounding yeast that had been undergoing autofermentation. Béchamp (VII., VIII., X., XI.) and Schützenberger (II.) were the first who learned to differentiate two separate processes in autofermentation: one leading to the decomposition of the carbohydrates into alcohol and carbon dioxide, and the other resulting in the decomposition of proteids and therefore worthy to rank as an actual digestion process. Béchamp's discovery of the hydrolysed proteids in the water used for washing yeast led him to formulate a physiological theory of fermentation: "In yeast, as in the case of all living organisms, we observe a double series of phenomena. First the phenomena of nutrition and assimilation induced by the presence of their foodstuffs (sugar, nitrogenous substances, and mineral salts), these various substances entering the cells endosmotically and being there transformed and utilised in the construction of tissues for the new-born cells Side by side with these phenomena of nutrition, however, but reversed, occur the phenomena of disassimilation, whereby the tissues are transformed into excrementitious substances, which are no longer beneficial to the life of the cell, and are ejected." (He classes alcohol and carbon dioxide in this category.) More recently, Boullanger (I.), Beijerinck (XXXI.), Artari (I.), Wehmer (XII.), and especially Will (XXXV.) have more closely investigated the proteolytic processes in yeast cultures, after the theory of the autodigestion (autolysis) of yeast has been firmly established by Salkowski (II.), by the digestion of yeast in chloroform water (*see* p. 175, vol. ii.). M. Hahn (I.) succeeded

in preparing a cell-free solution of the enzyme, after demonstrating the presence of a strongly proteolytic enzyme in the expressed juice obtained from yeast by the method of Buchner and Hahn (*see* p. 459, vol. ii.), and studying the properties of this enzyme (which he named "yeast endotryptase") in collaboration with L. Geret (I. and II.). The fission products of autodigestion were afterwards more closely examined by Fr. Kutscher (III.), chiefly by the Kossel method.

The existence of endotryptase can be proved in a most convenient and striking manner with yeast juice prepared by the method of Buchner and Hahn. A few cubic centimetres of the expressed juice distributed on thymol- or carbol-gelatin (*see* p. 270, vol. i.), or an ordinary nutrient gelatin with an addition of thymol, in a test-glass, produce a decided liquefaction of the gelatin in twenty-four hours at 22° C., the whole being liquefied in two to three days when 10 c.c. have been taken. The autolysis of the pressed yeast juice is equally convincing and undeniable; whereas the freshly prepared juice is strongly coagulated by boiling, a decided diminution of the coagulum is observed on boiling the juice after it has been kept, in presence of a little toluol or chloroform, for twenty-four hours at 37° C., a precipitate, however, forming without boiling. The formation of a coagulum is, moreover, almost entirely prevented by storing the juice for six to seven days at 37° C., or ten to fourteen days at room temperature; whilst a deposit of amino-acids (leucin particularly) is formed. Of course the dried pressed juice, mentioned on p. 463, vol. ii., can be used for the same purpose, after being dissolved in water and treated with an antiseptic. The detection of endotryptase can also be effected with the recently introduced permanent yeast (*see* p. 481, vol. ii.) on stirring the latter up to a thin pap with water and spreading it on gelatin in presence of toluol. This method, however, never produces such rapid liquefaction as can be obtained by the use of expressed yeast juice. Carmine fibrin (*see* p. 301, vol. i.) suspended in pressed yeast juice also dissolves in twenty-four hours at 37° C., and stains the liquid dark red; but the liquefaction of coagulated egg albumen takes longer to accomplish. Will (XXXI.) also observed the liquefaction of gelatin in living cultures, with stab cultures of various species of *Saccharomycetes* (*see* p. 555, vol. ii.) in wort-gelatin, and kept for eighteen to eighty days at 20° C., or for forty-five to two hundred and forty days at 13° C., the liquefaction usually beginning in the path of the stab. The quantitative determination of the effect produced by endotryptase can also be performed in a most convenient manner with pressed yeast juice, the coagulum produced by boiling the fresh and digested juice being dried and weighed, or else (which is preferable) the increase of nitrogen in the filtrate is ascertained. With this object 10 c.c. of yeast juice are diluted with water, treated with about 5 c.c. of a saturated solution of common salt,

neutralised, heated to boiling, acidified with a few drops of acetic acid, and filtered through a dry filter after having been made up to a definite volume on cooling. The nitrogen in an aliquot part of the filtrate is then determined by the Kjeldahl method. By performing the determination with fresh and digested juice, the increased amount of nitrogen in the filtrate gives a very accurate representation of the progress of digestion. According to Salkowski (II.) the determination is effected in precisely the same manner in the case of yeast suspended in water, the nitrogen being determined in the filtrate (separated from the coagulum) before and after the digestion of the yeast in water containing a little chloroform or toluol. The proteid nitrogen may be determined either in the coagulum, or else, according to Iwanoff (II.), in the precipitate furnished by copper oxide (*Stutzer*).

Chief among the properties of endotryptase ranks its practically valuable behaviour toward high and low temperatures. Temperature is the decisive factor, not only for the decomposition of proteids during fermentation (as already mentioned several times in chap. lxiii.), but also for the activity of alcoholase, which enzyme may, in certain circumstances, be injuriously affected by the endotryptase that is acting concurrently. All experiments on the properties of endotryptase may be carried out in a most satisfactory manner with expressed meat juice which, to some extent, represents a solution of this enzyme, and forms the best material from which the action of the enzyme can be quantitatively determined. Geret and Hahn obtained the accompanying particulars (*see* Tables on next page) with regard to the optimum and destruction temperatures of the enzyme.

The optimum temperature seems therefore to lie between 40° and 45° C., whilst the enzyme is completely destroyed by heating at 60° C. for an hour. In the dry state, both in pressed yeast juice and permanent yeast, endotryptase naturally exhibits higher powers of resistance to heat. On the other hand, at low temperatures (3° to 7° C.)—as was shown by an experiment continued for fourteen days by Hahn and Geret—the digestion, though by no means entirely prevented, is so considerably delayed as to demonstrate the advantage of carrying out ordinary yeast fermentations at low temperatures, in view of the protection of the alcoholase (*see* p. 473, vol. ii.).

As in the case of other proteolytic enzymes, the influence of gases on the action of endotryptase seems to be very slight. Lack of oxygen was shown by the experiments of Geret and Hahn (I. and II.) to have no such favourable influence on the proteolysis of pressed yeast juice as was reported by Will (XXXV.) in the case of the living cells. On the contrary, the passage of air or oxygen was found rather to stimulate the digestion of protein; whilst the operation proceeded unhindered by the passage of a current of carbon dioxide or hydrogen. So far as carbon dioxide

Temperature ° C.	Percentage of Coagulum.	
	Before digestion.	After digestion for 20 hours.
1. At 3°–7°	2.72	2.57
2. „ 22	4.71	3.93
3. „ 37	4.71	2.71
4. „ 48	4.71	2.42

After heating for 1 hour.	Percentage of Coagulum.	
	Before digestion.	After digestion for 20 hours at 37° C.
1. To 50° C.	3.4	1.36
2. „ 55°	3.4	1.99
3. „ 60°	3.4	3.36
4. Unheated control expert. to 1-3	3.4	0.69
5. To 65° C.	2.72	2.57
6. Unheated control expert. to 5 .	2.72	0.87

is concerned, this latter observation is also of practical importance to the fermentation process.

Weak antiseptics (chloroform, thymol, toluol, 0.2 per cent. salicylic acid, 0.1 per cent. formaldehyde) do not appear to have any influence on the action of endotryptase. On the other hand, powerful precipitants (3 per cent. phenol, 0.1 per cent. sublimate) naturally stop the digestion, which, however, contrary to the statements of Schär with regard to enzymatic action, is not restricted by 1 per cent. hydrocyanic acid. Even in concentrated solutions up to 5 per cent., according so T. Gromow (I.), of neutral salts, such as sodium, potassium and calcium chloride, stimulate proteolysis, whilst saturated solutions have a powerfully restrictive influence. Additions of 50 per cent. glycerine and saccharose also retard proteolysis considerably, and therefore preserve the alcoholase (*see* p. 477, vol. ii.), as well as the proteids. These discoveries of Geret and Hahn were supplemented by the observation of T. Gromow (I.), to the effect that, not only glycerine and saccharose, but also mannitol, glucose, lactose, and glycocoll restrict the proteolysis of permanent yeast suspended in water. In the case of saccharose, this effect is already apparent on the addition of 5 per cent., whilst the proteolysis almost entirely ceases in presence of 35 per cent. Saccharose has a more powerfully

restrictive action than glycerine or glycocoll in isotonic solutions; consequently, in this case the retarding effect must not be regarded as a purely physical process.

Pressed yeast juice, concentrated to one-third its original volume *in vacuo*, exhibited a considerably lessened auto-digestion, the same effect being produced when permanent yeast was stirred up to a thick pap in water, instead of being suspended therein. Whether, as opined by T. Gromow (I.), the accumulation of metabolic products constituted the retarding factor has not yet been proved.

The influence of alcohol, which is always present, more or less, in yeast cultures, is also a matter of practical importance. Experiments by Geret and Hahn have demonstrated that the proteolysis of expressed yeast juice is slightly retarded by the presence of 5 per cent. alcohol, seriously so by 10–20 per cent. (this was confirmed by T. Gromow), and stopped by 30 per cent. alcohol. Hence, even in the advanced stages of fermentation of wine and beer, the complete suppression of the action of endotryptase by alcohol can hardly be expected to occur. According to Iwanoff (II.), the restriction of proteolysis during fermentation is effected, not by alcohol, the action of which (*see* p. 477, vol. ii.), does not become apparent until the concentration exceeds 4 per cent.; but by other volatile products, aldehydes and esters (fruit ethers). He states that the proteids are not decomposed by fermenting yeast in pure nutrient solutions; but in the ordinary complete nutrient solutions, in which all physiological processes are in full swing, even a small quantity of acid phosphates, as was found by Iwanoff himself, is able to entirely neutralise the restrictive action of the fermentation products on proteolysis, and in fact accelerate the latter.

Weak acids favour the action of endotryptase, the optimum effect being produced by the presence of 0.2 per cent. hydrochloric acid in pressed yeast juice, or by an equimolecular amount of sulphuric acid, whilst the same strength of acetic acid seems to act still more favourably. Boric acid (1 per cent.) or sodium borate (1 per cent.), does not retard proteolysis, whilst borax (*see* p. 244, vol. ii.) and all alkalis, even as weak as 0.1–0.2 per cent., diminish proteolysis considerably by the neutralisation of the pressed yeast juice.

The action of endotryptase is not confined merely to the nucleins of the yeast cell, but extends also to other proteids. Boullanger (I.) and Beijerinck (XXXI.) observed the digestion of casein by yeast; and Beijerinck obtained a similar result with glutin, albumen, and fibrin, whilst confirmation was furnished by Geret and Hahn (I. and II.) in respect of casein, glutin-casein, and albumen. In his experiments with pressed yeast, Schütz (I.) ascertained that endotryptase attacks yeast nuclein and gelatin the most, euglobin and serum albumen being far less

powerfully decomposed, whilst in two cases out of three, pseudo-globulin was left intact.

With regard to the fission products resulting from the auto-digestion of yeast, the older statements must be accepted with a certain degree of care, because it cannot always be safely deduced from them that the putrefactive influence of bacteria was precluded; whilst in some cases the method of experiment adopted was such as might induce the decomposition of the proteids. Thus, in their investigations on the chemical composition of yeast, Nägeli and Loew (II.) found 2 per cent. of peptones, which Loew subdivided into *a*-, *b*- and *c*-peptone (Meissner). This result, however, was obtained with an aqueous extract of yeast obtained by eleven successive prolonged boilings, so that Nägeli himself was obliged to admit that the boiling water might have produced hydrolysis. According to Geret and Hahn (I. and II.), albumoses and peptone are not detected in expressed yeast juice digested at 37° C.; and in fact, even when albumoses and peptone are added to the juice, the biuret reaction soon disappears at a higher temperature. On the other hand, when the digestion is retarded by the temperature of the ice-chest, albumoses appear, these being chiefly deuteroalbumoses, whereas true peptones, in the sense indicated by Kühne, cannot be identified. Moreover, since F. Kutscher (III.) observed the occurrence of the biuret reaction for a period of 8–14 days, during the digestion of yeast with chloroform water—a process in which the enzyme only gradually issues from the cells and comes into action—the possibility of the formation of small quantities of albumoses during the protracted action of endotryptase must be admitted. Attention was also directed, at an early date, to other fission products occurring in the autodigestion of yeast. Thus, Liebig in 1868 referred to the occurrence of leucin during the autofermentation of yeast; the appearance of tyrosin among the fission products was observed by Béchamp in 1872; and both workers contemporaneously noticed a copious exudation of phosphoric acid from the yeast cell. Then followed Schützenberger's discovery of butalanin, alloxuric bases, carnin, sarkin, xanthin, and guanin (as well as tyrosin and leucin) in the aqueous extract from self-fermenting yeast. This worker regarded all these fission products as derivatives of albumen. The experiments of Kossel (III.) and Salkowski (II.) showed that the phosphoric acid and alloxuric bases should be regarded as fission products of the nuclein substances of the yeast cell, whereas the leucin and tyrosin probably originate in the decomposition of other proteids. This result is also confirmed by the autodigestion of expressed yeast juice. Geret and Hahn found that four-fifths to five-sixths of the phosphorus (mostly in the form of organic compounds) in expressed yeast juice is converted by this digestion into phosphoric acid, and that the greater part of the phosphorus can be identified as present in this

form after digestion has been proceeding for an hour at 37° C. The amount of sulphuric acid, on the other hand, increases but slightly. The nitrogenous constituents of the expressed yeast juice undergo decomposition in such a manner that, at the close of the autodigestion process, about 30 per cent. of the nitrogen in the digestion products is in the form of bases, 70 per cent. being allocated to the amino acids, the proportion being the same as that in which these bodies are found in the fresh yeast juice freed from albumen. The xanthin bodies, which are present in far smaller amount (50–60 mgrms. per 100 c.c. of yeast juice), exhibit an interesting behaviour, inasmuch as, under normal conditions, they are still present in a latent form after the autodigestion of the yeast juice, and are only revealed by boiling with acids. This latent condition is probably due to the carbon dioxide appearing in the yeast juice in consequence of the fermentation.

The more intimate characterisation of the several fission products was effected by Kutscher, with the aid of Kossel's methods. In a recently published work, Kutscher and Lohmann (I.) give the following series of fission products, obtained by the autofermentation of beer yeast suspended in toluol water: guanin (abundant), adenin (abundant), xanthin (traces), hypoxanthin (traces), histidin, leucin, arginin, tyrosin, lysin, aspartic acid, glutamic acid (hitherto undetected), and ammonia (little). Shiga (I.) recently ascertained that the amount of xanthin increases continuously during the digestion of expressed yeast juice; but that guanin is decomposed, even when added in a fresh state; whilst adenin and hypoxanthin fluctuate. According to Shiga, the arginin is partially decomposed by an enzyme ("arginase") discovered in yeast juice, into urea and ornithin (α-δ-diaminovalerianic acid), a process that had been previously observed in the case of animal organs by Kossel and Dakin (I.).

Finally, Kutscher succeeded in also detecting cholin as a fission product of yeast lecithin. This disposes of the hypothesis that cholin always originates entirely in the unfermented liquid (mash, wort, molasses, and must). The discovery of cholin is important, inasmuch as it is also capable of furnishing an explanation of the appearance of glycerine, namely, that, as mentioned on p. 493, vol. ii., the same may result from the decomposition of lecithin into fatty acids, cholin, and glycerophosphoric acid. Whether the other organic bases found in fermented liquids or their distillates (*see* p. 510, vol. ii.) owe their formation to the action of endotryptase must be regarded as at least doubtful. To this category belong the organic bases discovered in white wine by Brücke in 1855, the trimethylamine found in wine by E. Ludwig (I.) in 1867, the alkaloidal bases found in fermenting solutions of saccharose by Oser (I.), in beer by Lermer (I.), and in white wines by Guérin (I.), as well as the collidin found by Krämer and A. Pinner (I.), and the bases—which are regarded by Brand and Stoehr (II.) as

probable derivatives of pyridin—observed by Schrötter (I.) in the fusel oil (*see* p. 505) of a molasses distillery. These discoveries cannot be attributed with certainty to the activity of the yeast. Other micro-organisms may have been concerned to some extent, and the products in question may have partly originated through the action of boiling heat on the residual proteids in the fermented liquids. The small amount of ammonia found to result from the pure autodigestion of yeast renders it at least improbable that any considerable production of volatile amine bases occurs, since most of these readily furnish ammonia. These fission products, however, really facilitate the more intimate characterisation of the proteolytic enzyme of yeast. As pointed out by Kutscher and Lohmann, they correspond exactly with those found by these authors in trypsin digestion. Salkowski, as well as Geret and Hahn, had already classed the enzyme as a tryptic enzyme, and regarded the identification of the monamino acids as sufficient for this characterisation. However, in spite of the identity of the fission products, it would not be advisable to assume that yeast endotryptase is the same as pancreatic tryptase, since two essential points of difference exist between them. In the first place, the action of yeast endotryptase is greatly facilitated by an acid reaction, and retarded by an alkaline reaction, in which respect its behaviour is the antithesis of that of pancreas tryptase; and, secondly, the autodigestion of expressed yeast juice yields substances that do not furnish the biuret reaction more than transitorily, if at all, the reaction quickly disappearing even when peptone and albumoses are added. Nevertheless, despite these differences, it must be maintained that we have here to deal with an enzyme belonging to the group of tryptases, more particularly because of the fission products obtained. For whether, as assumed by Lawrow (I.), such an extensive decomposition of the protein molecule can also be effected by peptases (the influence of which is favoured by an acid reaction) must still be considered doubtful, since in Lawrow's experiments with sliced pigs' stomachs it was impossible to preclude the occurrence of autolytic processes inseparable from the actual pepsin action.

The practically and theoretically important question of the conditions under which yeast endotryptase is formed and exerts its action is less easily answered. Will (XXXV.), who examined a large number of pure cultures of various species of yeast in wort-gelatin, and observed liquefaction (commencing in the path of the stab) within 18–20 days at 20° C., ascertained that the more rapidly liquefying species (*Mycoderma* and *Willia*) are in general more exacting as regards the supply of oxygen. When the inoculating material was mixed with the warmed (and thereby liquefied) gelatin, proteolysis was found to commence in 7–55 days, the time being proportional to the requirements of the species in respect of oxygen. On the basis of these and other

observations, which need not be investigated more closely here, Will formed the conclusion that air played a direct or indirect part in proteolysis by yeast, inasmuch as the presence of air either hinders the formation of a proteolytic enzyme, or destroys the same when already existing. According to Will, the liquefaction of gelatin is a function, not of moribund and decomposing cells, but of the normal cell, and is caused by a deficiency of nutriment, not merely a lack of dissolved substances in general, but of nitrogenous substances in particular, and of oxygen. Whereas Will regards dead yeast cells as merely an invariable concomitant of proteolysis, and denies the existence of any connection between proteolysis and the death of the cells, Beijerinck (XXXI.) assumes that the enzyme originates exclusively in cells that have perished in consequence of a scarcity of oxygen. Geret and Hahn, however, were able to show that lack of oxygen is not a decisive factor in the formation of the enzyme, inasmuch as they obtained an actively digestive expressed juice from fresh surface cultures of low-fermentation beer yeast, grown on wort agar-agar, under which conditions there was no lack of oxygen. Even fresh yeast cells, in all stages of growth, furnish leucin and other fission products, not only in the aqueous extract, but also in the fresh expressed juice, the proteid derivatives in the fresh cells being distributed among bases and amino acids in the same proportion as in the completely digested expressed juice. Hence, a proteolytic enzyme, or the zymogen of same, is present in yeast cells under all conditions; and, as opined by Kutscher, this enzyme probably exercises constructive functions, *i.e.*, it lessens the amount of the nitrogenous foodstuffs, prepared by the proteolytic enzymes of malt and diffused in the yeast cells, to such an extent that they can be utilised by the yeast cells for the elaboration of structural materials. Consequently the proteolytic enzyme is present as an intracellar inhabitant of every yeast cell.

The problem of the conditions under which the excretion of the enzyme occurs still remains to be discussed. That deprivation of oxygen does not form the decisive factor in this case also is most easily concluded from the circumstance that when the living cells are washed and lixiviated with distilled water, and are then left for twelve hours at the bottom of the vessel, this yeast, in a state of starvation as regards oxygen, cedes to the water an inverting enzyme, but not a proteolytic enzyme. On the other hand, the excretion of the enzyme and the process of autodigestion begin when the yeast is left without nitrogenous nutriment for some considerable time at a high temperature. In these circumstances, the corporeal substance of the starving yeast is attacked by the enzyme, probably the cell membrane first of all, the enzyme then acting destructively, as stated by Kutscher. It is, however, unnecessary to assume with Beijerinck that all

the enzyme-generating cells have already perished, since it may be easily conceived that the death of a relatively small number of cells and the enzyme to which they have given rise leads to a modification of the nutrient medium or of the cell membrane of the surviving cells, whereby these latter are induced to excrete the enzyme. This may also perhaps explain the circumstance that Will could only find relatively few dead cells in the liquefied gelatin cultures.

These statements harmonise completely with the fact that Kutscher failed to detect the characteristic degradation products of yeast in pure lager beer. During the actual process of fermentation at a low temperature the number of moribund or pathologically modified yeast cells will presumably be very small, owing to the prevalence of the favourable environment, and consequently the amount of endotryptase or fission products passing out of the yeast cells and into the fermenting liquid will be strictly limited. The conditions may, however, be entirely different when the liquid is either left for a long time in contact with the sedimental yeast, *i.e.*, is not drawn off in good time, or else fermentation has been conducted at a higher temperature. In both cases it is not impossible for the yeast to be acted on by the endotryptase (autodigestion), since both factors, prolonged contact of the liquid with the sedimental yeast and high temperature, favour the death of the yeast cells and the action of the endotryptase. Whether the increase in the proteid content of beer that has been fermented at a high temperature (20° C.), as was carried out by Hantke, has any connection with this subject is a matter that appears doubtful, in view of the energetic action of endotryptase on protein, which it quickly reduces to final products. On the other hand, one is bound to agree with K. Windisch (I.) in ascribing to this cause the peculiar flavour observed in low-fermentation beers that have been exposed to an unduly high temperature during primary fermentation (*see* p. 217, vol. ii.), and the flavour of digested expressed yeast juice is also indicative of the same thing.

Another important feature bearing on practice is the fact that alcoholase is destroyed by endotryptase (*see* p. 478, vol. ii.), so that in all cases where even a portion of the cells are dead, the fermentative power of the yeast may suffer, especially when the environment is unfavourable or the yeast is in a condition of famine, such as is particularly the case when the yeast is being watered or washed in the manufacture of pressed yeast. In this operation, in order to free the yeast from particles of grains or to classify it by sedimentation, it is left for a long time in contact with cold water; and, as a matter of fact, the makers often find that the yeast is weakened, a result generally attributed to bacterial activity, to prevent which the yeast is treated with antiseptics. More probably, however, the loss of fermentative power

may be explained by the action of endotryptase, for which the application of antiseptics affords no remedy. It will be a difficult matter to counteract this influence of endotryptase, though, certainly, the formation of endotryptase might be hindered, and the yeast consequently preserved, by improving the machinery so as to shorten the time occupied in washing. Again, it is not always right to assume that the rapid decomposition of the finished cakes of pressed yeast in the warm weather is due to the agency of bacteria, these latter very often only coming into action secondarily, after the way has been prepared for them by the death of the yeast cells and the action of the endotryptase.

§ 336.—Philothion.

J. de Rey-Pailhade (I.), in 1888, was the first to observe the faculty of an alcoholic extract of yeast to convert elementary sulphur into sulphuretted hydrogen. For this reason he named the active principle (enzyme) of the extract "philothion," which name he afterwards (VIII.) changed to "hydrogenase." An extract of this kind is easily prepared by treating yeast at ordinary temperature either with pure methylalcohol or with 86 per cent. ethylalcohol, the resulting yellow liquid being forced through a biscuit-ware filter in order to free it from cells. The extract becomes inactive on being kept at 70° C. for two hours. According to the same author (III.), the reducing power of this yeast enzyme is not restricted to sulphur, oxygen also coming within its sphere of activity, the yeast extract losing its powers when exposed to the air for a few days. The sensitivity of philothion, however, towards oxygen is not great, since it has been found by A. Wroblewski (I.) in an active condition in expressed yeast juice prepared without exclusion of air (*see* pp. 462, 463, vol. ii.). The negative results of G. Cossettini's (I.) attempt to confirm the reports of Rey-Pailhade seem attributable to the usual incapacity of enzymes to pass through the Chamberland filter (see p. 98, vol. i.) in certain circumstances.

Up to the present, philothion has not been isolated and prepared in a pure state; and it is known and characterised solely from its reactions. It belongs to the group of the reductases, another member of which has been mentioned on p. 374, vol. ii., namely jacquemase, and from the whole of which it differs by its characteristic action on free sulphur. Pozzi-Escott (II.) states that it is also able to convert phosphorus and selenium into their hydrogen compounds though it has no such action on tellurium or arsenic. According to Rey-Pailhade (IX.) free nitrous acid is destroyed by philothion very rapidly at 40° C., but more slowly at ordinary temperature. This power is crippled by dilute hydrochloric or sulphuric acid. In addition to being present in the cells of species of *Saccharomyces* and *Torula* (*see* pp. 397, 398, vol. ii.),

this enzyme has been observed by Rey-Pailhade (II. and VI.) in various animal tissues and in germinating seeds. According to Pozzi-Escott (V.), it is retained by the first-named cells during the period of rapid reproduction, and is not diffused into the nutrient medium until fermentation has culminated in the latter.

Abelous and Ribaut (I.) denied the existence of philothion as such, and attempted to explain the above characteristic production of sulphuretted hydrogen by referring to the capacity of many proteids for readily parting with a portion of their sulphur in that state of combination. Pozzi-Escott (III.), however, demonstrated that the yeast extracts containing philothion lose their power of producing this gas in abundance, on being boiled; which was soon afterwards confirmed by Rey-Pailhade (X.); and that very energetic extracts are also able to reduce sulphites. According to Pozzi-Escott (VI.) this enzyme affords the yeast a means of defence against the poisonous action of sulphurous acid, a statement, however, in direct conflict with the just previously mentioned formation of that poison by philothion. Probably this last worker was correct in his opposition to the assumption of Gimel (I.), who regarded oxydase as the protective agent, and stated that yeast which has been habituated to large doses of sulphurous acid (*see* p. 442, vol. ii.) is able to produce larger quantities of oxydase than before habituation.

On the basis of his observation that methylene-blue is a more sensitive and rapid indicator than indigo-carmine, &c., for the detection of reducing enzymes, H. Hahn (IV.) investigated more closely the reducing power of expressed yeast juice. This power disappears within a few days when the yeast has been stored in the ice-chest in presence of toluol; and almost entirely vanishes on the juice being kept at 55°–60° C. The optimum temperature for reduction is 40° C., this agreeing with that (30°–40° C.) given by Pozzi-Escott (VIII.). The reducing power is lowered by diluting the yeast juice with water, whereas meat broth has a favourable influence. The reduction proceeds most rapidly in old yeast juice. The further observation of a certain parallelism between the fermentative and reducing action of the juice recalls the opinion held by J. Grüss on the part played by hydrogenase (philothion) in alcoholic fermentation (*see* p. 488, vol. ii.), namely, that it is the hydrogen temporarily formed during fermentation (and not philothion) that acts on free sulphur.

According to Pozzi-Escott (VIII.) the reduction is hindered most powerfully by salts with an acid reaction—mercury chloride and silver nitrate in particular, the nitrates being rather less injurious. Chloroform and acids retard, whereas alkalis stimulate the action.

More exhaustive investigations on the reducing power of yeasts and the real cause thereof will not only increase the sum of our knowledge on the theory of enzymes, but may be of practical

utility to the fermentation industry. The appearance of sulphurous acid and sulphuretted hydrogen in fermenting musts, worts, and washes, and their occurrence in wine, beer, and spirits, were already known to fermentation technologists and analytical chemists at a time before any one had begun to speak of reductases. On this point the reader is referred to p. 234, vol. ii., and to the recent publications of FREW (I.), A. OSTWALDER (I. and II.), R. SCHANDER (IV.), W. SEIFERT (V. and VI.), H. WILL and H. WANDERSCHECK (I.), W. WINDISCH (VII.) and J. WORTMANN (XXI.)

BIBLIOGRAPHY.

As will be readily seen, the following bibliography not only gives the original publications, but also references to the more widely distributed and easily accessible journals, as many readers may wish to go deeper into one or more branches of the subject than they could do within the limits of *Technical Mycology*. These references, on account of their frequently critical importance, will often be useful even to those who have the original papers at their command. The authors of *Technical Mycology* cannot claim to belong to this category. The titles of the most important journals are abbreviated in order to economise space, and the following list will explain them:

CONTRACTIONS.

A. f. Hygiene	=	Archiv für Hygiene.
Abt.	=	Abteilung = Section.
Ann. Inst. Past.	=	Annales de l'Institut Pasteur.
Ber. d. D. Bot. Ges.	=	Berichte der Deutschen Botan. Gesellschaft.
Ber. d. D. Chem. Ges.	=	Berichte d. Deutschen Chemischen Gesellschaft.
Bot. Ztg.	=	Botanische Zeitung.
Ch. C.	=	Chemisches Centralblatt.
C. f. B.	=	Centralblatt für Bakteriologie.
C. R.	=	Comptes rendus de l'Académie des Sciences, Paris.
K. J	=	Koch's Jahresbericht über die Fortschritte in der Lehre von Gärungsorganismen.
Pflüger's Archiv	=	Archiv für die gesamte Physiologie (Pflüger).
Ref.	=	Referred to in.
Vers.-Stat.	=	Die landwirthschaftl. Versuchs-Stationen (Nobbe).
W. f. Br.	=	Wochenschrift für Brauerei.
Z. f. Hygiene	=	Zeitschrift für Hygiene und Infektionskrankheiten.
Z. f. physiolog. Chemie	=	Zeitschrift für physiolog. Chemie.
Z. f. Spiritusind.	=	Zeitschrift für Spiritusindustrie.
Z. g. Br.	=	Zeitschrift für das gesamte Brauwesen.

Abba, Francesco,

I. Riconoscimento dell' arsenico in una farina per mezzo del penicillium brevicaule. — Rivista d'Igiene e Sanità pubblica. 1893. p. 831.—Hygien. Rundschau. 1894. IV. 325.—Ch. C. 1894. II. 112.

II. Über die Feinheit der biologischen Methode beim Nachweis des Arseniks.—C. f. B. 2. Abt. 1898. IV. 806.—Ch. C. 1898. II. 1281.

Abel, Rudolf,

I. Über die Brauchbarkeit der Schild'schen Formalinprobe zur Differentialdiagnose des Typhusbacillus.—C. f. B. 1894. XVI. 1041. — Ch. C. 1895. I. 277.

Abel, Rudolf, und Buttenberg, Paul,
I. Uber die Einwirkung von Schimmelpilzen auf Arsen und seine Verbindungen.—Z. f. Hygiene. 1899. XXXII. 449. —C. f. B. 2. Abt. 1900. VI. 187.—Ch. C. 1900. I. 428.

Abel, Rudolf, und Dräer, Arthur,
I. Das Hühnerei als Kulturmedium für Choleravibrionen.—Z. f. Hygiene. 1895. XIX. 61.—Ch. C. 1895. I. 697.

Abeles, Hans,
1. Zur Frage der alkoholischen Gärung ohne Hefezellen.—Ber. d. D. Chem. Ges. 1898. XXXI. 2261.—C. f. B. 2. Abt. 1899. V. 40.—Ch. C. 1898. II. 894. K. J. IX. 315.

Abelous, A., et H. Ribaut,
I. C. R. 1903. CXXXVII. 95, 3268.

Aberson, J. H.,
See Giltay und Aberson.

Adametz, Leopold,
I. Die Bakterien der Trink- und Nutzwässer.—Mitteilungen der österr. Versuchsstation für Brauerei u. Mälzerei in Wien. I. Heft. p. 19.
II. Die Bakterien normaler und abnormaler Milch. — Österr. Monatsschrift f. Tierheilkunde und Tierzucht. 1890. V. 1.— C. f. B. 1890. VIII. 109.
III. Über die Ursachen und Erreger der abnormalen Reifungsvorgänge beim Käse.—Milchzeitung. 1891. XX. 237; 1892. XXI. 205.—K. J. II. 196; III. 184.
IV. Über einen Erreger der schleimigen Milch, Bacillus lactis viscosus.—Milchzeitung. 1889. XVIII. 941.—C. f. B. 1890. VII. 767.
V. Untersuchungen über Bacillus lactis viscosus, einen weit verbreiteten milchwirtschaftlichen Schädling.—Landw. Jahrbücher. 1891. XX. 185.—C. f. B. 1891. IX. 698.—K. J. II. 182.
VI. Bakteriologische Untersuchungen über den Reifungsprozess der Käse.—Landw. Jahrbücher. 1889. XVIII. 227.—C. f. B. 1889. VI. 78.
VII. Über Micrococcus Sornthalii. —C. f. B. 2. Abt. 1895. I. 465. —K. J. VI. 248.
VIII. Kritische Bemerkungen über F. Baumann's Beiträge zur Erforschung der Käsereifung.— Deutsche Molkerei-Zeitung. 1893. No. 16.—K. J. IV. 213.
IX. Untersuchungen über die niederen Pilze der Ackerkrume. Dissert. Leipzig 1886.—C. f. B. 1887, I. 8.
X. Saccharomyces lactis, eine neue Milchzucker vergärende Hefeart. —C. f. B. 1. Abt. 1889. V. 116.
XI. C. f. B. 1889. V. 110.
XII. Über die Ursachen und Erreger abnormalen Reifungsvorgänge beim Käse. Bremen 1893.
XIII. Untersuchungen ü.d. niederen Pilze d. Ackerkrume. Dissert. Leipzig 1886, p. 39.

Adametz, L., und Wilckens, Martin,
I. Milchwirtschaftliche Untersuchungen, etc.—Landwirtsch. Jahrbücher. 1892. XXI. 131.— C. f. B. 1892. XII. 98.—K. J. III. 178.

Aderhold, Rudolf,
I. Die Morphologie der deutschen Saccharomyces ellipsoideus-Arten. — Landw. Jahrbücher. 1894. XXIII. 587.—C. f. B. 2. Abt. 1895. I. 410.—K. J. V. 159.
II. Über den Einfluss der Kohlensäure auf die normale Gärung störende Organismen, mit Bemerkungen über die Konservierung des Weines.—Mitteilungen über Weinbau und Kellerwirtschaft. 1892. IV. 132.
III. Studien über eine gegenwärtig in Mombach bei Mainz herrschende Krankheit der Aprikosenbäume und über die Erscheinungen der Blattranddürre. —Landw. Jahrb. 1893. XXII. 435.—Bot. Ztg. 1893. II. 267.
IV. Landw. Jahrbücher. 1899. XXVIII. 69.
V. C. f. B. 2. Abt. 1899. V. 511.

Aeby, J. H.,
I. Beitrag zur Frage der Stickstoffernährung der Pflanzen.— Vers.-Stat. 1896. XLVI. 409. —Ch. C. 1896. 818.—K. J. VII. 206.

Ahlburg,
I. *See* Korschelt, II.

Ahrens, Felix B.,
I. Ein Beitrag zur zellenfreien Gärung.—Zeitschr. f. angewandte Chemie. 1900. XIV. 483.—C. f. B. 2. Abt. 1900. VI. 744.—Ch. C. 1900. II. 52.—K. J. XI. 368.

Albert, Friedrich,
I. Die Umfrage über die Braunheuherstellung. — Mitteil. d. deutsch. Landwirtsch-Gesellsch. pro 1893-94. II. 14.
II. Untersuchungen über Grünpressfutter.—Jahrb. d. deutsch. Landwirtsch.-Gesellsch. Bd. VI. Tl. I. S. 149. Berlin 1891.—(Gives the prineipal literature thereon.)

Albert, Robert,
I. W. f. Brauerei. 1899. XVI. 485.

Albert, Rudolf,
I. Einfacher Versuch zur Veranschaulichung der Zymase-Wirkung.—Ber. d. D. Chem. Ges. 1900. XXXIII. 3775.—C. f. B. 2. Abt. 1901. VII. 473.—Ch. C. 1900. I. 469.—K. J. XI. 364.
II. Über künstliche Anreicherung der Hefe an Zymase. — Ber. d. D. Chem. Ges. 1899. XXXII. 2372.—C. f. B. 2. Abt. 1900. VI. 89.—Ch. C. 1899. II. 915. —K. J. X. 312.
III. Erfahrungen bei der Herstellung von Hefepresssaft aus untergäriger Bierhefe der Versuchs- und Lehrbrauerei zu Berlin.—W. f. Br. 1899. XVI. 485. K. J. X. 310.

See also Albert u. Buchner.

Albert, R., und Buchner, Ed.,
I. Hefepresssaft und Fällungsmittel.—Ber. d. D. Chem. Ges. 1900. XXXIII. 266, 971. W. f. Br. 1900. XVII. 49, 189.—C. f. B. 2. Abt. 1900. VI. 373, 536.—Ch. C. 1900. I. 677, 1034.—K. J. XI. 365, 367.
II. Ber. d. D. Chem. Ges. 1899. XXXII. 2372.
III. *Ibid.* 1900. XXXIII. 266; 971.

Albert, R., Buchner, E. u. Rapp, R.,
I. Ber. d. D. Chem. Ges. 1902. XXXV. 2376.

Ali-Cohen, Ch. H.,
I. Eigenbewegung bei Mikrokokken.—C. f. B. 1889. VI. 33.
II. Die Chemotaxis als Hilfsmittel der bakteriologischen Forschung. —C. f. B. 1890. VIII. 161.—K. J. I. 8.

Allik, A. K.,
I. Die chemische Analyse des Kumys. Dissert. Dorpat. 1895. —K. J. VI. 223.

Altehoefer,
I. Über die Desinfectionskraft von Wasserstoffsuperoxyd auf Wasser —C. f. B. 1890. VIII. 129.—(Also gives the literature prior to date.)

Altenburg,
I. Quoted by Kobert, III.

Altmann, Richard,
I. Über Nucleinsäuren.—Archiv d. Anatomie u. Physiologie. 1889. Physiologie. Abt. S. 524.—Ch. C. 1890. I. 126.

Alvarez,
I. Sur un nouveau microbe, déterminant la fermentation indigotique et la production de l'indigo bleu.—C. R. 1887. CV. 286.—C. f. B. 1887. II. 441.

Amand, A.,
I. La Cellule. 1902. XX. 225.
II. *Ibid.* 1904. XXI. 329.

Amann, P.,
I. Pleochroismus gefärbter Bakterienzellen.—C. f. B. 1893. XIII. 775.—K. J. IV. 54.

Amthor, Karl,
I. Über den Saccharomyces apiculatus.—Z. f. physiolog. Chemie. 1888. XII. 558; reprinted from Z. g. Br. 1888. XI. 370.—Ch. C. 1888. S. 1120.
II. Beobachtungen über den Saccharomyces apiculatus.—Chem.-Zeitg. 1891. S. 670.—Ch. C. 1891. I. 1077.—K. J. II. 141.
III. Uber Hefeweine und den Ammoniak-Gehalt in Most und Wein.—Zeitschr. f. angewandte Chemie. 1890. p. 27.—Ch. C. 1890. I. 237.—K. J. I. 66.
IV. Z. f. physiolog. Chemie. 1888. XII. 64.
V. Z. f. angew. Chemie. 1892. 319.

Anclés, Louis Edgar,
I. Das Konservieren der Nahrungs- und Genussmittel. Wien. 1895. Hartleben.

André, G.,
See Berthelot et André.

Andreasch, F.,
I. Gärungserscheinungen in Gerbbrühen.—Der Gerber. 1895. S. 193; 1896. S. 3.—Zeitschrift f. angewandte Chemie. 1896. S. 216.

Andrlik, K.,
I. Das Verhalten der Raffinose bei der Vergärung von Melasse.—Zeitschrift f. Zucker-Industrie in Böhmen. 1898. XXIII. I.—Ch. C. 1898. II. 1273.

Ansai,
I. Mitt. d. mediz. Gesellsch. zu Tokio. 1904. XVI. No. 6, p. 1.

Appert, M.,
I. Le livre de tous les ménages ou l'art de conserver, pendant plusieurs années, toutes les substances animales et végétales. Quatrième édition. Paris. 1831.

Apostoli et Laquerrière,
I. De l'action polaire positive du courant galvanique constant sur les microbes et en particulier sur la bactéridie charbonneuse.—C. R. 1890. CX. 918.—K. J. I. 44.

Araki, T.,
I. Über das Chitosan.—Z. f. physiolog. Chemie. 1895. XX. 498. (It gives the antecedent literature relating to the formulæ for Chitin.)—Ch. C. 1895. I. 1179.

Arata,
I. Über die Veränderungen, denen die flüchtigen Säuren der Butter beim Ranzigwerden derselben unterworfen sind, und über die Wirkung der ranzigen Butter auf den Organismus.—Annalen des Inst. f. Experimentalhygiene in Rom. 1893.—K. J. IV. 181.

Arcangeli, G.,
I. Sulla fermentazione panaria.—Atti della Società toscana di scienze naturali residente in Pisa. 1888. IX. 1.—C. f. B. 1888. III. 717.—Ch. C. 1888. 974.

Archangelski,
I. Über Milzbrand.—Centralblatt f. d. med. Wissenschaften. 1883. 257.

Archleb, Josef,
I. Uber den Einflüss der Konzentration der Nährflüssigkeiten auf die Vermehrung der Alkoholfermente und den Vergärungsgrad. 1887. Neustadt a. d. Mettau.—Z. f. Spiritusind. 1888. XI. 243.

Arloing, S.,
I. De l'influence des filtres minéraux sur les liquides contenant des substances d'origine microbienne.—C. R. 1892. CXIV. 1455.—C. f. B. 1892. XII. 882.—K. J. III. 21.

Arnheim, J., u. Rosenbaum, J.,
I. Z. f. physiolog. Chemie. 1903. XI. 220.

Aronson, Hans,
I. Uber die antiseptischen Eigenschaften des Formaldehydes.—Berliner klinische Wochenschrift. 1892. XXIX. No. 30.—Ch. C. 1892. II. 579.

Arsonval, A. d',
I. La pression osmotique et son rôle de défense contre le froid dans la cellule vivante.—C. R. 1901. CXXXIII. 84.—Ch. C. 1901. II. 433.

Arsonval, A. d', et Charrin, A.,
I. Electricité et microbes.—Comptes rendus de la société de biologie. Paris. 1893, p. 467.—K. J. IV. 114.
II. Concurrence vitale entre le bacille pyocyanique et la levure de bière.—*Ibid.*, p. 70 *et seq.*—K. J. IV. 116 u. f.

Artari, A.,
I. Abhandlungen d. Nat. Ges. zu Halle. 1897. XXI. 113.

Artari, Alexander,
I. Über einen im Safte der Zuckerfabriken in Gemeinschaft mit Leuconostoc schädlich auftretenden, den Zucker zu Alkohol und Säuren vergärenden Saccharomyces (S. Zopfii).—Abhandlungen d. Naturforschenden Ges. zu Halle. 1897. XXI. 113.—C. f. B. 2. Abt. 1897. III. 529.—K. J. VIII. 101.

Arthus, M., et Pagès, C.,
I. Recherches sur l'action du lab et la coagulation du lait dans l'estomac et ailleurs.—Arch. de physiologie. V^ème^ série. 1890. II. 331.—K. J. I. 173.

II. Sur le labferment de la digestion du lait. Arch. de physiologie. Vème série. 1890. II. 540.—K. J. I. 174.

Arustamoff, M.,
I. Uber die Natur des Fischgiftes. —C. f. B. 1891. X. 113.

Asbóth, Alexander von,
I. Uber das Vorkommen von Pyridin in manchen Amylalkoholen. —Chemiker-Zeitg. 1889. XIII. 871.—Ch. C. 1889. II. 423.

Ascoli, Alberto,
I. Uber die Plasminsäure. —Z. fur physiolog. Chemie. 1899. XXVIII. 426.—Ch. C. 1900. I. 43.
II. Uber den Phosphor der Nucleinstoffe.—*Ibid.* 1900. XXXI. 156.—Ch. C. 1901. I. 127.
III. Uber ein neues Spaltungsprodukt des Hefenucleins.—*Ibid.* 1900. XXXI. 161.—Ch. C. 1901. I. 127.

Aso,
I. Quoted by Kozai, II.

Astier, Charles Benoit,
I. Rapport des expériences faites sur le sirop et le sucre de raisin. —Annales de Chimie. 1813. LXXXVII. 27.
II. Sur la transmutation du sirop de raisin en vin.—Journal des propriétaires ruraux pour le midi de la France. XVII.

Atkinson, R. W.,
I. Sur la diastase du Koji.—Moniteur scientifique de Quesneville. 1882, p. 7.

Aubry, Louis,
I. Das Antinonnin im Dienste der Bierbrauerei.—Z. g. Br. 1893. XVI. 141.
II. Ein Beitrag zur Klärung und Richtigstellung der Ansichten über reine Hefe.—*Ibid.* 1885. VIII. 133.
III. Z. g. Br. 1894. XVII. 1.
IV. *Ibid.* 1897. XX. 631.

Axenfeld, D.,
I. Centralbl. f. Physiologie. 1903. XVII. 268.

Babes, Victor,
I. Über isoliert färbbare Anteile von Bakterien.—Z. f. Hygiene. 1888. IV. 173.

Babo, A. Freiherr von, und Mach, Edmund,
I. Handbuch des Weinbaues und der Kellerwirtschaft. 3rd Ed. I. Band. Weinbau. 22*s.*; II. Band: Kellerwirtschaft. 24*s.* Berlin, P. Parey. 1896.

Bach, Karl,
I. Die Verarbeitung und Konservierung des Obstes und der Gemüse. Stuttgart, Ulmer. Bound 3*s.*

Bachmann, Hans,
See Bachmann, Johann.

Bachmann, Johann,
I. Einfluss der äusseren Bedingungen auf die Sporenbildung von Thamnidium elegans Link.—Bot. Ztg. 1895. 1. Abt. LIII. 107.
II. Mortierella van Tieghemi nov. spec.—Jahrb. für wissenschaftl. Botanik. 1899. XXXIV. 279.

Baeyer, A.,
I. Ber. d. D. Chem. Ges. 1870. III. 63.

Baginsky, Adolf,
I. Rote Milch.—Deutsche Medizinal-Zeitung. 1889. No. 9.—C. f. B. 1. Abt. 1889. V. 448.
II. Zur Biologie der normalen Milchkotbakterien.—Z. f. physiolog. Chemie. 1888. XII. 434; 1889. XIII. 352.—C. f. B. 1. Abt. 1888. IV. 201; 1889. VI. 16.—Ch. C. 1888. S. 975; 1889. 1. 522.
III. Zum Grotenfelt'schen Bacillus der roten Milch. — Deutsche. mediz. Wochenschrift. 1889. XV. 212.—C. f. B. 1. Abt. 1889. VI. 137.—Ch. C. 1889. I. 691.
IV. Uber das Vorkommen von Xanthin, Guanin und Hypozanthin.—Z. f. physiolog. Chemie. 1884. VIII. 395.

Bail, O.,
I. C. f. B. 2. Abt. 1902. VIII. 567.

Bail, Theodor,
I. Über Hefe. — Reprinted from Kunst- und Gewerbeblatt d. polytechn. Vereins f. Bayern. 1857. p. 11.
II. Über die Entstehung der Hefe. —Journal f. prakt. Chemie. 1867. CI. 47.
III. Flora. 1857. XL. 417.

Bainier,
I. Bull. Soc. Mycol. de France 1905. XXI. 126.

Balcke, Julius,
I. Bakterientrübung im Bier.—W. f. Br. 1884. I. 181.
Balard,
I. Ann. Chem. et de phys. 1844. (3.) XII. 294.
Balland,
I. Observations sur les farines.—C. R. 1894. CXIX. 565.—Ch. C. 1894. II. 895.
Balland et Masson,
I. Sur la stérilisation du pain et du biscuit sortant du four.—C. R. 1893. CXVII. 797.—Ch. C. 1894. I. 178.
Ballard, Antoine Jérome,
I. Sur une altération spontanée de certains vins.—C. R. 1861. LIII. 1226.
Balling, C. J. N.,
I. Die Gärungschemie. 3rd Ed. 1865. II. 239.
Balogh, N. von,
See Funk und Balogh.
Bamberger, E., und A. Einhorn,
I. Ber. d. D. Chem. Ges. 1897. XXX. 224.
Bang, Ivar,
I. Die Guanylsäure der Pankreasdrüse und deren Spaltungsprodukte. — Z. für physiolog. Chemie. 1898. XXVI. 133.—Ch. C. 1898. II. 1210.
Banning,
1. C. f. B. 2. Abt. 1902. VIII. 395.
Barba, G.,
See Kayser et Barba.
Barbet,
I. Bull. de l'assoc. des Chimistes. 1900. XIX. 1107.
Barfoed, C.,
I. J. f. prakt. Chem. 1873. VI. 334.
Barker,
I. Ann. of Botany. 1903. XVII. 187.
Barker, B. T. P.,
I. Proc. Roy. Soc. 1901. LXVIII. Phil. Trans. 1901. CXCIV. 467.
Barker, P.,
I. Ann. of Botany. 1900. XIV. 215.
Barth, G.,
I. Z. g. Br. 1900. XXIII. L. 909.
II. Ber. d. D. Chem. Ges. 1878. XI. 474.
Bartel, A.,
See Schroeder und Bartel.
Barth, Max,
I. Die Obstweinbereitung mit besonderer Berücksichtigung der Beerenobstweine.—4. Ed. Stuttgart. 1897. E. Ulmer. 1*s.* 4*d.*
Bary, Anton de,
I. Vergleichende Morphologie und Biologie der Pilze, Myeetozoen und Bakterien.—Leipzig. 1884. Engelmann.
II. Über einige Sclerotinien und Sclerotienkrankheiten.—Bot. Ztg 1886. XXXIV. 377.
III. Morphologie und Physiologie der Pilze, Flechten und Myxomyeeten. 1866.
IV. Uber die Fruchtentwicklung der Aseomyeeten. 1863. Leipzig.
V. Vorlesungen über Bakterien. 2. Ed. 1887. Leipzig.
VI. Beiträge zur Morphologie und Physiologie der Pilze. 1. Series. Frankfurt a. M. 1864.
VII. *Ibid.*, II. Series. 1866.
VIII. *Ibid.*, 3rd Series. 1870 2. Abt. 18.
IX. Bot. Ztg. 1854. 425.
Basenau, Fritz,
I. Über die Ausseheidungen von Bakterien dureh die thätige Milchdrüse und über die sogen. bakterieiden Eigensehaften der Milch.—A. f. Hygiene. 1895. XXIII. 44.—Ch. C. 1895. I. 1070.
Basile, G.,
I. Mannitgärung der Weine in Sieilien.—Staz. sperim. agrar. ital. 1894. XXVI. 451.—Ch. C. 1894. II. 498.—K. J. V. 194.
Bau, Arminius,
I. Über die seheinbare Zunahme des Dextringehaltes in Bierwürzen während der Gärung, sowie über die Bestimmung der Dextrose und des Dextrins in denselben.—W. f. Br. 1890. VII. 1069.—C. f. B. 1891. IX. 99. —Ch. C. 1890. II. 863.—K. J. I. 69.
II. Über die Zusammensetzung der Bierwürzen in Bezug auf Kohlehydrate.—W. f. Br. 1891. VIII. 1.—Ch. C. 1891. I. 513.—K. J. II. 139.

III. Die Bestimmung von Maltose, Dextrose und Dextrin in Bierwürze und in Bier mittelst Reinkulturen von Gärungsorganismen.—C. f. B. 1891. IX. 825.—Ch. C. 1891. II. 92.—K. J. II. 141.
IV. Zur quantitativen Bestimmung der Isomaltose.—W. f. Br. 1892. IX. 1421.—Ch. C. 1893. I. 233 u. 960.—K. J. III. 127.
V. Über die Verwendung der Hefe zur quantitativen Bestimmung gärfähiger Substanzen.—Chemikerzeitg. 1893. XVII. 392.—Ch. C. 1893. I. 802.—K. J. IV. 135.
VI. Der Sammelbegriff Saccharomyces cerevisiae.—W. f. Br. 1894. XI. 1366.—C. f. B. 2 Abt. 1895. I. 88.—Ch. C. 1894. II. 1067.
VII. Über den Nachweis von Unterhefe in obergäriger Presshefe. —Z. f. Spiritusind. 1894. XVII. 374.—Ch. C. 1895. I. 91.—K. J. V. 159.
VIII. Prüfung der Presshefe auf eine Beimengung von Unterhefe. —Z. f. Spiritusind. 1895. XVIII. 372.—C. f. B. 2. Abt. 1896. II. 98.—K. J. VI. 166.
IX. Über Schwergärigkeit der Melassen.—Z. f. Spiritusind. 1894. XVII.—K. J. V. 158.
X. Über Raffinose oder Melitriose und über ihre Abwesenheit im Bier.—W. f. Br. 1894. XI. 1439.—K. J. V. 141.
XI. Über das Verhalten der Oberhefe gegenüber der Isomaltose und der Raffinose.—W. f. Br. 1894. XI. 113.—K. J. V. 140.
XII. Über ein neues Enzym der Hefe.—Chemikerzeitg. 1895. XIX. 1873.—C. f. B. 2. Abt. 1895. I. 887.—Ch. C. 1895. II. 1049.—K. J. VI. 319.
XIII. Über Melitriose und deren quantitative Bestimmung.—Chemikerzeitg. 1894. XVIII. 1794. (Also gives the history of this sugar.) Ch. C. 1895. I. 27.
XIV. Über Melibiose.—Chemikerzeitg. 1897. XXI. 186.—Ch. C. 1897. I. 744.
XV. Beiträge zur Vergärbarkeit und zur analytisehen Verwertung der Melitriose.—W. f. Br. 1898. XV. 389.—Ch. C. 1898. II. 682.
XVI. Über krystallisierte Melibiose. —W. f. Br. 1899. XVI. 397.—Ch. C. 1899. II. 526 u. 644.
XVII. Über die Vergärbarkeit der Galactose. Z. f. Spiritusind. 1896. XIX. 303.—C. f. B. 2. Abt. 1896. II. 653.—K. J. VII. 97.
XVIII. Über Gärversuche mit Trehalose.—W. f. Br. 1899. XVI. 305.—C. f. B. 2. Abt. 1899. II. 871.—Ch. C. 1899. II. 130.
XIX. Über Obergärung und Reinzucht.—W. f. Br. 1892. IX. 1057.—K. J. III. 158.
XX. W. f. Brauerei. 1891. VIII. 592.
XXI. *Ibid.* 1892. IX. 1185.
XXII. Z. f. Spiritusind. 1904. XXVII. 317.
XXIII. Z. f. Spiritusind. 1896. XIX. 303.
XXIV. Chem. Zeitg. 1892. XVI. 143. W. f. Brauerei. 1892. XIX. 193.
XXV. W. f. Brauerei. 1902. XIX. 44.
XXVI. W. f. Brauerei. 1903. XX. 560.
XXVII. Beitr. d. Vereins a. d. Rüben - Zuckerindustrie. 1904. LIV. 481.
XXVIII. Chem. Beitr. 1897. XXI. 185.
XXIX. Z. f. Spiritusind. 1898. XXI. 241.
XXX. W. f. Brauerei. 1900. XVII. 698.
XXXI. Chem. Ztg. 1892. XVI. 1473. 1893. XVII. 392.
XXXII. W. f. Brauerei. 1895. XII. 431.

Bauer,
I. Abgeänderter Sterilisator.—Z. g. Br. 1895. XVIII. 85.—K. J. VI. 11.
II. J. f. prakt. Chem. 1884. XXX. 283.

Bauer, Emil,
I. Über die Vergärbarkeit der Melasse.—Z f. Spiritusind. 1894. XVII. No. 14.—K. J. V. 157.
II. Ungar. Patent No. 12,075, Aug. 12, 1898.
III. Untersuchungen über Gärung, Ernährung und Vermehrung von Hefe.—Z. f. Spiritusind. 1901. XXIV. 309.—C. f. B. 2. Abt. 1902. VIII. 650.

Bauer, H.,
See C. Pohl und H. Bauer.
Bauke, H.,
I. Beiträge zur Kenntnis der Pyknіden. I.—Nova acta academiae caesareae Leopoldino-Carolinae germanicae naturae euriosorum. 1876. XXXVIII.
II. Zur Entwicklungsgeschichte der Ascomyceten.—Bot. Ztg. 1877. XXXV. 313.
Baumann, E.,
I. Über die Bildung von Phenol bei der Fäulnis.—Ber. d. D. Chem. Ges. 1877. X. 685.—Ch. C. 1877. 391.
II. Bildung der Hydroparacumarsäure aus Tyrosin.—Ber. d. D. Chem. Ges. 1879. XII. 1450. —Ch. C. 1879. 599.
See also Baumann und Brieger.
Baumann, E., und Brieger, L.,
I. Über die Entstehung von Kresolen bei der Fäulnis.—Z. f. physiolog. Chemie. 1879. III. 149.—Ch. C. 1879. 329.
Baumann, Fritz,
I. Beiträge zur Erforschung der Käsereifung.—Landw. Versuchsstationen. 1893. XXXXII. 181. —C. f. B. 1893. XIV. 494.—K. J. IV. 209.
Baumgarten, P.,
I. Lehrbuch der pathologischen Mykologie. 2 Bände. Braunschweig. H. Bruhn. 1890.
Bay, J. Chr.,
I. Tubercular Infectiousness of Milk.—Annual Report of the Iowa State Dairy Commissioner. Des Moines. 1896.—K. J. VII. 166.
II. The American Naturalist. 1893. XXVII. 693.
III. C. f. B. 2. Abt. 1896. II. 259.
IV. Ber. d. D. Bot. Ges. 1894. XII. 90.
Béchamp, A.,
I. Les Mikrozymas dans leurs rapports avec l'hétérogenie, l'histogénie, la physiologie et la pathologie. 992 pp.—Paris 1883.
II. Sur la viscose ou substance gommeuse de la fermentation visqueuse : équation de cette fermentation. — C. R. 1881. LXXXXIII. 78.—Z. g. Br. 1881. IV. 482.
III. De la réduction des nitrates et des sulfates dans certaines fermentations. — C. R. 1868. LXVI. 547.—Ch. C. 1868. S. 942.
IV. Gärung der Apfelsäure.—Bulletin de la Société chimique de Paris. 1894. XI. 466.—Ch. C. 1894. II. 100.
V. Gärung der Bernsteinsäure und der Brenzweinsäure.—Bulletin de la Société chimique de Paris. 1894. XI. 418.—Ch. C. 1894. II. 47.
VI. Nouvelle méthode d'incinération des matières végétales et animales ; application au dosage des éléments minéraux de la levure. C. R. 1871. LXXIII. 337.
VII. Sur l'épuisement physiologique et la vitalité de la levure de bière.—C. R. 1865. LXI. 689.
VIII. Sur la cause de la fermentation alcoolique par la levure de bière et sur la formation de la leucine et de la tyrosine dans cette fermentation.—C. R. 1872. LXXIV. 184.
IX. Recherches sur la nature et l'origine des ferments.—Annales de chimie et de physique. 4e sér. 1871. XXIII. 442.
X. Nouvelles recherches sur l'épuisement physiologique de la levure de bière et remarques à l'occasion d'une récente Communication de M. Schützenberger sur le même sujet.—C. R. 1874. LXXVIII. 645.
XI. Faits pour servir à l'histoire de la levure de bière et de la fermentation alcoolique.—C. R. 1879. LXXXVIII. 866.—Z. g. Br. 1879. II. 356.
XII. C. R. 1864. LIX. 496.
XIII. C. R. 1872. LXV. 1036.
XIV. C. R. 1863. LVI. 969, 1184.
Bechhold, Jakob,
I. Untersuchungen an dem Klärbeckenschlamm zu Frankfurt a. M.—Z. f. angew. Chemie. 1899. S. 849. — Ch. C. 1899. II. 726.
Beck, M.,
See Proskauer und Beck.

Becker, C.,
I. Über Schichtung und Färbbarkeit der Membran der Hefezellen.—Z. g. Br. 1899. XXII. 597.—C. f. B. 2. Abt. 1900. VI. 24.

Becker, H.,
I. Neuerungen auf dem Gebiete des Gärungswesens.—Z. g. Br. 1897. XX. 437.
See also Popp und Becker.

Behr, P.,
I. Über eine nicht mehr farbstoffbildende Rasse des Bacillus der blauen Milch.—C. f. B. 1890. VIII. 485.—K. J. I. 40.

Behrend, Paul,
I. Beiträge zur Chemie des Obstweines und des Obstes., p. 57.—Hohenheim 1892.—Ch. C. 1893. I. 327.

Behrend, P., u. Morgan, A.,
I. Über die Veränderungen, welche die stickstoffhaltigen Verbindungen der süssen Maische durch die Gärung erfahren. — Vers.-Stat. 1880. XXIV. 171.

Behrens, Johann,
I. Der Ursprung des Trimethylamins im Hopfen und die Selbsterhitzung desselben.—Karlsruhe. 1894. O. Nemnich.—K. J. V. 76.
II. Weitere Beiträge zur Kenntnis der Tabakpflanze.—VII. Die Fermentation. — Vers.-Stat. 1894. XXXXIII. 293.—Ch. C. 1894. I. 429.—K. J. IV. 253.
III. IX. Über Mikroorganismen des Tabakes nach der Ernte.—Vers.-Stat. 1895. XXXXVI. 163.—C. f. B. 2. Abt. 1896. II. 34.
IV. Die Infektionskrankheiten des Weines.—C. f. B. 2. Abt. 1896. II. 213.
V. Trockene und nasse Fäule des Tabaks. Der Dachbrand.—Z. f. Pflanzenkrankheiten. 1893. III. 82.—C. f. B. 1894. XVI. 315.
VI. Die Beziehungen der Mikroorganismen zum Tabakbau und zur Tabakfabrikation.—C. f. B. 2. Abt. 1896. II. 514.
VII. Phytopathologische Notizen. —Z. für Pflanzenkrankheiten. 1895. V. 136.
VIII. Studien über die Konservierung und Zusammensetzung des Hopfens.—W. f. Br. 1896. XIII. 802 u. 946.—Ch. C. 1896. II. 764.—K. J. VII. 61.
IX. Beiträge zur Kenntnis der Obstfäulnis.—C. f. B. 2. Abt. 1898. IV. 514. Also gives prior literature. — Ch. C. 1898. II. 1027.—K. J. IX. 296.
X. Der Bakteriengehalt des ausländischen Getreides. —Wochenblatt d. landw. Vereins im Grossherz. Baden. 1897. No. 24. S. 381.
XI. Der Einfluss der Düngung auf das Faulen des Tabaks.—Vers.-Stat. 1899. L. 223.
XII. Untersuchungen über den Wurzelschimmel der Reben.—C. f. B. 2. Abt. 1897. III. 584.
XIII. Weinlaube. 1903. 412.
XIV. C. f. B. 1892. XI. 110.
XV. W. f. Br. 1895. XII. 802.
XVI. Jahresber. pro 1903 der Landw. Versuchsanstalt Augustenburg. 1904. 43.
XVII. Bot. Ztg. 1901. 2. Abt. LIX. 4.

Behring, E.,
I. Über Desinfektion, Desinfektionsmittel und Desinfektionsmethoden.—Z. f. Hygiene. 1891. IX. 395.—Ch. C. 1891. I. 323. —C. f. B. 1891. X. 636.

Beijerinck, M. W.,
See Beyerinck.

Beissenhertz,
I. Berliner Jahresber. 1818. 158.

Bellamy,
See Lechartier et Bellamy.

Bělohoubek, Anton,
I. Das Shoyn.—Z. g. Br. 1889. XII. 433.
II. Studien über Presshefe. Prag. 1876.

Bendix, Ernst,
I. Über die Gärung sehwer vergärbarer Zuckerarten.—Z. für diätet. u. physikal. Therapie. 1900. III. 587.—C. f. B. 2. Abt. 1900. VI. 503.—Ch. C. 1900. I. 1136.—K. J. XI. 118.

Bendixen, N.,
I. Improvements in propagating apparatus for developing pure culture of yeast and bacteria.—Engl. Patent 14,106 of 9. 6. 1897. —K. J. IX. 100.

Benecke, F.,
I. Über die Ursachen der Veränderungen, welche sich während des Reifungsprozesses im Emmenthaler Käse vollziehen.—C. f. B. 1887. I. 521.
II. Über die Mykorrhiza.—C. f. B. 1888. IV. 753.

Benecke, F., und Schulze, E.,
I. Untersuchungen über den Emmenthaler Käse und über einige andere Schweizerische Käsesorten.—Landw. Jahrbücher. 1887. XVI. 318.—C. f. B. 1887. I. 521.

Benecke, Wilhelm,
I. Ein Beitrag zur mineralischen Nahrung der Pflanzen.—Ber. d. D. Bot. Ges. 1894. XII. 105.—C. f. B. 2. Abt. 1896. II. 157.—Ch. C. 1895. I. 792.—K. J. V. 70.
II. Die zur Ernährung der Schimmelpilze notwendigen Metalle.—Pringsheim's Jahrb. f. wissensch. Botanik. 1895. XXVIII. 487.—Ch. C. 1896. I. 266.—K. J. VI. 67.
III. Die Bedeutung des Kaliums und des Magnesiums für Entwicklung und Wachstum des Aspergillus niger v. Th. sowie einiger anderer Pilzformen.—Bot. Ztg. 1896. LIV. 1. Abt. 97.—C. f. B. 2. Abt. 1897. III. 675.—K. J. VII. 46.

Bérard, Jacques Étienne,
I. Sur la maturation des fruits.—Annales de Chimie et de Physique. 1821. XVI. 152.

Bergh, Axel,
See Jörgensen und Bergh.

Bergmann, E.,
I. Das putride Gift und die putride Intoxikation. Dorpat. 1866.

Bergmann, E., und Schmiedeberg, O.,
I. Über das schwefelsaure Sepsin.—Centralblatt f. d. med. Wissenschaften. 1868. p. 394.

Bergmann, Emil,
I. Untersuchungen über das Vorkommen der Ameisensäure und Essigsäure in den Pflanzen und über die physiologische Bedeutung derselben im Stoffwechsel.—Bot. Ztg. 1882. XL. 731. Gives prior literature.—Z g. Br. 1884. VII. 86.

Berlese, Amedeo,
I. Prima contribuzione allo studio della morfologia e biologia di Cladosporium e Dematium.—Revista di Patologia vegetale. Firenze. 1895. III. 2.—Zeitschr. f. Pflanzenkrankheiten. 1896. VI. 104.
II. Verhalten der Saccharomyceten an den Weinstöcken.—I.-III. Abhandlg.—Rivista di Patologia vegetale. 1897. VII.—C. f. B. 2. Abt. 1897. III. 592.—K. J. VIII. 116.
III. Bull. Soc. Mycologique de France. 1895. XI. 34.
IV. Rivista di Patologia vegetale. 1896–97. V. Nos. 5–12; 1897. VI. Nos. 6–10.

Berlese et Sostegni,
I. Recherches sur l'action des sels de cuivre sur la végétation de la vigne et sur le sol.—La Revue internationale de viticulture et œnologie.—C. f. B. 2. Abt. 1895. I. 770.

Bernard, Claude,
I. Sur le mécanisme de la formation du sucre dans le foie.—C. R. 1855. XLI. 461; 1857. XLIV. 578.
II. Leçons sur les phénomènes de la vie. 1878.

Bernheim, H.,
I. Taschenbuch für den bakteriologischen Praktikanten. 2. Ed. 1891. Würzburg. Stuber. 1*s*.
II. Die parasitären Bakterien der Cerealien.—Münchn. mediz. Wochenschrift. 1888. S. 743.—C. f. B. 1889. V. 126.—Z. g. Br. 1888. XI. 502.

Bernheimer, O.,
I. Allgem. Weinztg. 1895.—Ch. C. 1895. II. 650.

Bernstein, Alexander,
I. Die Herstellung eines neuen Getränkes aus Milch.—Milch-Zeitung. 1895. XXIV. 85 u. 942.—Ch. C. 1895. I. 707; 1896. I. 317.—K. J. VI. 224.
II. Umwandlung des Caseins der Milch in Albumosen und Peptone mittelst einer Bakterie. German Patent, No. 80, 451.—K. J. VI. 224.

Bert, Paul,
I. Influence de l'air comprimé sur les fermentations.—C. R. 1875. LXXX. 1579.

BERTHELOT, Marcelin,
I. Fixation directe de l'azote atmosphérique libre par certains terrains argileux.—C. R. 1885. CI. 775; 1886. CII. 951; 1887. CIV. 205 et 625; 1889. CVIII. 700; CIX. 277; 1892. CXV. 569.—Ch. C. 1885. 904; 1886. 460; 1887. 304, 418; 1889. I. 725, II. 563.
II. Recherches nouvelles sur les microorganismes fixateurs de l'azote.—C. R. 1893. CXVI. 842.—K. J. IV. 230.
III. Recherches sur la fermentation.—C. R. 1856. XLIII. 238.
IV. Faits pour servir à l'histoire du raffinose.—C. R. 1889. CIX. 548.—Z. g. Br. 1889. XII. 481. —Ch. C. 1889. II. 869.
V. Chimie organique, fondée sur la synthèse. Paris. 1860. Tome II. p. 621.
VI. C. R. 1857. XLIV. 702.
VII. Ann. de Chimie et de Physique 1865. 4ème série. VI. 329; 399.
VIII. C. R. 1863. LVII. 797.
IX. C. R. 1860. L. 980.
X. *Ibia.* 1855. XLI. 392.

BERTHELOT, M., et ANDRÉ, G.,
I. Sur l'odeur propre de la terre.—C. R. 1891. CXII. 598.—Ch. C. 1891. I. 891.

BERTRAND, Gabriel,
I. Préparation biochimique du sorbose.—C. R. 1896. CXXII. 900. —Ch. C. 1896. 1 1201.—K. J. VII. 228.
II. Sur le latex de l'arbre à laque.—C. R. 1894. CXVIII. 1215; CXX. 266.—Ch. C. 1894. II. 99; 1895. I. 606.
III. Sur les rapports qui existent entre la constitution chimique des composés organiques et leur oxydabilité sous l'influence de la laccase.—C. R. 1896. CXXII. 1132.—Ch. C. 1896. II. 93.—K. J. VII. 244.
IV. Sur une nouvelle oxydase, ou ferment soluble oxydant d'origine végétale.—C. R. 1896. CXXII. 1215.—Ch. C. 1896. II. 108.—K. J. VII. 245.
V. Sur la recherche et la présence de la laccase dans les végétaux. —C. R. 1895. CXXI. 166.—Ch. C. 1895. II. 496; 1896. I. 252.
VI. Sur l'intervention du manganèse dans les oxydations provoquées par la laccase.—C. R. 1897. CXXIV. 1032, 1355.—Ch. C. 1897. II. 47, 177.
VII. C. R. 1898. CXXVI. 842, 984.

BERTRAND, G., et MALLÈVRE, A.,
I. La Pectase.—C. R. 1894. CXIX. 1012; 1895. CXX. 110; CXXI. 726.—Ch. C. 1895. 1. 276, 482; 1896. 1. 123.

BERZELIUS, Jöns Jakob,
1. Lehrbuch der Chemie. 1840.

BESANA, Carlo,
1. Über das Grünwerden des Lodisaner Käses. — Jahrbuch der milchw. Versuchsstation zu Lodi. 1888.—Milch-Ztg. 1888. XVII. 364.

BEU, Hans,
1. Über den Einfluss des Räucherns auf die Fäulniserreger bei der Konservierung von Fleischwaren.—C. f. B. 1890. VIII. 513.—K. J. I. 43.

BEUMER,
1. Die Bakteriologie des Bodens.—Deutsche med. Wochenschrift. 1886. No. 27.

BEVAN, E. J.,
See CROSS and BEVAN; CROSS, BEVAN and SMITH.

BEYERINCK, M. W.,
I. Über Atmungsfiguren beweglicher Bakterien.—C. f. B. 1893. XIV. 827.—K. J. IV. 75.
II. Über Spirillum desulfuricans als Ursache von Sulfatreduktion. —C. f. B. 2. Abt. 1895. I. 1.—Ch. C. 1895. II. 169.
III. Über Thermotaxis bei Bacterium Zopfii.—C. f. B. 1894. XV. 799.—K. J. V. 88.
IV. Die filtrierende Wirkung der Chamberland'schen Bougies. — Wissenschaftl. Nachrichten d. Nieuwe Rotterd. Courier. 1890. —K. J. I. 17.
V. Verfahren zum Nachweise der Säureabsonderung bei Mikrobien.—C. f. B. 1891. IX. 781. —Ch. C. 1892. I. 635.—K. J. II. 11.
VI. Kulturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen.—Bot. Ztg. 1890. XXXXVIII. 725.—C. f. B. 1890. VIII. 460; 1893. XIII. 368.—K. J. IV. 83.

VII. Kulturversuche mit Amöben auf festem Substrate.—C. f. B. 1. Abt. 1896. XIX. 257.
VIII. L'auxanographie ou la méthode de l'hydrodiffusion dans la gélatine appliquée aux recherches mierobiologiques.—Archives Néerlandaises. 1889. XXIII. 367.—C. f. B. 1890. VII. 347.
IX. Qualitative und quantitative mikrobiochemische Analyse.—C. f. B. 1891. X. 723.—Ch. C. 1892. I. 142.—K. J II. 11.
X. Die Lebensgesehichte einer Pigmentbakterie.—Bot. Ztg. 1891. XXXXIX. 705.—C. f. B. 1892. XII. 862.—K. J. II. 78.
XI. Over liehtvoedsel en plastiseh voedsel van Lichtbakterien.—Verslagen en Mededeelingen der Koninklijke Akademie van Wetenschappen. Afdeeling Natuurkunde. 2de Reeks. Deel VII. 239.—C. f. B. 1890. VIII. 616.—K. J. I. 180.
XII. Über die Butylalkoholgärung und das Butylferment.—Verhandlungen der K. Akademie van Wetenschappen te Amsterdam. Tweede Sectie, Deel I, No. 10.—Archives Néerlandaises des sciences exactes et naturalles. 1895. XXIX.—C. f. B. 1894. XV. 171.—Ch. C. 1894. I. 963.—K. J. IV. 258.
XIII. Über Nachweis und Verbreitung der Glukase, das Enzym der Maltose.—C. f. B. 2. Abt. 1895. I. 221.—K. J. VI. 320.
XIV. Die Bakterien der Papilionaeeen-Knöllehen.—Bot. Ztg. 1888. XXXXVI. 725. With plate.—C. f. B. 1889. V. 804.
XV. Künstliche Infektion von Vieia Faba mit "Baeillus radicicola—Bot. Ztg. 1890. XXXXVIII. 837.—K. J. I. 128.
XVI. Über die Natur der Fäden der Papilionaceenknöllehen.—C. f. B. 1894. XV. 728.—K. J. V. 248.
XVII. Over ophooping van atmospherisehe Stickstof in culturen van Bacillus radieieola.—Verslagen en Mededeelingen der Koninkl. Akademie van Wetenschappen te Amsterdam. Afdel: Natuurkunde. 1891. 3de Reeks. VIII. 460.—C. f. B. 1892. XII. 687.—K. J. III. 203.
XVIII. Schizosaceharomyces oetosporus, eine achtsporige Alkoholhefe.—C. f. B. 1894. XVI. 49.—Ch. C. 1894. II. 614.—K. J. V. 38.
XIX. Sur le Kéfir.—Arehives Néerlandaises etc. 1889. XXIII. 428.—Ch. C. 1893. I. 619.—K. J. III. 182.
XX. Die Laetase, ein neues Enzym.—C. f. B. 1. Abt. 1889. VI. 44.—Ch. C. 1889. II. 461.
XXI. Zur Ernährungsphysiologie des Kahmpilzes.—C. f. B. 1. Abt. 1892. XI. 68.—Ch. C. 1892. I. 445.—K. J. III. 108.
XXII. Weitere Beobaehtungen über die Oetosporushefe.—C. f. B. 2. Abt. 1897. III. 449.—Ch. C. 1897. II. 868.—K. J. VIII. 98.
XXIII. Notiz über die Cholerarot-Reaktion.—C. f. B. 1. Abt. 1892. XII. 715.—Ch. C. 1893. I. 106.—K. J. III. 64.
XXIV. C. f. B. 1898. IV. 657.
XXV. Bot. Centralbl. 1892. LI. 384.
XXVI. C. f. B., 2. Abt. 1897. III. 454.
XXVII. *Ibid.* 1900. VI. 11.
XXVIII. *Ibid.* 1894. XVI. 51.
XXIX. Verhand. der. K. Akad. v. Wetensehappen te Amsterdam. Tweede Sectie, Deel I, 1893; Deel II. 1894. No. 40. Reeueil des travaux chim. des Pays-Bas. 1893. XII. 141.
XXX. Bull. de l'Assoc. Belge des Chimistes. 1890. XVI. 178.—Archives Néerlandaises. 1890. XXIII. 428.
XXXI. C. f. B. 2. Abt. 1897. III. 449, 521.
XXXII. *Ibid.* 1900. VI. 194.

Biedermann, W.,
I. Beiträge zur vergleiehenden Physiologie der Verdauung. I.—Pflüger's Archiv. 1898. LXXII. 105.—Ch. C. 1898. II. 370.

Biel, J.,
I. Studien über die Eiweissstoffe des Kumys und des Kefir.—Pharmae. Zeitschrift f. Russland. 1886. XXV. 161.—Milehzeitung. 1886. XV. 128.

II. Über Kefir, seine Eigenschaften und Bereitung.—*Ibid.* S. 267.—Ch. C. 1886. S. 845.

Biel, W.,
I. Über einen schwarzes Pigment bildenden Kartoffelbacillus.—Inaug.-Diss. Kiel. 1896.—C. f. B. 2. Abt. II. 137.—K. J. VII. 27.

Bienstock, Berthold,
I. Über die Bakterien der Fäces.—Zeitschrift für klinische Medizin. 1883. VIII. 1.—Ch. C. 1883. S. 809; 1884. S. 646.

Bierbrouwery d'Oranjeboom,
I. Beziehungen der Hefengabe zum Hefenwaehstum und zum Attenuationsverlauf während der Biergärung.—Z. g. Br. 1897. XX. 423.—K. J. VIII. 124.

Biernacki, E.,
I. Über die Eigenschaft der Antiseptica, die Alkohol-Gärung zu beschleunigen und über gewisse Abhängigkeit ihrer Kraft von der chemischen Baustruktur, der Fermentmenge und der Vereinigung mit einander.—Pflüger's Arehiv f. d. ges. Physiologie. 1891. XXXIX. 112.—Reproduced in Z. g. Br. 1891. XIV. 248.—C. f. B. 1891. X. 296.—Ch. C. 1891. II. 31.—K. J. II. 69.

Biffen, R. H.,
I. A fat-destroying Fungus.—Annals of Botany. 1899. XIII. 363.

Biginelli, P.,
I. Composizione e costituzione chimica del gas arsenicale delle tappezzerie.—Atti della R. Aecad. dei Lineei. Roma. 1900. [5]. IX. 210 e 242.—Ch. C. 1900. II. 1067 u. 1100.

Billroth, Theodor,
I. Untersuchungen über die Vegetationsformen von Coccobacteria septiea, etc. Berlin 1874. G. Reimer.

Biot et Persoz,
I. Ann. de Chim. et de Phys. 1833. LIII. 83.

Biourge, Ph.,
I. Recherches sur la fermentation alcoolique.—La Cellule. 1895. XI. 95.—Ch. C. 1896. II. 105.—K. J. VI. 149.

Birch-Hirschfeld,
I. Über die Züehtung von Typhusbacillen in gefärbten Nährlösungen.—A. f. Hygiene. 1888. VII. 341.—C. f. B. 1888. III. 447 u. 569.

Bischoff, C. A.,
I. Weitere Beiträge zur Kenntnis der substituierten Bernsteinsäuren.—Ber. d. D. Chem. Ges. 1891. XXIV. 1064.—Ch. C. 1891. I. 963.

Bissinger,
I. Über Bestandteile der Pilze Lactarius piperatus und Elaphomyces granulatus.—Archiv. der Pharmacie. 1883. p. 321.

Bittó, Béla von,
See Liebermann und Bitto.

Bizzozero, Giulio,
I. Sui microfiti dell' epidermide umana.—Atti Acc. Torino. 1894

Blachstein,
I. Contribution à la biologie du baeille typhique.—Archives de scienees biol. publ. par l'Institut imp. de méd. expér. à St. Pétersbourg. 1892. I. No. 1 et 2.—K. J. III. 80.

Bleisch, C., u. Schweitzer, R.,
I. Beiträge zur Kenntnis der Vorgänge beim Lüften der Würze.—Z. g. Br. 1899. XXII. 515.—Ch. C. 1900. II. 697.

Bleisch, M.,
I. Über bittere Milch und die Sterilisierung der Mileh durch Erhitzen unter Luftabschluss.—Z. f. Hygiene. 1893. XIII. 81.—K. J. IV. 182.

Blondeau, Ch.,
I. Etude ehimique du fromage de Roquefort.—Annales de ehimie et de physique. 4ème série.—1864. I. 208.—Ch. C. 1864. S. 333.
II. Sur la formation de l'humus et du nitre —C. R. 1863. LVII. 414.

Blondlot,
I. Sur la fermentation alcoolique du sucre de lait.—C. R. 1872. LXXIV. 534.

Blumenthal,
I. Virchow's Archiv. 1894. CXXXVII. 539.

Blumentritt,
I. Ber. d. D. Bot. Ges. 1901. XIX. 442.
II. *Ibid.* 1905. XXIII. 419.

Blunt,
See Downes and Blunt.
Bochicchio, Nicola,
I. Über einen Milchzucker vergärenden und Käseblähungen hervorrufenden neuen Hefepilz.—C. f. B. 1. Abt. 1894. XV. 546.—Le stazioni sperim. agrar. ital. 1894. XXVI. 339, 568.—Ch. C. 1894. II. 214.—K. J. V. 244.
Bodin,
I. Les parasites de l'Homme. Paris. 1902.
Boersch, C.,
I. Beitrag zur Kenntnis der Bakterien des Weines und zur Kenntnis der Hefen. Dissert. Erlangen. 1893.—K. J. IV. 127.
Böhmer,
I. Die Heubereitungsarten. — Deutsche landw. Presse. 1890. XVII. 103.
Boidin, A., and Rolants, E.,
I. The Utilisation of the Chinese Yeast, Amylomyces Rouxii, in European Industries.—La Bière. 1897. V. 33.—K. J. VIII. 131.
See also Collette and Boidin.
Bókay, A.,
I. Über die Verdaulichkeit des Nucleins und Lecithins.—Z. f. physiolog. Chemie. 1877. I. 157.
Bokorny, Theodor,
I. Über den Einfluss des Calciums und Magnesiums auf die Ausbildung der Zellorgane.—Botan. Centralblatt. 1895. LXII. 1.
II. Chemisch-physiologisches über die Hefe.—Pharmaceut. Centralhalle. 1900. XXXXI. 737.—Ch. C. 1901. I. 56.
III. Über die Kohlenstoffernährung der Sprosshefe.—Dingler's polytechn. Journal. 1897. CCCIII. 115.—C. f. B. 2. Abt. 1897. III. 372.—Ch. C. 1897. I. 553.—R. J. VIII. 86.
IV. Chem. Zeitung. 1901. XXV. 502; 1902. XXVI. 704; 1903. XXVII. 502.
Bolas, Th.,
I. Alkoholgehalt des Brotes.—Chemical News. 1873. XXVII. 271.—Ch. C. 1873. p. 676.
Bolley, H. L., and Hall, C. M.,
I. Cheese curd inflation. Its relation to the bacterial flora of fore milk.—C. f. B. 2. Abt. 1895. I. 788.
Boltshausen, H.,
See Kirchner und Boltshausen.
Bondzynski, Stephan,
I. Zur Kenntnis der chemischen Natur einiger Käsearten. — Landw. Jahrbuch der Schweiz. 1894. VIII. 189.
Bonhoff,
I. Die Einwirkung höherer Wärme grade auf Tuberkelbacillen-Reinkulturen. — Hygien. Rundschau. 1892. II. 1009.—C. f. B. 1893. XIII. 294.
II. Untersuchungen über Giftbildung verschiedener Vibrionen in Hühnereiern.—A. f. Hygiene. 1895. XXII. 351.
Bonnet, Charles,
I. Considérations sur les corps organisés. 1762. Amsterdam et Paris.
Bonnier et Mangin,
I. Recherches sur la respiration et transpiration des champignons. —Annales des sciences naturelles. Botanique. 6eme série. 1884. XVII. 210.
II. Recherches sur la respiration des tissus sans chlorophylle. *Ibid.* 1884. XVIII. 293.
Bonorden,
I. Handbuch d. allg. Mykologie. Stuttgart. 1851.
Boorsma, W. G.,
I. Ang-Khak. — Geneesk. Tijds. 1896. XXXV.—Nederlandsch Tijdschrift voor Pharmacie, etc. 1896. VIII. 126.—Ch. C. 1896. I. 1131.
Bordas,
I. Sur une maladie nouvelle du vin en Algérie.—C. R. 1888. CVI. 85.
II. C. R. 1904. CXXXVIII. 928.
Bordoni-Uffreduzzi, Guido,
I. Ein Fall von fuchsinähnlicher Bakterienfärbung des Fleisches —Hygien. Rundschau. 1894. IV. 12.—C. f. B. 1894. XV. 666.—Ch. C. 1894. I. 914.—K. J. V. 87.
II. Die biologische Untersuchung des Eises in seiner Beziehung zur öffentlichen Gesundheitspflege.—C. f. B. 1887. II. 489.
Borgeaud,
See Hess und Borgeaud.

Borgman,
I. Zeitg. f. analyst. Chem. 1886. XXV. 532.
Bornträger,
I. Zeitg. f. angew. Chem. 1894. 13.
II. Deutscher Chemiker - Ztg. 1893. VII. 377.
Borschtschoff, I.,
I. Die gallertigen Substanzen der Rübensäfte und Sirupe.—Annalen der Technischen Gesellschaft zu Kiew.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1876. S. 738.
Botkin, S.,
I. Über einen Baeillus butyricus.—Z. f. Hygiene. 1892. XI. 421.—C. f. B. 1892. XI. 628.—K. J. III. 233.
Bouchardat,
I. C. R. 1874. LXXVIII. 1145.
Bouffard, A.,
I. Sur le eassage des vins.—C. R. 1894. CXVIII. 827.—K. J. V. 194.
II. C. R. 1895. CXXI. 357.
Boullanger, E.,
I. Aetion des levures de bière sur le lait.—Ann. Inst. Past. 1897. XI. 720.—Ch. C. 1897. II. 1011.—K. J. VIII. 155.
II. Contribution à l'étude de quelques levures de bière.—Ann. Inst. Past. 1896. X. 598.—C. f. B. 2. Abt. 1897. III. 23.—K. J. VII. 95.
Bouquet, Robert,
I. Nouvelle hypothèse sur l'absorption de l'azote par les végétaux.—Journal de l'agrieulture pratique. 1888. I. 710.—Biedermann's Centralbl. 1891. S. 424.
Bourquelot, Émile,
I. Les Ferments solubles.—Paris. 1896. Soeiété d'éditions seientifiques.—K. J. VII. 247.
II. Sur l'hydrolyse de la raffinose (mélitose) par les ferments solubles.—Journal de Pharmaeie et de Chimie. 6e série. 1896. III. 390.—Ch. C. 1896. I. 1169.—K. J. VII. 238.
III. Présence et rôle de l'émulsine dans quelques ehampignons parasites des arbres ou vivant sur le bois.—Comptes rendus de la société de biologie. 1893, p. 804.—K. J. IV. 284.
IV. Présence d'un ferment analogue à l'émulsine dans les champignons parasites des arbres ou vivant sur le bois. C. R. 1893. CXVII. 383.—Ch. C. 1893. II. 825.—K. J. IV. 284.
V. C. R. 1893. CXVI. 826.
VI. Bull. Soe. Myeol. de France. 1893. IX. 230.
VII. Comptes rendus Soc. de Biologie. 1896. XLVIII. 205.
VIII. C. R. 1902. CXXXV. 349; Journ. de Pharm. et de Chimie. 1903. XVI. 578.
IX. Comptes rendus Soc. de Biologie. 1903. LV. 386.
X. *Ibid.* 1896. XLVIII. 896.
XI. C. R. Soc. de Biologie. 1893. XLV. 653, 804.
XII. C. R. Soc. de Biologie. XLV. 425.
XIII. Comptes rendus Soe. de Biologie. 1893. 653; C. R. 1893. CXVI. 1143; CXVII. 826.
XIV. C. R. 1903. CXXXVI. 762.
XV. C. R. 1898. CXXVI. 1045; 1901. CXXXIII. 690; 1902. CXXXV. 399.
Bourquelot, E., et Bertrand, G.,
I. La laecase dans les Champignons.—C. R. 1895. CXXI. 783.—Ch. C. 1896. I. 124.
II. Über die Färbung der Gewebe und des Saftes gewisser Pilze an der Luft.—Journal de Pharmaeie et Chimie. 1896. III. 177.—Ch. C. 1896. I. 756.—K. J. VII. 244.
Bourquelot et Graziani,
I. Bull. Soc. Mycol. de France. 1896. XII. 27.
Bourquelot, E., et Hérissey, H.,
I. Reeherehe et présence d'un ferment soluble protéohydrolytique dans les Champignons.—C. R. 1898. CXXVII. 666.—Journal de Pharmaeie et de Chimie. 1898. sér. VI. t. VIII. p. 448.—C. f. B. 2. Abt. 1899. V. 159.—Ch. C. 1898. II. 1274.
II. Journ. de Pharm. et de Chimie. 1902. XVI. 417.
III. *Ibid.* 1897. IX. 385.
IV. C. R. 1895. CXXI. 696.
V. *Ibid.* 1901. CXXXII. 571.
VI. C. R. 1899. CXXIX. 228.

Boussingault,
I. C. R. 1880. XCI. 373.
II. Ann. de Chim. et de Phys. 1881. [5]. XXII. 118.

Boutron-Chalard, Antoine François. *See* Frémy et Boutron-Chalard.

Boutroux, Léon,
I. Sur une fermentation nouvelle du glucose. — C. R. 1878. LXXXVI. 605; 1880. LXXXXI. 236.—Ch. C. 1880. S. 600.
II. Contribution à l'étude de la fermentation panaire.—C. R. 1883. LXXXXVII. 116.—Ch. C. 1883. S. 614.
III. Sur la fermentation panaire.—C. R. 1891. CXIII. 203.—C. f. B. 1892. XII. 153.—Ch. C. 1891. II. 549.—K. J. II. 227.
IV. Sur les causes qui produisent la couleur du pain bis.—C. R. 1895. CXX. 934.
V. Sur la conservation des ferments alcooliques.—Annales des sciences naturelles. Botanique. 6e série. 1884. XVII. 144.
VI. Sur la dissémination naturelle des levures de vin.—C. R. 1898. CXXVII. 1033.—C. f. B. 2. Abt. 1899. V. 311.
VII. Le pain et la panification. Paris. 1897. Baillière et fils. 4*s.* 2*d.*
VIII. Bull. de la Soc. Linn. de Normandie. 1883. VII.; Ann. d. Sc. Nat. Bot. 1884. XVII. 196.
IX. Bull. de la Soc. Linn. de Normandie. 1881. VI. 1883. VII.
X. A. d. Sci. Nat. Bot. 1884. XVII. 144.

Bovet, V.,
I. Ann. de Microgr. 1891. III. 353.

Bowhill, Thomas,
I. Zur bakteriolog. Technik.—C. f. B. 2. Abt. 1899. V. 287.

Boyce, R., and Evans, E.,
I. Upon the action of gravity on Bacterium Zopfii.—Proceedings of the Royal Society of London. 1893. LIV. 300.—C. f. B. 1894. XV. 568.—K. J. IV. 123.

Braconnot, Henri,
I. Sur la Jusée et l'Écorce de chêne.—Annales de chimie et de physique. 1832. L. 376.
II. De la Fongine, ou Analyse des Champignons. — Journal de Physique, de chimie, etc. 1811. LXXIII. 130.
III. Recherches analytiques sur la nature des champignons.—Annales de chimie. 1811. LXXIX. 265; LXXX. 272.

Bräutigam, Walter,
I. Über das Gelatinieren des Infusum foliorum Digitalis.—Pharm. Centralhalle. 1891. XXXII. 349, 427.—Ch. C. 1891. II. 489, 549.—K. J. II. 222.
II. Unters. üb. d. Mikroorganismen in Schlämpe u. Bierträben. Dissert. Leipzig. 1886.

Brand, Josef,
I. Krickenbier.—From "La Gazette du Brasseurs." 1897. XI. 61, noticed in Z. g. Br. 1897. XX. 543.—K. J. VIII. 140.
II. Über das Vorkommen von Furfurol im Malze, in der Würze und im Biere.—Z. g. Br. 1898. XXI. 255.—Ch. C. 1898. II. 146.—K. J. IX. 161.
III. Z. g. Br. 1893. XVI. 303; 1894. XVII. 131.

Brandes, P., und Stoehr, C.,
I. Über die Bildung von Pyrazin und Homologen aus Traubenzucker und Ammoniak.—Journal f. prakt. Chemie. 1896. CLXII. 481.—Ch. C. 1897. I. 381.
II. Z. f. prakt. Chem. 1896. CLXI. 501.

Brassier,
I. Sur les modifications que le fromage subit en vieillissant.—Ann. d. chim. et phys. 4ème s. 1865. V. 270.

Brault, A., et Loeper, M.,
I. Journ. de Physiol. et de Pathol. générale. 1904. VI. 720.

Braun, Richard,
I. Nachweis des Glycogenes in Hefenzellen.—Z. g. Br. 1901. XXIV. 397.—C. f. B. 2. Abt. 1902. VIII. 27.—Ch. C. 1901. II. 446.
II. Z. g. Br. 1902. XXV. 409.

Bréal, E.,
I. De la présence, dans la paille, d'un ferment aérobie, réducteur des nitrates.—C. R. 1892. CXIV. 681.—C. f. B. 1892. XII. 300.—K. J. III. 227.

Brefeld, Oskar,
I. Botanische Untersuchungen über Schimmelpilze. 1. Heft. 1872: Mucor Mucedo, etc. Zygomyceten
II. *Ibid.* II. 1874: Die Entwicklungsgeschichte von Penicillium.
III. *Ibid.* III. 1877: Basidiomyceten. I.
IV. *Ibid.* IV. 1881: Kulturmethoden zur Untersuchung der Pilze. Bacillus subtilis, etc.
V. *Ibid.* V. 1883: Die Brandpilze. 1. Darunter: Der morphologische Wert der Hefen.
VI. *Ibid.* VI. 1884: Myxomyceten. I. Entomophthoreen. II.
VII. *Ibid.* VII. 1888: Basidiomyceten. II.
VIII. *Ibid.* VIII. 1889: Basidiomyceten. III. Das natürliche System der Pilze.
IX. *Ibid.* IX. 1891: Die Hemiasci und die Ascomyceten.—C. f. B. 1. Abt. 1892. XI. 291.—K. J. III. 41.
X. *Ibid.* X. 1891: Ascomyceten. II.
XI. *Ibid.* XI. 1895: Die Brandpilze. II.
XII. *Ibid.* XII. 1895: Hemibasidii. Brandpilze. III.
XIII. Untersuchungen über Alkoholgärung.—Landw. Jahrbücher. 1874. III. 65.—Ber. d. D. Chem. Ges. 1874. VII. 281.—Botan. Jahresber. pro 1874. 341, 342.
XIV. *Ibid.* II.—Landw. Jahrbücher. 1875. IV. 405.—Botan. Jahresbericht pro 1875. S. 198.
XV. *Ibid.* III. — Landw. Jahrbücher. 1876. V. 281.—Botan. Jahresbericht pro 1876. S. 143.
XVI. Mucor racemosus und Hefe. —Flora. 1873. LVI. 385.
XVII. Landw. Jahrbücher. 1875. IV. 414; 1876. V. 332.
XVIII. Landw. Jahrbücher. 1876. LIX. 310.

Bremer,
I. Dissert. Münster. 1902.

Brevans, J. de,
I. Les Conserves alimentaires. Paris 1896. Baillière et fils.

Briant, Lawrence,
I. The Influence of Aeration on Fermentation.—Journal of the Federated Institutes of Brewing. 1895. I. 472.—Z. g. Br. 1896. XIX. 100.—K. J. VI. 136.
II. The Influence of Nitrates in Brewing.—Journ. Fed. Institutes of Brewing, 1899. V. 372.—K. J. X. 134.

Brieger, Ludwig,
I. Spaltungsprodukte der Bakterien.—Z. f. physiolog. Chemie. 1884. VIII. 306. 1885. IX. 1.—Ch. C. 1884. S. 456. 1885. S. 60.
II. Über die flüchtigen Bestandteile der menschlichen Exkremente.—Ber. d. D. Chem. Ges. 1877. X. 1027.—Journal f. prakt. Chemie. 1878. XVII. 124.—Ch. C. 1887. S. 469.
III. Über Ptomaïne. Berlin 1885. —Ch. C. 1885. S. 362.
IV. Zur Kenntnis der Fäulnisalkaloide.—Z. f. physiolog. Chemie. 1883. VII. 274.—Ch. C. 1883. S. 539.
V. Die Quelle des Trimethylamins im Mutterkorn.—Z. f. physiolog. Chemie. 1887. XI. 184.
See also Baumann und Brieger.

Brizi, Ugo,
I. Über die Fäulnis der Rebentriebe, durch Botrytis cinerea verursacht.—C. f. B. 2. Abt. 1897. III. 141.—Ch. C. 1897. I. 996.

Brochet, A.,
1. Sur la production de l'aldehyde formique gazeux pur.—C. R. 1896. CXXII. 201.—Ch. C. 1896. 1. 539.—K. J. VII. 15.
See also Cambier et Brochet.

Brodmeier, A.,
I. Über die Beziehung des Proteus vulgaris Hsr. zur ammoniakalischen Harnstoffzersetzung.—Dissert. Erlangen. 1896.—C. f. B. 1. Abt. 1895. XVIII. 380.—Ch. C. 1896. I. 316.

Brown, Adrian J.,
I. On an Acetic Ferment which forms Cellulose. — Journal of the Chemical Society. 1886. XXXXIX. 432.—Ch. C. 1886. S. 699; 1887. S. 804.
II. Note on Bacillus subtilis.—Journal of the Federated Institutes of Brewing. 1895. I. 423. —Z. g. Br. 1895. XVIII. 339.—W. f. Br. 1895. XII. 657.—K. J. VI. 206.

III. The Chemical Action of Pure Cultivations of Bacterium aceti. —Journal of the Chemical Society. 1886. XXXXIX. 172; 1887. LI. 638.—Ch. C. 1886. S. 200; 1887. S. 803.

IV. Influence of Oxygen and Concentration on Alcoholic Fermentation.—Journal of the Chem. Society. Transactions. 1892. LXI. 369.—C. f. B. 1. Abt. 1892. XII. 148.—Ch. C. 1892. I. 560. —K. J. III. 101.

V. Über das Fermentationsvermögen.—C. f. B. 2. Abt. 1897. III. 33.—Ch. C. 1897. I. 871. —K. J. VIII. 91.

VI. The Specific Character of the Fermentative Functions of Yeast-cells.—Brewing Trade Review. 1895. IX. No. 104.—K. J. VI. 57.

VII. Versuche über die numerische Vermehrung der Hefezellen.—Transactions of the Laboratory Club. 1890. III. 64.—Ch. C. 1890. I. 803.—W. f. Br. 1890. VII. 355.—K. J. I. 58.

VIII. Journ. Federated Inst. Brewing. 1901. VII. 93.

Brown, Horace T., and Morris, G. H.,

I. On Certain Functions of Hops used in the Dry-hopping of Beers. —Transactions of the Institute of Brewing. 1893. VI. No. 4. —K. J. IV. 137.

II. Über eine neue Methode der Bier-Analyse. — Transactions of the Laboratory Club. 1890.—K. J. I. 68.

III. Chem. Zeitg. 1893. XVII. 655.

IV. Liebig's Ann. 1885. CCXXXI. 134.

V. Journ. Chem. Soc. 1889. 449, 462.

Brown, Robert,

I. Observations on the Organs and Mode of Fecundation in Orchideæ and Asclepiadeæ. London. 1831.

Brücke, Ernst,

I. Die Elementarorganismen. — Sitzungsberichte der K. Akademie der Wissenschaften. Mathem.-naturw. Kl. 1861. XXXIV. Abt. II. S. 381.

Brunchorst, Jörgen,

I. Über die Knöllchen an den Legumnosenwurzeln.—Ber. d. D. Bot. Ges. 1885. III. 241.

II. Über die Wurzelanschwellungen von Alnus und den Elaeagnaceen. Dissert. Tübingen. 1886.—Untersuchungen a. d. Botan. Inst. zu Tübingen.

Brunner, Alfred,

See Morpurgo und Brunner.

Brunstein,

1. Beihefte z. Bot. Centralbl. 1901. X. 1.

Brusilowsky, E.,

I. Die Bedeutung der Mikroorganismen bei der Bildung des Buchtschlammes. — Wratsch 1890. S. 717. — Hygienische Rundschau. 1891. I. 240.—C. f. B. 1891. X. 194.—Ch. C. 1891. I. 672.—K. J. II. 227.

Bryilants, O.,

1. Milchztg. 1895. XXIV. 697.

Buchner, Eduard,

I. Über den Einfluss des Sauerstoffes auf Gärungen.—Z. f. physiolog. Chemie. 1885. IX. 380.—Z. g. Br. 1885. VIII. 268.—Ch. C. 1885. S. 555.

II. Alkoholische Gärung ohne Hefezellen.—Ber. d. D. Chem. Ges. 1897. XXX. 117.—C. f. B. 2. Abt. 1897. III. 251.—Ch. C. 1897. I. 422.

III. *Ibid.* II. Mittlg.—Ber. d. D. Chem. Ges. 1897. XXX. 1110. —C. f. B. 2. Abt. 1897. III. 527.—Ch. C. 1897. II. 46.—Z. g. Br. 1897. XX. 362.

IV. Notiz aus der Gärungschemie. —Ber. d. D. Chem. Ges. 1892. XXV. 1161.—Ch C. 1892. I. 860.—K. J. III. 241.

V. Zymase aus getöteter Hefe.—Ber. d. D. Chem. Ges. 1900. XXXIII. 3307.—C. f. B. 2. Abt. 1901. VII. 247.—Ch. C. 1901. I. 54.—K. J. XII.

VI. Bemerkungen zur Arbeit von A. Macfadyen, G. H. Morris und S. Rowland: "Über ausgepresstes Hefezellplasma (Buchner's Zymase)."—Ber. d. D. Chem. Ges. 1900. XXXIII. 3311.—C. f. B. 2. Abt. 1901. VII. 73 —Ch. C. 1901. I. 55.—K. J. XI. 375.

VII. Über die Zymase.—W. f. Br. 1901. XVIII. 197.—C. f. B. 2. Abt. 1901. VII. 845.—Ch. C. 1901. I. 1110.

VIII. Verfahren zur Gewinnung des flüssigen Zellinhaltes von Mikroorganismen in unveränderter Form. German Pat., No. 99,508, Jan. 12, 1897.—Ch. C. 1899. I. 320.—K. J. IX. 314.

IX. Verfahren zur Herstellung abgetöteter Dauerhefe. German Pat., No. 97,240, April 30, 1897.—Ch. C. 1898. II. 591.—K. J. IX. 315.

X. Zymasegärung. München und Berlin. 1903.

Buchner, E., u. Antoni, W.,

I. Z. f. physiol. Chem. 1905. XLIV. 206.

II. Z. f. physiol. Chem. XLVI. 136.

Buchner, u. Hahn,

I. Die Zymasegärung. 1903. 210.

Buchner, E., u. Meisenheimer, J.,

I. Zeit. f. physiolog. Chemie. 1903. XL. 167.

II. Ber. d. D. Chem. Ges. 1905. XXXVIII. 620.

III. *Ibid.* 1903. XXXV. 634.

IV. *Ibid.* 1904. XXXVII. 417.

Buchner, E., u. Mitscherlich, S.,

I. Z. f. physiol. Chem. 1904. XLII. 554.

Buchner, Ed., u. Rapp, R.,

I. Alkoholische Gärung ohne Hefezellen.—Ber. d. D. Chem. Ges. 1897. XXX. 2668.—C. f. B. 2. Abt. 1898. IV. 297.—Ch. C. 1898. I. 70.

II. *Ibid.* II.—Ber. d. D. Chem. Ges. 1898. XXXI. 209.—C. f. B. 2. Abt. 1898. IV. 522.—Ch. C. 1898. I. 681.—K. J. IX. 209.

III. *Ibid.* V. (should be III.) Mittlg.—Ber. d. D. Chem. Ges. 1898. XXXI. 1084.—C. f. B. 2. Abt. 1898. IV. 927.—Ch. C. 1898. II. 47.—K. J. IX. 312.

IV. *Ibid.* VI. (should be IV.) Mittlg.—Ber. d. D. Chem. Ges. 1898. XXXI. 1090.—C. f. B. 2. Abt. 1898. IV. 860.—Ch. C. 1898. II. 48.—K. J. IX. 312.

V. *Ibid.* VII. (should be V.) Mittlg.—Ber. d. D. Chem. Ges. 1898. XXXI. 1531.—Ch. C. 1898. II. 438.—K. J. IX. 314.

VI. *Ibid.* VIII. (VI.).—Ber. d. D. Chem. Ges. 1899. XXXII. 127.—C. f. B. 2. Abt. 1899. V. 312.—Ch. C. 1899. I. 531.—K. J. X. 313.

VII. *Ibid.* IX. (VII.).—Ber. d. D. Chem. Ges. 1899. XXXII. 2086—C. f. B. 2. Abt. 1899. V. 843.—Ch. C. 1899. II. 446.—K. J. X. 317.

VIII. *Ibid.* X. (VIII.).—Ber. d. D. Chem. Ges. 1901. XXXIV. 1523.—C. f. B. 2. Abt. 1901. VII. 809.—Ch. C. 1901. II. 140

IX. Ber. d. D. Chem. Ges. 1898. XXXI. 1085.

X. Ber. d. D. Chem. Ges. 1901. XXXIV. 1527.

XI. Ber. d. D. Chem. Ges. 1898. XXXI. 2671.

XII. Ber. d. D. Chem. Ges. 1897. XXXV. 2668.

Buchner, Ed., und Spitta, Alb.,

I. Zymasebildung in der Hefe.—Ber. d. D. Chem. Ges. 1902. XXXV. 1703.—Ch. C. 1902. I. 1371.

Buchner, Hans,

I. Über die vermeintlichen Sporen des Typhusbacillus.—C. f. B. 1888. IV. 353.

II. Über die experimentelle Erzeugungdes Milzbrandcontagiums aus den Heupilzen.—Nägeli's Untersuchungen über niedere Pilze. München. 1882. S. 140.

III. Kritisches und Experimentelles über die Frage der Konstanz der pathogenen Spaltpilze.—Nägeli's Untersuchungen, etc S. 272.

IV. Über den Einfluss des Lichtes auf Bakterien.—C. f. B. 1892. XI. 781; XII. 217.—K. J. III. 76.

V. Über den Einfluss des Lichtes auf Bakterien und über die Selbstreinigung der Flüsse.—Archiv f. Hygiene. 1894. XVII. 177.—C. f. B. 1894. XV. 515.

VI. Beiträge zur Morphologie der Spaltpilze.—Nägeli's Untersuchungen, etc. S. 205.

VII. Eine neue Methode zur Kultur anaerober Mikroorganismen.—C. f. B. 1888. IV. 149.

VIII. Notiz betreffend die Frage des Vorkommens von Bakterien im normalen Pflanzengewebe — Münch. Medic. Wochenschrift. 1888. XXXV. 906.—C. f. B. 1889. V. 341.—Z. g. Br. 1889. XII. 21.

Buchner, Hans, und Gruber, Max,
I. Verfahren zur Gewinnung von Hefeneiweiss mittelst Äther behufs Verwendung als Nährmittel. —German Pat. No. 113181, May 24, 1899.—Ch. C. 1900. II. 829.

Buchner, H., Longard, K., u. Riedlin, G.,
I. Über die Vermehrungsgeschwindigkeit der Bakterien.—C. f. B. 1887. II. 1.

Buchner, H., und Rapp, Rudolf,
I. Beziehungen des Sauerstoffes zur Gärtätigkeit der lebenden Hefezellen.—Zeitschrift f. Biologie. 1898. XXXVII. 82.—Ch. C. 1899. I. 133.—K. J. IX. 77.

Buchner, H., und Segall,
I. Über gasförmige antiseptische Wirkungen des Chloroformes, Formaldehydes und Kreolins.— Münchner medic. Wochenschrift. 1889. No. 20.—Ch. C. 1889. II. 460.

Bucholz, W.,
I. Pharmac. Zeitschr. f. Rüssland. 1867. 627, 686.

Bücheler, Max,
I. Beiträge zur Beurteilung der Reinhefe Rasse II.—Z. f. Spiritusind. 1894. XVII. No. 8.—K. J. V. 176.

Büsgen, Moritz,
I. Kulturversuche mit Cladothrix dichotoma.—Ber. d. D. Bot. Ges. 1894. XII. 147.—C. f. B. 1894. XVI. 860.—K. J. V. 57.
II. Über einige Eigenschaften der Keimlinge parasitischer Pilze.— Bot. Ztg. I. Abt. 1893. LI. 53. Gives the prior literature relating to irritability.
III. Die Entwicklung der Phycomyceten - Sporangien. Dissert. Strassburg. 1882. — Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1882. XIII. 253.
IV. Ber. d. D Bot. Ges. 1885. 66.

Bütschli, O.,
I. Über den Bau der Bakterien und verwandter Organismen. Vortrag. Leipzig. 1890. Winter.— Bot. Ztg. 1890. XXXXVIII. 463.—C. f. B. 1890. VII. 639.
II. Untersuchungen über mikroskopische Schäume und das Protoplasma. Leipzig. 1892. Engelmann.—C. f. B. 1893. XIII. 436.—K. J. IV. 54.

Bujwid, Odo,
I. Die Bakterien in Hagelkörnern. —C. f. B. 1888. III. 1.

Bulling, A.,
I. Spontane Lungentuberkulose einer Ziege.—Münchener medizin. Wochenschrift. 1896. S. 474. — Baumgarten's Jahresbericht. XII. 465.

Bungener,
1. Moniteur Scientifique. 1882. XXIV. 523, 829.

Bungener, H., und Weibel, L.,
1. Einges über die Zusammensetzung des Würze-Extraktes.— Allgem. Brauer- und Hopfen-Zeitung. 1891. S. 65.—Z. g. Br. 1891. XIV. 57.
II. Z. g. Br. 1891. XIV. 75.

Burci, E., e Frascani, V.,
I. Contributo allo studio dell' azione battericido della corrente continua.—Atti della Soc. Tosc. di Scienze nat. Mem. Vol. XII. 1891.—K. J. III. 76.

Burdon-Sanderson,
1. The Origin and Distribution of Microzymes (Bacteria) in Water, and the Circumstances which determine their Existence in the Tissues and Liquids of the Living Body.—Quarterly Journal of the Microscopical Society. 1871. XI.

Burgerstein, Alfred,
I. Untersuchungen über das Vorkommen und die Entstehung des Holzstoffes in den Geweben der Pflanzen.—Sitzgs.-Ber. d. Wiener Akademie. 1874. 1. Abt. LXX. 338.—Gives the antecedent literature.

Burkard u. Seifert,
1. Pharmac. Centralhalle. 1395. XXXVI. 365.

Burri, Robert,
1. Über einen milzbrandähnlichen Bacillus aus südamerikanischem Fleischfuttermehl.— Hygienische Rundschau. 1894. IV. No. 8. —C. f. B. 1894. XVI. 374.

Burri, R., Herfeldt, E., und Stutzer, A.,
I. Bakteriologisch-chemische Forschungen über die Ursachen der Stickstoff-Verluste in faulenden organischen Stoffen insbesondere im Stallmist und in der Jauche.—Journal für Landwirtschaft. 1894. XLII. 329.—C. f. B. 2. Abt. 1895. I. 284.

Burri, R., und Stutzer, A.,
I. Über einen interessanten Fall einer Mischkultur.—C. f. B. 1894. XVI. 815.—Ch. C. 1895. I. 96.—K. J. V. 88.
II. Über Nitrat zerstörende Bakterien und den durch dieselben bedingten Stickstoffverlust.—C. f. B. 2. Abt. 1895. I. 257; 1896. II. 473.—Ch. C. 1895. II. 571, 638.—K. J. VI. 285.
III. Über einen auf Nährgelatine gedeihenden nitratbildenden Bacillus.—C. f. B. 2. Abt. 1895. I. 721; 1896. II. 105.—Ch. C. 1896. I. 267, 1011.—K. J. VI. 278.

Burstert, H., und Herz, F. J.,
I. Rote Käse.—Mitteilungen des Milchwirtsch. Vereins im Allgäu. 1895. No. 6.—Chemikerzeitung Repertorium 1895. XIX. 274.

Buscaglioni,
See Fermi und Buscaglioni.

Buscalioni, L.,
I. Il Saccharomyces guttulatus.—Malpighia. 1896. X. 281.—K. J. VII. 29.

Buscalioni, L., e Casagrandi, O.,
I. Sul Saccharomyces guttulatus (Rob.) nuove osservazioni.—Malpighia. 1898. XII. 59.—C. f. B. 2. Abt. 1899. V. 311.

Busse, Otto,
I. Die Hefen als Krankheitserreger. Berlin. 1897.—C. f. B. 1. Abt. 1897. XXII. 349.

Butkewitsch, Wl.,
I. Über das Vorkommen proteolytischer Enzyme in gekeimten Samen und über ihre Wirkung.—Ber. d. D. Bot. Ges. 1900. XVIII. 185.—Ch. C. 1900. II. 386.
II. Jahrb. Wissensch. Bot. 1903. XXXVIII. 147.

Butlerow,
I. Liebig's Ann. 1868. CXLV. 277.

Buttenberg, Paul,
See Abel und Buttenberg.

Buxton, B. H.,
I. American Medicine. 1903. V. 137 Abst. in W. f. Br. 1904. XXI. 722.

Cagniard-Latour, Charles,
I. Sur la fermentation vineuse.—L'Institut. Nos. 158, 159, 164. 165, 166, 167, 185, 199.—Journal für prakt. Chemie. 1836. VIII. 415.
II. Mémoire sur la fermentation vineuse.—C. R. 1837. IV. 905.—More detailed in Annales de Chimie et de Physique. 1838. LXVIII. 206.—Journal für prakt. Chemie. 1839. XVI. 347.

Cahours,
I. Ann. de chim. et de phys. 1839. LXX. 81.

Cahours et Demarçay,
I. C. R. 1875. LXXX. 1568.

Calmette, A.,
I. Contribution à l'étude des ferments d'amidon. La levure chinoise.—Ann. Inst. Past. 1892. VI. 604.—C. f. B. 1. Abt. 1893. XIII. 273.—Ch. C. 1893. II. 86.—K. J. III. 111.
II. Revue Scientif. 1892. Feb. 27.

Cambier, R.,
I. Contribution à l'étude de la fermentation ammoniacale et des ferments de l'urée.—Annales de micrographie. 1893. V. 322.—K. J. IV. 285.

Cambier, R., et Brochet, A.,
I. Sur la production de l'aldéhyde formique gazeux destiné à la désinfection. — C. R. 1894. CXIX. 607.—Ch. C. 1894. II. 857.—K. J. V. 96.

Campbell, George F.,
See Osborne und Campbell.

Camus, L.,
I. Formation de lipase par le Penicillium glaucum.—Comptes rendus de la Société de Biologie. 1893. XXXXIX. 192.—K. J. VIII. 269.
II. De la lipase dans les cultures d'Aspergillus niger.—*Ibid.* p. 230.—K. J. VIII. 269.
III. Comptes rendus de Soc. de Biol. 1897. XLIX. 192.
IV. *Ibid.* p. 230.

CANDOLLE, Augustin Pyramus de,
I. Mémoires sur la famille des Légumineuses. Paris. 1825, p. 22.

CARLES, P.,
I. Über das Umschlagen der Weine. — Biedermann's Centralblatt. 1883. XII. 790; from Répertoire de pharmacie et journal de chimie médicale. 1883. XI. 59.
II. Sur la caracteristique des vins de figue.—C. R. 1891. CXII. 811.

CARO, O.,
I. Della maniera in cui i bacilli del carbonchio si comportano nel latte nelle prime 24 ore.—La Riforma med. 1893. p. 84.—C. f. B. 1893. XIV. 398.—Ch. C. 1894. I. 164

CASAGRANDI, O.,
I. Sulla morfologia dei Blastomiceti.—Naturalista Siciliano. 1897. No. 1 a 3.—Deutsch in C. f. B. 2. Abt. 1897. III. 563.

CASPARI, Theodor,
I. Über zwei- und dreierlei Früchte einiger Schimmelpilze (Hyphomyceten). — Monatsberichte d. Kgl. Akademie d. Wissenschaften. Berlin. 1855. S. 308.—Flora. 1855. XXXVIII. 483.

CATHCART, E., u. M. HAHN,
I. Archiv. f. Hyg. 1902. XLIV. 295.

CATHELINEAU, H., und LEBRASSEUR A.,
I. Über Anwendung der Fluoride in den Gärungsgewerben und über ihre toxische, therapeutische und physiologische Wirkung.—Revue internationale des falsifications, etc. 1895. VIII. 70.—Ch. C. 1895. I. 306.—Gives information relative to the principal works of Effront and others on the action of fluorides.

CATTANI, Giuseppina,
See TIZZONI e CATTANI.

CAZENEUVE, P., et HADDON,
I. Sur les causes de la coloration et de la coagulation du lait par la chaleur.—C. R. 1895. CXX. 1272.—Ch. C. 1895. II. 231.

CECH, C. O.,
I. Über den Ursprung der Hopfenkultur.—Z. g. Br. 1881 IV. 277.

CELLI, A.,
I. Die Kultur der Amöben auf festem Substrate.—C. f. B. I. Abt. 1896. XIX. 536.

CERKEZ, S. C.,
I. Über Braga.—Zeitschrift f. Untersuchung d. Nahrungs- und Genussmittel. 1899. II. 29.—Ch. C. 1899. I. 572.—K. J. X. 171.

ČERNY, Franz,
I. Über die Gärung der Bierwürze bei verschieden grossen Mengen des beigemengten Trubes. — Österr. Brauer- und Hopfenzeitg. 1895. VIII. 277.—Z. g. Br. 1896. XIX. 112.—K. J. VI. 162.
II. Über die Hefengabe und ihren Einfluss auf die Gärung.—Österr. Brauer- und Hopfenzeitg. 1895. VIII. 225.—Z. g. Br. 1896. XIX. 100.—K. J. VI. 141.

CHAMBERLAND, Ch.,
I. Recherches sur l'origine et le développement des organismes microscopiques. Thèse. Paris. 1879.
See also PASTEUR, JOUBERT et CHAMBERLAND.

CHANCEL,
I. C. R. 1853. XXXVIII. 410.

CHAPMAN, C.,
I. The Volatile Bye-products of Fermentation.—Journal of the Federated Institutes of Brewing. 1897. III. 240.—Ch. C. 1898. I. 72.—K. J. VIII. 101.

CHARPENTIER,
I. C. R. 1905. CXLI. 367; 429.

CHARRIN, A., et PHISALIX,
I. Abolition persistante de la fonction chromogène du Bacillus pyocyaneus.—C. R. 1892. CXIV. 1565.—C. f. B. 1893. XIV. 429.—K. J. III. 88.
See also D'ARSONVAL et CHARRIN.

CHESHIRE, Frank R., and CHEYNE, W. Watson,
I. The Pathogenic History and History under Cultivation of a New Bacillus (B. alvei), the Cause of a Disease of the Hive Bee hitherto known as Foul Brood.—Journal of the Royal Microscop. Society. Ser. II. 1885. V. 381.
See also F. C. HARRISON (I.)

Chevallier,
I. Note sur le pain moisi.—Annales d'Hygiene publique et de médecine légale. 1843. XXIX. 39.
Cheyne, W. Watson,
See Cheshire and Cheyne.
Chiaromonte, Tom.,
See Fonseca und Chiaromonte
Chicandard, G.,
I. Sur la fermentation panaire.—C. R. 1883. LXXXXVI. 1585; LXXXXVII. 616.—Ch. C. 1883. S. 439 u. 641.
Chodat und Bach,
I. Ber. d. D. Chem. Ges. 1902. XXXV. 1275.
Christmas,
I. Sur la valeur antiseptique de l'ozone.—Ann. Inst. Past. 1893. VII. 776.—C. f. B. 1894. XV. 1016.—K. J. IV. 110.
Chrzaszcz, T.,
I. Die Chinesische Hefe.—C. f. B. 2. Abt. 1901. VII. 326 u. 913.
II. C. f. B. 2. Abt. 1901. VII. 326.
Chuard, E.,
I. Essai de vinification avec les levures selectionnées.—Extract from the Chronique agricole du canton de Vaud. 1892.—K. J. III. 145.
Chuard, E., und Jaccard, M.,
I. Veränderungen der schwefligen Säure in den Weinen.—Ch. C. 1894. II. 129.
Chudiakow, N. von,
I. Untersuchungen über die alkoholische Gärung.—Landw. Jährbücher. 1894. XXIII. 391.—C. f. B. 2. Abt. 1895. I. 122 u. 188.—Bot. Ztg. 2. Abt. 1894. LII. 257 u. 289.—Ch. C. 1894. II. 291.—K. J. V. 123.
Cienkowski, L.,
I. Die Gallertbildungen des Zuckerrübensaftes. Charkow 1878.—German abstract in Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1878. S. 1017. Gives the plate.
II. Die Pilze der Kahmhaut.—Biological Selections from the Bulletin de l'Académie imp. des sciences de St. Pétersbourg. 1872. VIII. 566.
III. Bull. de l'Acad. imp. de St. Pétersbourg. 1872. XVII. 513.
Claessen, Heinrich,
I. Über einen indigoblauen Farbstoff erzeugenden Bacillus aus Wasser.—C. f. B. 1890. VII. 13.
Claudon, Edouard, et Morin, E. Ch.,
I. Produits de fermentation du sucre par la levure elliptique.—C. R. 1887. CIV. 1109.—C. f. B. 1. Abt. 1887. II. 655.
II. Sur la présence de l'alcohol butylique normal dans une eau-de-vie de Cognac. C. R. 1887. CIV. 1187.
Claussen, N. H.,
I. W. f. Brauerei. 1904. XXI 370.
Clautriau, M.,
I. Etude chimique du Glycogène chez les Champignons et les Levures.—Mémoires couronnés and other Mémoires published by the Acad. Roy. des Sciences, des Lettres et des Beaux-Arts de Belgique. Collection in 8°. 1895-1896. LII. 1.—C. f. B. 2. Abt. 1896. II. 429.—Z. g. Br. 1897. XX. 232 u. 245.—K. J. VI. 51.
Clerfeyt, Charles,
I. Expériences sur l'accoutumance héréditaire des Levures aux solutions salines concentrées.—Bulletin de l'Académie Royale de Belgique. Classe des Sciences. 1901. p. 337.
Cnopf, R. Th.,
I. Quantitative Spaltpilzuntersuchungen in der Kuhmilch.—Tageblatt der 62. Versammlung D. Naturforscher und Ärzte in Heidelberg. 1889. S. 494.—C. f. B. 1889. VI. 553.
Coates, Charles E., and Dodson, W R.,
I. Stickstoffassimilation der Baumwollpflanze.—Journal of the Amer. Chem. Soc. 1896 XVIII. 428.—Ch. C. 1896. II. 45.—K. J. VII. 206.
Cohn, Ferdinand,
I. Untersuchungen über Bakterien. I.—Beiträge zur Biologie der Pflanzen. Bd. I., Heft 2. S 127. Breslau 1870 bis 1875.
II. *Ibid.* II.—*Ibid.* Bd. I. Heft 3. S. 141.
III. *Ibid.* III.—*Ibid.* S. 208.

IV. Versuch eines natürlichen Systems der Kryptogamen.—Hedwigia. 1872. No. 1.
V. Über die Entwicklungsgeschichte mikroskopischer Algen und Pilze.—Nova acta Academiae Carol. Leop. nat. cur. 1853. XXIV. I. 123.
VI. Über Bakterien, die kleinsten lebenden Wesen.—Virchow und Holtzendorff's Sammlung gemeinverst. Vorträge. 7. Serie. Heft 165. Berlin. 1872.
VII. Untersuchungen über Bakterien. IV.—Beiträge zur Biologie der Pflanzen. 1877. Bd. II. Heft 2. S. 249.
VIII. Über thermogene Bakterien. —Ber. d. D. Bot. Ges. 1893. XI. 66.—C. f. B. 1893. XV. 424.—K. J. IV. 81.
IX. Über Wärmeerzeugung durch Schimmelpilze und Bakterien. Vortrag. Breslau. 1890.—K. J. I. 40.
X. Über thermogene Wirkung von Pilzen.—Jahresbericht d. Schlesiseh. Ges. f. vaterländ. Kultur. 1888. S. 150.—C. f. B. 1889. VI. 351.—K. J. I. 40.
XI. Über den Brunnenfaden (Crenothrix polyspora). — Beiträge zur Biologie der Pflanzen. 1870. I. Band. I. Heft. S. 108.
XII. Über zwei neue Beggiatoen.—Hedwigia. 1865. S. 81.
XIII. Jahresb. d. Schles. Ges. f. vaterländsche Kultur. 1883. Breslau. 1884. 226.
XIV. *Ibid.* 1888. 156.
XV. *Ibid.* 1890.
XVI. *Ibid.* 1883. LXI. 226.

Cohn, F., und Mendelsohn, B.,
I. Über Einwirkung des elektrischen Stromes auf die Vermehrung von Bakterien.—Beiträge zur Biologie der Pflanzen. 1879. III. 141.

Cohnheim,
I. Z. f. physiol. Chemie. 1903. XXXIX. 336.

Colin,
I. Ann. d. chim. et de physique. 1824. XXVII. 128; XXX. 42.

Collette, August, und Boidin, August,
I. Verfahren zur Gewinnung von Alkohol aus stärkehaltigem Material unter Benutzung aseptiseher Verzuckerung und Vergärung mittelst Mucedineen.—German Patent No. 99253, Aug. 17, 1897. —Z. f. Spiritusind. 1899. XXI. Ergänzungsheft I. 63.—Ch. C. 1899. I. 155. — K. J. VIII. 262.
II. Zusatz-Patent zu I.—German Patent No. 100129, Jan. 28, 1898. —Z. f. Spiritusind. 1899. XXI. Ergänzungsheft. I. 64.—Ch. C. 1899. I. 576.
III. Engl. Patent No. 13053, June 10, 1898.—K. J. IX. 288.
IV. Engl. Patent No. 19858, Aug. 28, 1897.—K. J. IX. 288.
V. Engl. Patent No. 1155, Jan. 15, 1897.—K. J. IX. 287.

Conn, H. W.,
I. Cream Ripening with Bacillus No. 41.—C. f. B. 2. Abt. 1895. I. 385.
II. The Relation of Pure Cultures to the Acid, Flavour and Aroma of Butter.—C. f. B. 2. Abt. 1896. II. 409.—Ch. C. 1896. II. 636. —K. J. VII. 182.
III. The Isolation of Rennet from Bacteria Cultures.—Fifth Report of the Storrs Agricultural School of the Experiment Station, Middletown, Connecticut. 1892. p. 106.—C. f. B. 1892. XVI. 914.—K. J. III. 259.
IV. Über einen bittere Milch erzeugenden Micrococcus.—C. f. B. 1891. IX. 654.—K. J. II. 185.

Conn, Thom, Bosworth, Stocking, and Issajeff,
I. (Chap. 56) Storrs Agric. Exp. Station, Storrs, Conn. 1905. Bull. No. 35.
II. (Chap. 57) U.S. Dept. of Agriculture. Bull. No. 71. Bureau of Animal Industries, Washington. 1905.

Cordier J. A.,
I. Contribution à la biologie des levures de vin.—C. R. 1898. CXXVII. 628.—C. f. B. 2. Abt. 1899. V. 105.

Cornu, Max,
I. Etudes sur le Phylloxera. Paris. 1878, p. 159.

Cossettini, G.,
I. Bolletino chim. farm. 1901. XL. 75.

Costantin, J.,
I. Recherches sur le Cladosporium herbarum.—Journal de Botanique. 1889.
II. Sur les variations des Alternaria et Cladosporium.—Revue générale de Botanique. 1889.
III. Les mueédinées simples. Paris. 1888. P. Klineksieck. With 190 figures.
IV. Bull. Soc. Bot. de France. 1893. 236.

Costantin et Lucet,
I. Bull. Soc. Mycol. de France. 1903. XIX. 33.
II. Ann. d. Sei. Nat. Bot. 1905. 9th Series. II. 119.

Costantin et Ray,
I. C. R. de la Soc. de Biologie. 1898. 10th Series. V. 504.

Coupin,
I. C. R. 1904. CXXXVIII. 389.

Coupin et Friedel,
I. C. R. 1904. CXXXVIII. 1118.

Cramer, E.,
I. Die Zusammensetzung der Bakterien in ihrer Abhängigkeit vom Nährmaterial. — Habilitationssehrift, Heidelberg.—A. f. Hygiene. 1892. XVI. 151.—C. f. B. 1. Abt. 1893. XIV. 12.—Ch. C. 1893. I. 543.—K. J. IV. 67.
II. Die Zusammensetzung der Cholerabacillen.—A. f. Hygiene. 1895. XXII. 167.—C. f. B. 1. Abt. 1895. XVII. 611.—Ch. C. 1895. I. 850.
III. Die Zusammensetzung der Sporen von Penieillium glaueum und ihre Beziehung zu der Widerstandsfähigkeit derselben gegen äussere Einflüsse.—A. f. Hygiene. 1894. XX. 197.—C. f. B. 2. Abt. 1895. I. 499.—Ch. C. 1894. II. 54.—K. J. V. 86.
IV. Die Ursache der Resistenz der Sporen gegen troekene Hitze.—A. f. Hygiene. 1891. XIII. 71.—C. f. B. 1. Abt. 1892. XI. 451.—Ch. C. 1891. II. 760.—K. J. II. 85.

Cramer, Carl,
I. Quoted by Kabsch (I) from the Vierteljahrssehrift d. naturforseh. Ges. in Zürieh. 1858. III. 1.
II. Vierteljahr. d. naturf. Ges. in Zürich. 1859. 325.

Cremer, Max,
I. Über die Umlagerung der Zuckerarten unter dem Einflusse von Ferment und Zelle.—Zeitschr. f. Biologie. 1894. XXXI. 183.—Ch. C. 1894. II. 245.—K. J. V. 112.
II. Das Verhalten einiger Zuckerarten im tierisehen Organismus.—Zeitsehr. f. Biologie. 1893. XXIX. 484.—Ch. C. 1893. II. 693.
III. Demonstration des Hefeglycogens in den Zellen. — Münchener med. Wochenschrift. 1894. No. 26.—Ch. C. 1894. II. 245.—K. J. V. 113.
IV. Über Hefe- und Leberzelle.—Münchener med. Woehensehrift. 1894.—Ch. C. 1894. II. 245.—K. J. V. 113.
V. Zucker und Zelle.—Zeitschr. f. Biologie. 1895. XXXII. 1.—K. J. VI. 55.
VI. Über Glycogenbildung im Hefepresssaft.—Ber. d. D. Chem. Ges. 1899. XXXII. 2062.—C. f. B. 2. Abt. 1900. VI. 90.—Ch. C. 1899. II. 447.
VII. Sitz. d. Ges. f. Morphologie u. Physiologie München. 1894. Hft. 1.

Crochetelle, J.,
See Dumont et Crochetelle.

Cross, C. F., and Bevan, E. J.,
I. Die Kohlenhydrate der Gerste.—Journal of the Federated Institutes of Brewing. 1897. III. 2.—Ch. C. 1897. II. 1028.

Cross, C. F., Bevan, E. J., and Smith, Claud,
I. Über einige chemische Vorgänge in der Gerstenpflanze.—Ber. d. D. Chem. Ges. 1895. XXVIII. 2604.—Ch. C. 1895. II. 1129.

Crouzel,
I. Sehwefelwasserstoff bildende Hefe.—L'union pharmac. 1891. T. 32. 1892. T. 33, p. 60.—C. f. B. 1892. XI. 800.—K. J. II. 135; III. 119.
II. Journ. de pharm. et chimie. 1891. (5.) XXIII. 309.

Cuboni, G.,
I. Über die dureh Botrytis einerea bedingte Fäulnis der Rebentriebe.—Bollettino di Notizie agrarie. 1896. II. 487.—C. f. B. 2. Abt. 1897. III. 330.

II. Sulla probabile origine dei Saccaromiceti.—Rivista di viticoltura ed enologia italiana. 1885. IX. No. 12-13.—Just's Botan. Jahresbericht pro 1885. I. 277.

Cugini, G.,
I. Sulla alimentazione delle piante cellulari.—Nuovo giornale botan. Ital. 1876. VIII. 77, 261.—Just's Bot. Jahr. pro 1876. S. 113.

Cuisinier, Léon,
I. Une nouvelle matière sucrée diastasique la Céréalose et pour sa fabrication. French Patent No. 171958.—La sucrerie indigène et coloniale. 1886. XXVII. 241.—C. f. B. 2. Abt. 1895. I. 329.—Ch. C. 1887. S. 614.

Curtis,
I. Contribution à l'étude de la Saccharomycose humaine.—Ann. Inst. Past. 1896. X. 449.

Czapek, Friedrich,
I. Über Orseillegärung.—C. f. B. 2. Abt. 1898. IV. 49.—Ch. C. 1898. I. 684.
II. Zur Biologie der holzbewohnenden Pilze.—Ber. d. D. Bot. Ges. 1899. XVI. 166.—C. f. B. 2. Abt. 1899. V. 872.
III. Beitr. z. Chem. Phys. u. Path. 1902. I. 538; II. 557; III. 62.—Ber. d. D. Bot. Ges. 1902. XIX. 130.
IV. Biochem. d. Pflanz. Jena, 1905.
V. Ber. d. D. Bot. Ges. 1899. XVII. 166.

Czaplewski, E.,
I. Zur Anlage bakteriologischer Museen.—C. f. B. 1889. VI. 409.

Czéh, Andreas, u. Müller-Thurgau, H.,
I. Welche Vorgänge finden während der Gärung und der Weiterentwicklung des Weines statt?—Weinbau und Weinhandel. 1888. VI. 121.

Dachnjewski,
I. Vergleichende Wertprüfung der Filter von Chamberland und von Berkefeld.—Wratsch. 1893. No. 13.—C. f. B. 1894. XVI. 664.—K. J. V. 19.

Dahlen, H. W.,
I. Neuere Beobachtungen über den sogen. Edelfäulepilz (Botrytis cinerea).—Weinbau und Weinhandel. 1894. XII. 306.

Dahmen, Max,
I. Die bakteriologische Wasseruntersuchung. — Chemikerzeitung. 1892. XVI. 861.—C. f. B. 1892. XII. 302, 620.

Dangeard, P. A.
I. Observations sur le groupe des bactéries vertes.—Annales de micrographie. 1895. V. 67.
II. Sur la structure histologique des levures et leur développement.—C. R. 1893. CXVII. 68.—K. J. IV. 51.
III. C. R. 1908. CXXXVII. 1281.

Danilewsky, A., u. Radenhausen, P.
I. Zur chemischen Konstitution der Milch.—Schweiz. Zeitschrift f. Pharmacie. 1880. No. 22.—Ch. C. 1880. S. 519.

Le Dantec, Felix,
I. Etude de la morue rouge.—Ann. Inst. Past. 1891. V. 636.—C. f. B. 1892. XI. 198.—K. J. II. 234.

Darexy, Prosper,
I. Recherches sur la matière grasse de la levure de bière.—Toulouse. 1896. Marquès & Co. 47 pp.
See also Gérard et Darexy.

Dastre,
I. C. R. 1883. XCVI. 932.

Davaine,
I. Recherches sur les Vibrioniens.—C. R. 1864. LIX. 393, 629.

Dean,
I. Botan. Gazette. 1903. XXXV. Jan.

Decaisne, E.,
I. Sur l'oïdium aurantiacum du pain.—C. R. 1871. LXXIII. 507.
II. Sur l'oïdium aurantiacum du pain au point de vue pathologique.—C. R. 1871. LXXIII. 684.

Dehérain, P. P.,
I. Sur la fabrication du fumier de ferme. — C. R. 1884. LXXXVIII. 377; LXXXIX. 45.—Ch. C. 1884. S. 262 u. 709.
II. Über die Buttersäuregärung in den Diffusionsgefässen der Zuckerfabriken.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie. 1884. XXI. 269.—Ch. C. 1884. S. 403.

III. Sur la composition des eaux de drainage d'hiver des terres nues et amblavées.—C. R. 1893. CXVII. 1041.—K. J. IV. 235.
IV. Le travail de la terre et la nitrification. — C. R. 1893. CXVI. 1091.—K. J. IV. 234.

Delacroix, E.,
I. Darstellung von Milchsäure aus Milchserum. — Journal de Pharmacie et de Chimie. 1891. (5.) XXIII. 287.—Ch. C. 1891. I. 698.—K. J. II. 178.
II. Bull. Soc. Mycol. de France. 1891. VII. 107.

Delbrück, Max,
I. Grundlagen für ein Preisausschreiben zur Lösung der Schaumgärungs-Frage.—Z. f. Spiritusind. 1892 u. 1893.—K. J. IV. 155, 157.
II. Fortschritte im Arbeiten mit Reinhefe.—Z. f. Spiritusind. 1895. XVIII. Ergänzungsheft. S. 25.—K. J. VI. 178.
III. Das Pilzmaischverfahren.—Z. f. Spiritusind. 1899. XXII. Ergänzungsheft. II. 52.
IV. Die Bewegung des Stickstoffs in der gärenden Maische als Massstab der Gärungsführung.—Z. f. Spiritusind. 1879. II. 310, 351.
V. Über die physiologische Methode der Eiweissbestimmung für Würze und Bier und ihre praktische Bedeutung.—W. f. Br. 1893. X. 810.—Ch. C. 1894. I. 846.—K. J. IV. 139.
VI. Der Einfluss der Lüftung auf Hefe und Gärung und ihre Benutzung zur Vermehrung der Hefeausbeute in der Presshefefabrikation und zur Vergärung der Dickmaischen.—Z. f. Spiritusind. 1890. XIII. Ergänzungsheft. S. 31.—K. J. I. 59.
VII. Alkoholische Gärung ohne Hefezellen.—W. f. Br. 1897. XIV. 363.—Ch. C. 1898. I, 70. —K. J. VIII. 307.
VIII. Die Carlsberger reingezüchtete Hefe.—W. f. Br. 1885. II. 126.
IX. W. f. Br. 1890. VII. 636.
X. *Ibid.* 1898. XV. 650.
XI. *Ibid.* 1902. XIX. 25.
XII. *Ibid.* 1903. XX. 66.
XIII. Chem. Zeitg. 1899. XXIII. 176.
See also Hayduck u. Delbrück.

Delffs,
I. Poggendorff. Annalen. 1851. LXXIV. 505.

Demme, R.,
I. C. f. Bakt. 1890. IX. 271.

Denayer, A.,
I. Engl. Patent No. 13032 of 1898.

Denys, J., et Martin, J.,
I. Sur les rapports du Pneumobacille de Friedländer, du ferment lactique et de quelques autres organismes avec le Bacillus aërogenes et le Bacillus typhosus.—La Cellule. 1893. IX. 261.—C. f. B. 1894. XVI. 127.

Deschamps,
I. Über das Lab.—Journal de Pharmacie. 1840, p. 412.—Dingler's polyt. Journal. 1840. LXXVIII. 445.

Desmazières,
I. Ann. d. Sci. Nat. Bot. 1834. 2nd Series. II. 71.

Destrem, A.,
See Schützenberger et Destrem.

Diakonow, N. W.,
I. La respiration intramoléculaire et la fermentation des champignons moisissures. — Archives slaves de Biologie. 1886. I. 531. —Ber. d. D. Bot. Ges. 1886. IV. 2.—Z. g. Br. 1886. IX. 153
II. Sur le rôle de la substance nutritive fermentescible dans la vie de la cellule végétale.—Archives slaves de Biologie. 1887. IV. 31.—Ber. d. D. Bot. Ges. 1887. V. 380.—Z. g. Br. 1888. XI. 460.

Dickinson, W. F.,
See Lea and Dickinson.

Dierkx,
I. Ann. Soc. Scient. de Bruxelles. 1901. XXV.

Dieudonne, A.,
I. Beiträge zur Kenntnis der Anpassungsfähigkeit der Bakterien an ursprünglich ungünstige Temperaturverhältnisse. — Arbeiten aus dem Kaiserl. Gesundheitsamte. 1894. IX. 492.—C. f. B. 1894. XVI. 965.—K. J. V. 86.

II. Eine einfache Vorrichtung zur Erzeugung von strömenden Formaldehyd-Dämpfen für Desinfektionszwecke.—Arbeiten aus d. K. Gesundheitsamte. 1895. XI. 534.—Ch. C. 1895. II. 607.—K. J. VI. 15.

Dodson, W. R.,
See Coates and Dodson.

Domens, A.,
I. Keimgehalt ausschankreifer Flaschenbiere.—Allgem. Brauer- u. Hopfenzeitung. 1892. S. 2037.—Ch. C. 1893. II. 303.—K. J. IV. 141.

Dönitz, W.,
I. Über das Verhalten der Cholera-vibrionen im Hühnerei.—Z. f. Hygiene. 1895. XX. 31.—Ch. C. 1895. II. 172.

Doepping, O.,
See Schlossberger und Doepping.

Dokkum, L.,
I. Über giftige Bestandteile von faulendem Käse.—Ch. C. 1894. II. 485.

Donath, E.,
I. Z. f. Naturwissenschaften. 1894. LXVII. 179.
II. Chem. Ztg. 1891. XV. 597.
III. Ber. d. D. Chem. Ges. 1875. VIII. 795.
IV. Chem. Ztg. 1892. XVI. 459.

Dormeyer, Carl,
I. Die rationelle Verwertung der Bierhefe.—W. f. Br. 1899. XVI. 557.—C. f. B. 2. Abt. 1900. VI. 375.—K. J. X. 162.
II. Verfahren zur Gewinnung der Eiweissstoffe aus Hefe. German Patent No. 111915, Apr. 18, 1899. —Ch. C. 1900. II. 607.

Dossios,
I. Jahresb. Chemie. 1866. 384.

Downes,
I. On the Action of Sunlight on Micro-organisms, with Demonstration of the Influence of Diffused Light.—Proceedings of the R. Society. 1886. XL. 14.

Downes and Blunt,
I. Researches on the Effect of Light upon Bacteria and other Organisms.—Proceedings of the R. Society. 1877. XXVI. 488.
II. On the Influence of Light on Protoplasma.—Proceedings, etc. 1878. XXVIII. 197, 199.

Dräer, Arthur,
See Abel und Dräer.

Dreyfuss, Isidor,
I. Über das Vorkommen von Cellulose in Bacillen, Schimmel- und anderen Pilzen.—Z. f. physiolog. Chemie. 1893. XVIII. 358.—C. f. B. 1894. XV. 909.—Ch. C. 1893. II. 941.—K. J. IV. 73.

Drouin, R.,
See Gautier et Drouin.

Dubois, Raphael,
I. Extinction de la luminosité du Photobacterium sarcophilum par la lumière.—Comptes rendus de la Société de Biologie. 1893. II. 160.—K. J. IV. 128.
II. Sur le prétendu pouvoir digestif du liquide de l'urne des Népenthes.—C. R. 1890. CXI. 315.—K. J. I. 173.
III. C. R. 1890. CXI. 655.

Dubourg, E.,
I. De la fermentation des saccharides.—C. R. 1899. CXXVII. [?CXXVIII.] 440.—C. f. B. 2. Abt. 1899. V. 657.—Ch. C. 1899. I. 701.—K. J. X. 298.
II. Contribution à l'étude des levures de vin.—Revue de Viticulture. 1897, p. 467.—K. J. VIII. 100.
See also Gayon et Dubourg.

Dubrunfaut,
I. Sur la distillation des betteraves et la fermentation dite nitreuse. —C. R. 1868. LXVI. 275.—Ch. C. 1868. 941.
II. Mémoire sur la saccharification des fécules. Paris. 1882.
III. Sur la fermentation et le ferment alcooliques.—C. R. 1871. LXXIII. 263.
IV. C. R. 1856. XLII. 945.
V. *Ibid.* 1846. XXIII. 38. 1856. XLII. 902.

Duclaux, Emile Pierre,
I. Sur l'action antiseptique de l'acide formique.—Ann. Inst. Past. 1892. VI. 593.—C. f. B. 1893. XIII. 75.—K. J. III. 74.
II. Sur les analogies entre les procès de fermentation et de combustion solaire.—Ann. Inst. Past. 1893. VII. 751.—C. f. B. 1894. XVI. 119.—K. J. IV. 85.
III. Action de l'Electricité sur les Microbes. — Ann. Inst. Past. 1890. IV. 677.

IV. Influence de la lumière du Soleil sur la vitalité des germes de microbes.—C. R. 1885. C. 119; CI. 395.—Ch. C. 1885. 166, 794.
V. Die Selbstreinigung der Flüsse. —Ann. Inst. Past. 1894. VIII. 117, 178.—Ch. C. 1894. I. 783, 1007.
VI. Sur le rôle protecteur des microbes dans la crème et les fromages. — Ann. Inst. Past. 1893. VII. 304. — K. J. IV. 208.
VII. Le lait. Etudes chimiques et microbiologiques. Paris. 1887. Ballière et fils.
VIII. Action de la présure sur le lait.—C. R. 1884. LXXXXVIII. 526.—Ch. C. 1884. 343.
IX. Deuxième Mémoire sur le lait. —Annales de l'Institut national agronomique. 1883. VIII.
X. Traité de chimie biologique. Paris. 1883, p. 613.
XI. Nutrition sans bactéries.—Ann. Inst. Past. 1895. IX. 896.—Ch. C. 1896. II. 502.
XII. Sur le vieillissement des vins. —Ann. Inst. Past. 1893. VII. 537.—K. J. IV. 141.
XIII. Fabrication, maturation et maladies du fromage du Cantal. —Annales de l'Institut national agronomique. 1879. No. 5, p. 23.—Milchzeitung. 1879. 724, 740.
XIV. Fermentation du sucre de lait.—Ann. Inst. Past. 1887. I. 573.—C. f. B. I. Abt. 1888. III. 525.
XV. Traité de microbiologie. Tome I. et II. 1899. Tome III. 1900. —C. f. B. 2. Abt. 1899. V. 773. 1900. VI. 255.
XVI. Sur la fermentation alcoolique.—C. R. 1864. LVIII. 1114; LIX. 450.
XVII. Sur l'absorption d'ammoniaque et la production d'acides gras volatiles pendant la fermentation alcoolique.—Annales de l'école normale supérieure. 1866. II.
XVIII. Pouvoir ferment et activité d'une levure.—Ann. Inst. Past. 1896. X. 119.—C. f. B. 2. Abt. 1896. II. 556.—Ch. C. 1896. I. 1169.—K. J. VII. 87.
XIX. Sur la conservation des levures.—Ann. Inst. Past. 1889. III. 375.—C. f. B. I. Abt. 1889. VI. 412.
XX. Ann. Pasteur. 1892. 341.
XXI. Chimie biologique. 1883. 142, 193, 219.
XXII. Ann. Pasteur. 1889. III. 97.
XXIII. Ann. Pasteur. 1887. I. 573. 1889. III. 201.
XXIV. Traité de Microbiologie. Paris. 1898.
XXV. Journ. de la Brasserie. 1900. —Abst. in Chem. Centralbl. 1900. II. 54.
XXVI. Ann. Pasteur. 1897. XI. 348.
XXVII. Thèse. 1865.
XXVIII. C. R. 1874. LXXVIII. 1160.
XXIX. Traité de Microbiologie. II. 222.
XXX. Ann. Pasteur. 1889. III. 67.
XXXI. Thèse. 1865. 44.

Dujardin, Felix,
I. Histoire naturelle des Zoophyte Paris. 1900.

Dujardin, J.,
I. Recherches rétrospectives sur l'art de la distillation. Historique de l'alcool, de l'alambic et de l'alcoométrie. Paris. 1900.

Düll, G.,
I. Zur Kenntnis des Bierextraktes. —Chemikerzeitung. 1892. XVI. 1178.—Ch. C. 1892. II. 764.
II. Chem. Beitg. 1895. XIX. 216.
III. Z. f. d. Ges. Brauwesen. 1894. XVII. 79.
See also Lintner und Düll.

Dumas, Jean Baptiste,
I. Traité de chimie appliquée aux arts. Paris. 1828–45.
II. Sur la conversion de l'ammoniaque en acide nitrique.—C. R. 1846. XX. 1020.
III. Recherches sur la fermentation alcoolique. 1872.
IV. Traité de chimie appliquée aux arts. VI. 316.
V. C. R. 1872. LXXV. 277.
VI. Ann. de chimie et de phys. 1874. 5th Series. III. 57.
VII. C. R. 1872. LXXV. 295.

Dumas et Boullay,
I. Ann. de chimie et de phys. 1828. (2.) XXXVII. 45.

Dumont, J., et Crochetelle, J.,
I. Sur la nitrification des terres de prairie. C. R. 1893. CXVII. 670.—Ch. C. 1894. I. 97.—K. J. IV. 233.
II. Influence des sels de potassium sur la nitrification.—C. R. 1894. CXVIII. 604.—C. f. B. 2. Abt. 1895. I. 508.
III. De l'influence des chlorures sur la nitrification.—C. R. 1894. CXIX. 92.—Ch. C. 1894. II. 492.—K. J. V. 263.

Duncker, J.,
I. Die physikalische Prüfung der Desinfektion mit Wasserdampf. — Deutsche Medicinalzeitung. 1892. No. 85–91.—K. J. III. 27.

Dünnenberger, Karl,
I. Bakteriologisch-chemische Untersuchung über die beim Aufgehen des Brotteiges wirkenden Ursachen.—Botan. Centralblatt. 1888. XXXIII. 245.—Archiv d. Pharmacie. 1888. XXVI. 544. —Ch. C. 1888. 667.

Dupetit, G.,
See Gayon et Dupetit.

Durin, E.,
I. De la fermentation cellulosique du suere de canne.—C. R. 1876. LXXXIII. 128.—Zeitschrift des Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1876. 752.
II. Bull. de l'assoc. des Chimistes. 1887. VI. 239.
III. *Ibid.* 1890. VIII. 296.

Durst, Otto,
I. Handbuch der Presshefe-Fabrikation. 2. Ed. Berlin. 1896. P. Parey. 16*s.* (bd.).

Dusch, Th. von,
See Schröder u. Dusch.

Duval, J.,
I. Nouveaux faits concernant la mutabilité des germes microscopiques.—Journal de l'anatomie et de physiologie. 1874, 489.

Ebstein, W.,
I. Einfluss der Kohlensäure auf die diastatischen Fermente.—Naturwissenschaftliche Rundschau. 1889. IV. 557.—Ch. C. 1889. II. 1028.

Eck,
I. Neue Zeitschr. f. deutsche Spiritusfabrikanten. 1877. XI. 149.

Eckenroth, Hugo, und Heimann, R.,
I. Über Hefe und Schimmelpilze an den Trauben.—C. f. B. 2. Abt. 1895. I. 530.—Ch. C. 1895. II. 875.—K. J. VI. 39.

Effront, Jean,
I. Action des acides minéraux dans la saccharification par le malt et la fermentation des matières amylacées. — Moniteur scientifique. 1890. IV. 449.—K. J. I. 72.
II. Etudes sur les levures.—Moniteur scientifique. 1891. V. 1137. —K. J. II. 156.
III. De l'influence des fluorures sur l'accroissement et le développement des cellules des levures alcooliques. — Moniteur scientifique. 1891. V. 254.—K. J. II. 154.
IV. Sur certaines conditions chimiques de l'action des levures de bière.—C. R. 1893. CXVII. 559.—K. J. IV. 164.
V. Nouvelle méthode pour la purification des levures.—Bulletin de la Société chimique de Paris. 1891. (3ème série.) V. 705.—Moniteur scientifique. 1891. V. 1137.—K. J. II. 156.
VI. Les Enzymes. Paris. Carré et Naud. I. Bd. 1899. 7*s.* 6*d.* German translation by M. Bücheler. Wien. 1899. 7*s.*—C. f. B. 2. Abt. 1900. VI. 176, 231.
VII. Studien über die Vergärbarkeit der Melasse.—Moniteur scientifique. 4e série. 1894. VIII. 161.—Ch. C. 1894. I. 786.—K. J. V. 155.
VIII. Action de l'oxygène sur la levure de bière.—C. R. 1898. CXXVII. 326.—Ch. C. 1898. II. 727.—K. J. IX. 79.
IX. Verfahren zur Gewöhnung von Hefe an die Dextringärung. German Patent No. 102631, April 3, 1898.—Engl. Patent No. 9615, April 26, 1898.—Z. f. Spiritusind. 1899. XXII. 126. Ch. C. 1899. II. 500.—K. J. X. 110.
X. C. R. 1892. XCV. 1324.
XI. *Ibid.* 1894. CXIX. 92.

XII. *Ibid.* 1894. CXIX. 169.
XIII. *Ibid.* 1897. CXXV. 38, 116.
XIV. Bull. de l'Ass. des Chimistes de Sucrerie et Distillerie. 1905. XXIII. 393.
XV. Z. f. Spiritusindustrie. 1898. XXI. 298. 1899. XXII. 126.

Ehlers, T.,
I. Untersuchungen über den Rauschbrandpilz. Dissert. Rostock. 1884.

Ehrenberg, Alexander,
I. Experimentelle Untersuchungen über die Frage nach dem Freiwerden von gasförmigem Stickstoff bei Fäulnisprozessen.—Zeitschrift f. physiolog. Chemie. 1886. XI. 145, 438.—Ch. C. 1887. 15, 864.

Ehrenberg, Christian Gottfried,
I. Die Infusionstierchen als vollkommene Organismen. Leipzig. 1838.

Ehrich, E.,
I. Über die Darstellung schaumhaltiger und vollmundiger Biere.—Der Bierbrauer. 1897. 65.—K. J. VIII. 126.

Ehrlich,
I. Zeits. d. Vereins d. D. Zuckerindustrie. 1905. LV. 539.

Eichelbaum, Georg,
I. German Patent No. 116127, Dec. 15, 1899.—Ch. C. 1901. I. 80.

Eichengrün, A.,
I. Die neuen Arzneimittel im ersten Halbjahr 1899.—Zeitschrift f. angew. Chemie. 1899. 1147.—Ch. C. 1900. I. 144.

Eidam, Eduard,
I. Die Einwirkung verschiedener Temperaturen und des Eintrocknens auf die Entwicklung von Bacterium Termo.—Cohn's Beiträge zur Biologie der Pflanzen. 1875. I. 3. Heft. 208. 1883. III. 128.
II. Über die Entwicklung von Sphaerotilus natans.—Jahresber. d. schlesischen Ges. f. vaterländ. Kultur. 1876.
III. Zur Kenntnis der Entwicklung. bei den Ascomyceten.—Cohn's Beiträge zur Biologie der Pflanzen. 1883. III. 377.

Eijkman, C.,
I. Lichtgevende Bakterien.—Jaarverslag van het Labor. voor pathol. Anat. en Bact. te Weltevreden. 1891.—C. f. B. 1. Abt. 1892. XII. 656.—K. J. III. 71.
II. Mikrobiologisches über die Arrakfabrikation in Batavia.—C. f. B. 1. Abt. 1894. XVI. 97.—Ch. C. 1894. II. 614.—K. J. V. 149.

Eisenberg, James,
I. Bakteriologische Diagnostik. 3rd Ed. 1891. Hamburg. Voss.

Eisenschitz, Siddy,
I. Beiträge zur Morphologie der Sprosspilze. Berner Dissert. Wien. 1895.—Z. g. Br. 1895. XVIII. 379.
II. Über die Granulierung der Hefezellen.—C. f. B. 2. Abt. 1895. I. 674.

Eitner, W.,
I. Der Gerber. 1898. XXIV. 4.

Ekenstein, van,
I. C. R. 1897. CXXV. 719.
II. Recueil des travaux chim. des Pays-Bas. 1896. XV. 221.

Elfving, Fredrik,
I. Studien über die Einwirkung des Lichtes auf die Pilze.—Helsingfors. 1890.
II. Über Saccharomyces glutinis (Fres.) Cohn.—Öfversigt af Finska Vet. Soc. Förhandl. 1886. XXVIII.

Elion, H.,
I. Studien über Hefe.—C. f. B. 1893. XIV. 53.—K. J. IV. 142.
II. Die Bestimmung von Maltose, Dextrose und Dextrin in Bierwürze und Bier mittelst Reinkulturen von Gärungsorganismen—C. f. B. 1891. IX. 525.—Ch. C. 1891. II. 281.—K. J. II. 141.
III. Züchtung von Ascosporen auf Tonwürfeln.—C. f. B. 1. Abt. 1893. XIII. 749.—K. J. IV. 39.
IV. Reinhefe in der Brauerei.—Z. g. Br. 1888. XI. 33.—C. f. B. 1. Abt. 1892. XI. 192.—K. J. II. 149.
V. Zeitschr. f. angew. Chemie. 1890. 321, 322.

Elliesen, Max,
I. Einfluss des Vegetationszustandes verschiedener Hefen auf ihr Vermehrungs und Gärvermögen.—C. f. B. 2. Abt. 1901. VII. 497.

Elsner, Fritz,
I. Untersuchungen von Lebensmitteln und Gebrauchsgegenständen. Berlin. 1878.—Zeitschrift gegen Verfälschung der Lebensmittel. 1878. No. 3.—Z. g. Br. 1878. 217, 219.
Elwart,
I. Bull. de l'assoc. des Chimistes. 1901. XX. 562.
Emmerich, Rudolf,
I. Untersuchungen über die Pilze der Cholera asiatica.—A. f. Hygiene. 1885. III. 291.
Emmerling, O.,
I. Zur Frage, wodurch die Giftigkeit arsenhaltiger Tapeten bewirkt wird.—Ber. d. D. Chem. Ges. 1896. XXIX. 2728. 1897. XXX. 1026.—Ch. C. 1897. I. 119, 1218.—K. J. VII. 231.
II. Über armenisches Mazun.—C. f. B. 2. Abt. 1898. IV. 418.—Ch. C. 1898. II. 441.
III. Zur Kenntnis des Sorbosebakteriums.—Ber. d. D. Chem. Ges. 1899. XXXII. 541.—C. f. B. 2. Abt. 1899. V. 657.—Ch. C. 1899. I. 853.
IV. Über Schimmelpilzgärung.—Ber. d. D. Chem. Ges. 1897. XXX. 454.—Ch. C. 1897. I. 767.—C. f. B. 2. Abt. 1897. III. 322.—K. J. VIII. 107.
V. Ber. d. D. Chem. Ges. 1902. XXXV. 2289.
VI. C. f. B. 2. Abt. 1903. X. 273.
VII. Ber. d. D. Chem. Ges. 1902. XXXV. 694.
VIII. *Ibid.* 1899. XXXII. 544.
IX. *Ibid.* 1896. XXIX. 2726.
X. *Ibid.* 1897. XXX. 451.
XI. Z. f. Spiritusindustrie. 1904. XXVII. 477.
XII. Ber. d. D. Chem. Ges. 1901. XXXIV. 3810.
XIII. *Ibid.* XXXIV. 600.
Engel,
I. Les ferments alcooliques. Thèse. Paris. 1872.
II. *Ibid.* Thèse. Paris. 1872. 52.
Engelke,
I. Hedwigia. 1902. XLI. Dec.
Engelmann, Th. W.,
I. Bacterium photometricum. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes.—Pflüger's Archiv. 1883. XXX. 95. With plate.
II. Neue Methode zur Untersuchung der Sauerstoff - Ausscheidung pflanzlicher und tierischer Organismen. — Bot. Ztg. 1881. XXXIX. 441. — Ch. C. 1881. 561.
III. Über Sauerstoff-Ausscheidung von Pflanzenzellen im Mikrospectrum.—Bot. Ztg. 1882. XL. 419, 321.
IV. Die Erscheinungsweise der Sauerstoff-Ausscheidung chromophyllhaltiger Zellen im Licht bei Anwendung der Bakterienmethode. With plate. Pflüger's Archiv. 1894. LVII. 375.—Ch. C. 1894. II. 481.—K. J. V. 27.
V. Die Purpurbakterien und ihre Beziehungen zum Licht.—Bot. Ztg. 1888. XXXXVI. 661.—C. f. B. 1889. V. 569.—Ch. C. 1889. I. 80.
VI. Über Bacteriopurpurin und seine physiologische Bedeutung. —Pflüger's Archiv. 1888. XXXXII. 183.—Ch. C. 1888. 581.
VII. Zur Biologie der Schizomyceten.—Bot. Ztg. 1882. XL. 321.
Engler, Ad.,
I. Über die Pilzvegetation des weissen oder toten Grundes der Kieler Bucht.—Bericht d. Kommission z. Erforschung der deutschen Meere. 1881.—Ber. d. Botan. Ver. d. Prov. Brandenburg. 1882. 19.
Engler, A., u. Prantl, K.,
I. Die natürl. Pflanzenfamilien. 1900. Lief. 196 and 197, 454.
Epstein,
I. Arch. f. Hygiene. 1902. XLV. 354.
Erdmann, O.,
I. Bildung von Anilinfarben aus Proteinkörpern.—Journal f. prakt. Chemie. 1866. LXXXXIX. 385.
Eriksson, Jakob,
I. Studier öfver leguminosernas rotknölar.—Acta Univ. Lund. 1873. X.; bez. Akademisk Afhandlingar. Lund. 1874.
Erlenmeyer u. Planta, A. v.,
I. Biedermann's Centralbl. 1875. VII. 25.

Ermann,
I. Beitrag z. Kenntnis der Fettwachsbildung. — Vierteljahrsschrift für gerichtl. Medicin. 1882.
Ermengem, E. van,
I. Sterilisation der Wässer durch Ozon.—Ann. Inst. Past. 1895. IX. 673.—Ch. C. 1895. II. 999.
Ermengem, E. van, und Sugg,
I. Über die desinfizierende Wirkung des Formalins.—C. f. B. 1. Abt. 1896. XIX. 91.—Ch. C. 1896. I. 934.—K. J. VII. 72.
Ernst, Paul,
I. Über den Bacillus xerosis und seine Sporenbildung.—Z. f. Hygiene. 1888. IV. 25.—C. f. B. 1888. II. 47.
II. Über Kern- und Sporenbildung in Bakterien.—Z. f. Hygiene. 1889. V. 428.—C. f. B. 1889. V. 796.
III. Über einen neuen Bacillus des blauen Eiters.—Z. f. Hygiene. 1887. II. 369.—C. f. B. 1887. II. 276.—Ch. C. 1887. 1168.
Errera, Léo,
I. Expériences relatives à l'action des rayons X sur un Phycomycees. —C. R. 1896. CXXII. 787.
II. L'épiplasme des Ascomycètes et le glycogène des végétaux.—Thèse d'agrégation. Bruxelles. 1882.
III. Sur le glycogène chez les Mucorinées.—Bulletin de l'Acad. Roy. de Belg. 1882. 3e sér. IV. 451.
IV. Sur le glycogène chez les Basidiomycètes. — Mémoires de l'Ac. Roy. de Belgique; Collection in 8°. 1885. XXXVII.—Bot. Ztg. 1886. XXXXIV. 200, 316.
V. Sur l'existence du glycogène dans la levure de bière.—C. R. 1885. CI. 253.—Z. g. Br. 1885. VIII. 333. – Ch. C. 1885. 685.
VI. Les réserves hydrocarbonnées des Champignons.—C. R. 1885. CI. 391.—Z. g. Br. 1885. VIII. 369.
VII. Anhäufung und Verbrauch von Glycogen bei Pilzen.—Ber. d. D. Bot. Ges. 1887. V. 500. —Z. g. Br. 1888. XI. 37.
VIII. Die grosse Wachstumsperiode bei den Fruchtträgern von Phycomyces. — Bot. Ztg. 1884. XXXXII. 497.
IX. On the Cause of Physiological Action at a Distance.—Annals of Botany. 1892. VI. No. 24.
Erxleben,
I. Die Güte und Stärke des Bieres. Prag. 1818. 69.
Esaulow, Nikolai,
I. Über Kefir, eine bakteriolog. u. chem. Untersuchung. Dissert. Moskau. 1895. In Russian.—Pharmac. Zeitschrift f. Russland. 1895. XXXIV. 232.—Ch. C. 1895. I. 1072.—K. J. VI. 222.
Eschenhagen, Franz,
I. Über den Einfluss von Lösungen verschiedener Konzentration auf das Wachstum von Schimmelpilzen. Ein Beitrag zur Kenntnis der Rolle, welche der Turgor in niederen Organismen spielt.—Leipziger Dissert. 1889. Stolp. —Ch. C. 1890. II. 250.
Escherich, Theodor,
I. Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung. Stuttgart. 1886.—Fortschritte der Medicin. 1885. No. 16 and 17.—Baumgarten's Jahresbericht. 1885. I. 169.
Escombe, F.,
I. Beitrag zur Chemie der Membranen der Flechten und Pilze.—Z. f. physiolog. Chemie. 1896. XXII. 288.—C. f. B. 2. Abt. 1897. III. 195.—Ch. C. 1897. I. 89.
Esmarch, Erwin von,
I. Über die Reinkultur eines Spirillum.—C. f. B. 1887. I. 225.
II. Der Henneberg'sche Desinfektor.—Z. f. Hygiene. 1887. II. 342.—C. f. B. 1887. II. 234.
III. Über eine Modifikation des Koch'schen Plattenverfahrens zur Isolierung und zum quantitativen Nachweis von Mikro-organismen.—Z. f. Hygiene. 1886. I. 293.—Ch. C. 1886. 699.
Etard, A., et Olivier, L.,
I. De la réduction des sulfates par les êtres vivants.—C. R. 1882. XCV. 846.
Eugling, Wilhelm,
I. Studien über das Kasein der Kuhmilch und über die Labfermentwirkung — D. Landw. Versuchsstationen. 1885. XXXI. 391.

Euler, H.,
I. Z. f. physiolog. Chemie. 1905. XLIV. 53.

Evans,
I. Die Beseitigung der Salpetersäure im Brauwasser und deren Einfluss auf die Hefe.—Journal of the Federated Institutes of Brewing. 1896. II. 195.—W. f. Br. 1896. XIII. 521.—Ch. C. 1896. II. 218.—K. J. VII. 92.

Ewart, J. Cossar,
I. The Life-History of Bacterium termo.—Proceedings of the Royal Society of London. 1878, p. 474.

Fabian, A.,
See Nencki und Fabian.

Fabrizio ab Aquapendente, Geronimo,
I. De visione, voce et auditu. Venetia. 1600.

Faget,
I. Liebig's Ann. 1853. LXXXVIII. 325.
II. *Ibid.* 1862. CXXIV. 355.

Falke, Friedrich,
I. Die Braunheu-Bereitung.—Arbeiten der D. Landwirtschafts-Gesellschaft. 1895. Heft 9.

Famintzin, A.,
I. Eine neue Bakterienform, Nevskia ramosa.—Bulletin de l'Acad. de St. Pétersbourg. Nouvelle série (II). 1891. XXXIV. 481.—Ch. C. 1892. I. 636.—K. J. II. 42.

Fedorolf, A. K.,
I. Der Einfluss des Chlorlithiums auf Bakterien.—Wratsch 1895. XVI. 1084.—Ch. C. 1896. I. 266.

Fehling,
I. Dingler's Journ. 1853. CXXX. 77.

Feilitzen, Hjalmar von, und Tollens, B.,
I. Gärungsversuche mit Torf.—Journal f. Landwirtschaft. 1898. XXXXVI. 23.—Ber. d. D. Chem. Ges. 1897. XXX. 2577.—Ch. C. 1898. I. 35.—K. J. VIII. 131.

Feltz, E.,
I. Über das Auftreten gallertartiger Stoffe bei den Walzenpressen.—Sucrerie indigène. 1874. No. 10. 1876. No. 14. — Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1875. 109. 1876. 749. 1877. 464.

Fermi, Claudio,
I. Über die Reinigung der Abwässer durch Elektrizität.—A. f. Hygiene. 1891. XIII. 206.—K. J. II. 95.
II. Die Leim und Fibrin lösenden und die diastatischen Fermente der Mikroorganismen.—A. f. Hygiene. 1890. X. 1.—C. f. B. 1890. VII. 469.—K. J. I. 159.
III. Weitere Untersuchungen über die tryptischen Enzyme der Mikroorganismen.—C. f. B. 1891. X. 401.—K. J. II. 254.
IV. Die Leimgelatine als Reagens zum Nachweis tryptischer Enzyme.—A. f. Hygiene. 1891. XII. 240.—K. J. II. 252.

Fermi, Cl., und Buscaglioni,
I. Die proteolytischen Enzyme im Pflanzenreiche.—C. f. B. 2. Abt. 1899. V. 24.—Ch. C. 1899. I. 501.

Fermi, Cl., und Montesano, G.,
I. Die von den Mikroben bedingte Inversion des Rohrzuckers.—C. f. B. 2. Abt. 1895. I. 482.—Ch. C. 1895. II. 712.

Fermi, Cl., und Pernossi, L.,
I. Über die Enzyme.—Z. f. Hygiene. 1894. XVIII. 83.—C. f. B. 1894. XV. 229; XVI. 830.—Ch. C. 1894. I. 965.—K. J. V. 280.

Fermi, Cl., u. Pomponi, E.,
I. C. f. B. 2. Abt. 1896. II. 574.

Fernbach, A.,
I. De l'absence des microbes dans les tissus végétaux.—Ann. Inst. Past. 1888. II. 567.—C. f. B. 1888. IV. 713.
II. Der Amylomyces Rouxii und seine Verwendung in der Brennerei.—Z. f. Spiritusind. 1899. XXI. Ergänzungsheft. I. 57.
III. C. R. 1900. CXXXI. 1214.
IV. Annales de la brasserie et distillerie. 1899. II. 409.
V. Recherches sur la sucrase. Paris. 1890.
VI. Ann. Pasteur. 1890. IV. 641.
VII. *Ibid.* 1889. III. 473, 531.
VIII. *Ibid.* 1890. IV. 1.

Fernbach, A., et Hubert, L.,
I. Sur la diastase protéolytique du malt.—C. R. 1900. CXXXI. 1783.—Ch. C. 1900. II. 391.

Ferrier,
I. Considérations générales sur le pléomorphisme des cils vibratiles de quelques bactéries mobiles.—Archives de médecine expérimentale et d'anatomie pathologique. Série I. T. VII.—C. f. B. 2. Abt. 1895. I. 497.

Fesca, Max, und Imai,
I. Über die Bestandteile des Tabakes und über die für die Beurteilung und Untersuchung desselben wichtigsten Momente.—Landw. Jahrbücher. 1888. XVII. 329.—Ch. C. 1888. 1121.

Fick, A.,
I. Zu P. Walther's Abhandlung über Fick's Theorie der Labwirkung, etc.—Pflüger's Archiv. 1891. XXXXIX. 110.—K. J. II. 258.

Fiechter,
I. Wirkung d. Blausäure auf Fermente. Dissert. Basel. 1875.

Fisch, C.,
I. Über die Pilzgattung Ascomyces.—Bot. Ztg. 1885. XXXXIII. 33.

Fischel, F.,
I. Untersuchungen über die Morphologie und Biologie des Tuberkulose-Erregers.—Fortschritte d. Medizin. 1892. X. No. 22.—C. f. B. 1893. XIII. 134.

Fischer, A.,
I. Liebig's Ann. 1860. CXV. 247. 1861. CXVIII. 307.

Fischer, Alfred,
I. Die Plasmolyse der Bakterien.—Ber. d. K. sächs. Ges. d. Wissenschaften. Mathem.-phys. Klasse. Leipzig. 1891.—K. J. II. 63.
II. Untersuchungen über Bakterien.—Jahrbücher für wissenschaftl. Botanik. 1894. XXVII. 163.—C. f. B. 2. Abt. 1895. I. 701.—K. J. V. 45.
III. Die Pilze Deutschlands, Osterreichs und der Schweiz. IV. Abt.: Phycomycetes. Leipzig. 1892. Ed. Kummer.

Fischer, B., u. Brebeck, K.,
I. Zur Morphologie, Biologie, u. Systematik d. Kahmpilze, d. Monilia candida Hansen u. d. Soorpilzes. Jena. 1894.

Fischer, Bernhard,
I. Bakterienwachstum bei 0°, sowie über das Photographieren von Kulturen leuchtender Bakterien in ihrem eigenen Lichte.—C. f. B. 1888. IV. 89.
II. Über einen neuen lichtentwickelnden Bacillus.—C. f. B. 1888. III. 105.

Fischer, Bernhard, und Proskauer, B.,
I. Über die Desinfektion mit Chlor und Brom.—Mitteil. a. d. K. Gesundh.-Amte. 1884. II. 228.—Ch. C. 1884. 222.

Fischer, Ed.,
I. Die Pilze Deutschlands, Österreichs und der Schweiz. V. Abt.: Tuberaceen und Hemiasceen. Leipzig. 1897.
II. In Engler-Prantl. Nat. Pflanzenfamilien. Teil I. 1900. 537.

Fischer, Emil,
I. Synthesen in der Puringruppe.—Ber. d. D. Chem. Ges. 1899. XXXII. 435.—Ch. C. 1899. I. 834.
II. Ber. d. D. Chem Ges. 1890. XXIII. 270.
III. *Ibid.* 1895. XXVIII. 1432.
IV. *Ibid.* 1894. XXVII. 3481.
V. *Ibid.* 1891. XXIV. 1836.
VI. W. f. Br. 1897. XIV. 363.
VII. Ber. d. D. Chem. Ges. 1894. XXVII. 2988, 3251.
VIII. *Ibid.* 1895. XXVIII. 3037.
IX. Verhandl. d. Ges. Naturforscher u. Aerzte. Wien. 1895. Bericht. 109.
X. Ber. d. D. Chem. Ges. 1887. XX. 1089, 3384. 1888. XXI. 2634, 1889. XXII. 97, 106.
XI. *Ibid.* 1890. XXIII. 2226.
XII. *Ibid.* 1895. XXVIII. 1429.
XIII. *Ibid.* 1890. XXIII. 3687.
XIV. *Ibid.* 1895. XXVIII. 3024.
XV. *Ibid.* 1894. XXVII. 2985.
XVI. *Ibid.* 1894. XXVII. 2031.
XVII. *Ibid.* 1889. XXII. 3218.

Fischer, Emil, u. Armstrong,
I. Ber. d. D. Chem. Ges. 1902. XXXV. 3151.

Fischer, Emil, und Lindner, Paul,
I. Über die Enzyme einiger Hefen.—W. f. Br. 1895. XII. 959.—C. f. B. 2. Abt. 1895. I. 889.—Ch. C. 1895. II. 1048.

II. *Ibid.* II.—Ber. d. D. Chem. Ges. 1895. XXVIII. 3034.—C. f. B. 2. Abt. 1895. I. 889.—Ch. C. 1896. I. 265.—K. J. VI. 323.
III. Ber. d. D. Chem. Ges. 1895. XXVIII. 3037.
IV. *Ibid.* 1895. XXVIII. 984.

Fischer, E., u. Niebel,
I. Sitz. d. Preuss. Akad. d. Wissen. 1896. Jan. 30.

Fischer, Emil, und Roeder, G.,
I. Synthese des Thymins und anderer Uracile.—Sitzgsber. d. Akad. d. Wiss. Berlin. 1901. XII. 268.—Ch. C. 1901. I. 887.

Fischer, Emil, und Thierfelder, H.,
I. Verhalten der verschiedenen Zucker gegen reine Hefen.—Ber. d. D. Chem. Ges. 1894. XXVII. 2031.—C. f. B. 2. Abt. 1895. I. 121.—Ch. C. 1894. II. 360.—K. J. V. 145.

Fischer, H.,
I. C. f. B. 2. Abt. 1902. IX. 353, 385.
II. *Ibid.* 1903. X. 452.

Fischer von Waldheim, Alexander,
I. Über Heliotropismus bei niederen Pilzen und speziell bei Pilobolus.—Arbeiten des bot. Laboratoriums d. Kais. Universität Warschau. 1875. Heft I.—Bot. Ztg. 1876. XXXIV. 479.

Fittig,
I. Zeits. f. Chemie. 1867. II. 44.

Fitz, Albert,
I. Über Schizomyceten-Gärungen.—Ber. d. D. Chem. Ges. 1876. IX. 1348.
II. *Ibid.* II.—*Ibid.* 1877. X. 276.
III. *Ibid.* III.—*Ibid.* 1878. XI. 42.
IV. Über Spaltpilzgärungen. IV. *Ibid.* 1878. XI. 1890.—Dingl. Journal. 1879. CCXXXI. 191.
V. *Ibid.* V.—*Ibid.* 1879. XII. 474.
VI. *Ibid.* VI.—*Ibid.* 1880. XIII. 1309.
VII. *Ibid.* VII.—*Ibid.* 1882. XV. 867.
VIII. Notizen über die Gärung.—Annalen der Önologie. 1870. I. 437.
IX. Über alkoholische Gärung durch Mucor mucedo.—Ber. d. D. Chem. Ges. 1873. VI. 48.
X. Über alkoholische Gärung durch den Schimmelpilz Mucor racemosus.—Ber. d. D. Chem. Ges. 1875. VIII. 1540.
XI. Über alkoholische Gärung.—Ber. d. D. Chem. Ges. 1876. IX. 1352.
XII. Ann. d. Önologie. 1872. II. 428.
XIII. Ber. d. D. Chem. Ges. 1877. X. 278.
XIV. *Ibid.* 1880. XIII. 36.

Fleck, H.,
I. Quoted by Will, I.
II. Ber. d. Chem. Centralstelle. Dresden. 1876.

Fleischer, M.,
I. Über Bodenimpfung, ihre Ergebnisse und ihre Aussichten.—Chemikerzeitg. 1893. XVII. 900.—K. J. IV. 222.

Fleischmann, W.,
I. Lehrbuch der Milchwirtschaft. 1893. Bremen. M. Heinsius Nachf.

Flemming,
I. Zellsubstanz, Kern und Zellteilung. Leipzig. 1882.

Flückiger, F. A.,
I. Pharmakognosie des Pflanzenreiches. 1891. 3rd Ed. Berlin. R. Gaertner.

Flügge, C.,
I. Die Mikroorganismen. 3rd Ed. 1896. Zwei Bände. Leipzig. Vogel.
II. Die Aufgaben und Leistungen der Milchsterilisierung gegenüber den Darmkrankheiten der Säuglinge.—Z. f. Hygiene. 1894. XVII. 272.—Ch. C. 1894. II. 385.—K. J. V. 226.

Flügge, C., und Sirotinin,
I. Studien über die Abschwächung virulenter Bakterien und die erworbene Immunität.—Z. f. Hygiene. 1888. IV. 208, 262.—C. f. B. IV. 593, 636.

Flühler, Adalbert,
I. Die Säurebildung beim Mälzen und Brauen.—Der bayr. Bierbrauer. 1872. VII. 167. 1873. VIII. 24.

Fodor, J. von,
I. Bakterien im Blute lebender Tiere.—A. f. Hygiene. 1886. IV. 129.—Ch. C. 1886. 922.

Fokker, A. P.,
I. Über das Milchsäureferment.—Fortschritte der Medizin. 1889. No. 11.—C. f. B. 1889. VI. 293.—K. J. I. 83.

II. Onderzoekingen over melkzuur gisting.—Weekblad van het Ned. Tijdschrift voor Geneeskunde. 1890. 88, 509.—C. f. B. 1890. VIII. 426.—K. J. I. 83.

Fonseca, Ant., und Chiaromonte, Tom.,
I. Der Zusatz von Säuren zum Most und Wein.—Staz. sperim. agrar. ital. 1894. XXV. 20.—Ch. C. 1894. I. 246.

Fonssagrives,
I. Sur l'Oïdium aurantiacum.—C. R. 1871. LXXIII. 781.

Fordos,
I. Recherches sur la matière colorante des suppurations bleues: pyocyanine.—C. R. 1860. LI. 215.—Journal f. prakt. Chemie. 1862. LXXXV. 249.

Forster, J.,
I. Über einige Eigenschaften leuchtender Bakterien.—C. f. B. 1887. II. 337.
II. Über die Entwicklung von Bakterien bei niederen Temperaturen.—C. f. B. 1892. XII. 431.—K. J. III. 63.
III. Über den Einfluss des Räucherns auf die Infektiosität des Fleisches perlsüchtiger Rinder.—Münchener medizinische Wochenschrift. 1890. No. 16.—C. f. B. 1890. VIII. 79.

Forster, J., und Man, C. de,
I. Über die Einwirkung hoher Temperaturen auf Tuberkel-Bacillen.—Hygien. Rundschau. 1892. II. 869. 1893. III. 669.—Archiv. f. Hygiene. 1893. XVIII. 134.—Ch. C. 1893. II. 941.—C. f. B. 1893. XIII. 299.

Förster, K.,
I. Über den Furfurolgehalt gegorener Flüssigkeiten.—Ber. d. D. Chem. Ges. 1882. XV. 322.

Forti, Cesare,
I. Relazione sugli studi zimotecnici. I. e II.—Bolletino di Notizie agrarie. 1896. XVIII. 363.—C. f. B. 2. Abt. 1897. III. 122.—K. J. VII. 131.

Foth, G.,
I. W. f. Br. 1887. IV. 73, 305.
II. *Ibid.* 1889. VI. 263.

Foureur, A.,
See Wurtz et Foureur.

Foutin, W. M.,
I. Bakteriologische Untersuchungen von Hagel. — Wratsch. 1889. No. 49 u. 50.—C. f. B. 1890. VII. 372.

Fox, Joseph. J.,
See Frankland and Fox.

Fraenkel, Carl,
I. Die Einwirkung der Kohlensäure auf die Lebenstätigkeit der Mikroorganismen.—Zeitschrift f. Hygiene. 1888. V. 332. Gives anteeedent literature.—C. f. B. 1889. V. 208. — Ch. C. 1889. I. 49.
II. Beiträge zur Kenntnis des Bakterienwaehstums auf eiweissfreien Nährböden. — Hygien. Rundschau. 1894. IV. 769.—C. f. B. 2. Abt. 1895. I. 252.——K. J. V. 13.
III. Untersuchungen über das Vorkommen von Mikroorganismen in versehiedenen Bodenschichten.—Z. f. Hygiene. 1887. II. 521.—C. f. B. 1888. III. 235.—Ch. C. 1887. 1170.
IV. Über die Kultur anaerober Mikroorganismen.—C. f. B. 1888. III. 735.—Ch. C. 1888. 1036.
V. Über den Bakteriengehalt des Eises.—Z. f. Hygiene. 1886. I. 302.—Ann. Inst. Past. 1887. I. 136.

Fraenkel, C., u. Pfeiffer, R.,
I. Mikrophotographischer Atlas der Bakterienkunde. 2nd Ed. 1893. Berlin. A. Hirschwald. 60*s.*

Fraenkel, S.,
See Kerry und Fraenkel.

Francke, G.,
I. Über Bakterientrübung im Bier.—W. f. Br. 1884. I. 727.

Frank, Albert Bernhard,
I. Mitteilung betreffs in einem Rohzucker-Nachprodukt vorgefundener, gefärbter Pilze.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. D. Reiches. 1891. 662.—C. f. B. 1892. XII. 661.—K. J. II. 225.
II. Die Krankheiten der Pflanzen. 2nd Ed. I. Bd.: Die durch anorgan. Einflüsse hervorgerufenen Krankheiten. 6*s.*—II. Bd.: Die Pilzkrankheiten der Pflanzen. 10*s.* 10*d.*—Breslau. Trewendt.

III. Über die Parasiten in den Wurzelanschwellungen der Papilionaceen. — Bot. Ztg. 1879. XXXVII. 832.
IV. Die Assimilation des freien Stickstoffes bei den Pflanzen.—Landw. Jahrb. 1892. XXI. 1.—Bot. Ztg. 1. Abt. 1893. LI. 139.—Ch. C. 1894. I. 172.—K. J. III. 194; IV. 224.
V. Sind die Wurzelanschwellungen der Erlen und Eleagnaceen Pilzgallen?—Ber. d. D. Bot. Ges. 1887. V. 50.—C. f. B. 1887. I. 606.
VI. Über die Pilzsymbiose der Leguminosen.—Ber. d. D. Bot. Ges. 1889. VII. 332.—Landw. Jahrbücher. 1890. XIX. 523.—C. f. B. 1890. VII. 413. 1891. IX. 629.—K. J. I. 120.
VII. Über den Dimorphismus der Wurzelknöllchen der Erbse.—Ber. d. D. Bot. Ges. 1892. X. 170.—C. f. B. 1892. XII. 271.—K. J. III. 200, 203.
VIII. Uber die auf den Gasaustausch bezüglichen Einrichtungen und Tätigkeiten der Wurzelknöllchen der Leguminosen.—Ber. d. D. Bot. Ges. 1892. X. 271.—K. J. III. 198.
IX. Über die Ursache der Nitrifikation der Ammoniaksalze im Erdboden.—Deutsche landw. Presse. 1887. XIV. No. 104.

Frankland, Percy F.,
I. Über den Einfluss der Kohlensäure und anderer Gase auf die Entwicklungsfähigkeit der Mikroorganismen. — Z. f. Hygiene. 1889. VI. 13.—C. f. B. 1889. VI. 261.
II. Die Wirkung einiger Mikroorganismen auf Salpetersäure.—Chemical News. 1888. LVII. 89.—Ch. C. 1888. 553.
III. The Nitrifying Process and its Specific Ferment.—Transactions of the Royal Society of London. 1890. CLXXXI. 107.—K. J. I. 107.
IV. Trans. Chem. Soc. 1891.—Chem. News. 1891. LXIII. 136.
V. Journ. Chem. Soc. 1891. LIX. 253. 1892. LX. 432, 737.
VI. *Ibid.* 1892. LX. 254.

Frankland, P. F., and Fox, J. J.,
I. On a Pure Fermentation of Mannite and Glycerin.—Proceedings of the Royal Society. 1889. XXXXVI. 345.—C. f. B. 1890. VII. 241. — Ch. C. 1889. II. 1027.

Frankland, P. F., and Frew, W.,
I. The Fermentation of Calcium Glycerate by the Bacillus ethaceticus.—Journal of the Chemical Society. 1891. LIX.-LX. 81.—C. f. B. 1892. XII. 724.—K. J. II. 237.
II. A Pure Fermentation of Mannitol and Dulcitol.—Journal of the Chemical Society. Transactions. 1892, p. 254.—C. f. B. 1892. XII. 252.—K. J. III. 229.
III. An Optically Active Glyceric Acid.—Journal of the Chemical Society. 1891. LIX.-LX. 96.—Ch. C. 1891. I. 528.—K. J. II. 238.

Frankland, P. F., and Lumsden, J. S.,
I. Decomposition of Mannitol and Dextrose by the Bacillus ethaceticus.—Journal of the Chemical Society. Transactions. 1892, p. 432.—Ch. C. 1893. I. 46.—K. J. III. 231.

Frankland, P. F., and MacGregor, J.,
I. Fermentation of Arabinose with the Bacillus ethaceticus.—Journal of the Chemical Society. Transactions. 1892, p. 737.—C. f. B. 1892. XII. 725.—Ch. C. 1892. II. 532.—K. J. III. 232.

Frankland, P. F., Stanley, A., and Frew, W.,
I. Fermentations induced by the Pneumococcus of Friedländer. Transactions of the Chemical Society. 1891.—Chemical News. 1891. LXIII. 136.—C. f. B. 1891. X. 222.—Ch. C. 1891. I. 704. 1892. I. 217.—K. J. II. 234.

Frankland, P. F., and Ward, M. H.,
I. First Report to the Water Research Committee of the Royal Society on the Present State of our Knowledge concerning the Bacteriology of Water, with Especial Reference to the Vitality of Pathogenic Schizomycetes in Water.—Proceedings of the Royal Society. 1892. LI. 183.

II. Second Report: The Vitality and Virulence of Bacillus anthracis and its Spores in Potable Waters.—Proceedings, etc. 1893. LIII. 164.

Fremlin,

I. Vergleichende Studien an Bacterium coli commune verschiedener Provenienz. — A. f. Hygiene. 1893. XIX. 295.—C. f. B. 1894. XV. 693.—Ch. C. 1894. I. 162. —K. J. IV. 87.

Frémy, Edmond,

I. Recherches sur la composition chimique des tissus des végétaux. —Journal de Pharmacie et de chimie. 1859. XXXVI. 5.—Compare Kabsch (I.).

Frémy, Edmond, et Boutron-Charlard, Ant. Franç.,

I. Sur la fermentation lactique.—C. R. 1841. XII. 728.—Ann. de Chim. et de Phys. (3.) 1841. II. 257.—Journal f. prakt. Chemie. 1841. XXIV. 51, 364.

Frenzel, J.,

I. Über den Bau und die Sporenbildung grüner Kaulquappen-Bacillen.—Z. f. Hygiene. 1891. XI. Heft 2.—K. J. II. 47.

Fresenius,

I. Beiträge z. Mykologie. Frankfurt. 1850–1863.

II. *Ibid.* Frankfurt. 1853. I. 22.

III. Z. f. analyt. Chem. 1891. XXX. 669.

Freudenreich, Eduard von,

I. Über den jetzigen Stand der bakteriologischen Forschung auf dem Gebiete des Käsereifungsprocesses.—C. f. B. 2. Abt. 1895. I. 854.—Ch. C. 1896. I. 451.

II. De la teneur du lait en bactéries. —Annales de micrographie. 1890. II. 115.—K. J. I. 82.

III. Bakteriologische Untersuchungen über den Reifungsprocess des Emmenthaler Käses.—Landw. Jahrbuch d. Schweiz. 1891. V. 16.—C. f. B. 1892. XII. 334.—K. J. II. 195.

IV. Weitere bakteriologische Untersuchungen über den Reifungsprocess des Emmenthaler Käses. —Landw. Jahrbuch d. Schweiz. 1894. VIII. 207.—C. f. B. 2. Abt. 1895. I. 168.—K. J. V. 240.

V. Recherches préliminaires sur le rôle des bactéries dans la maturation du fromage d'Emmenthal.—Annales de micrographie. 1890. II. 257.—K. J. I. 92.

VI. Sur quelques bactéries produisant le boursouflement des fromages.—Annales de micrographie. 1890. II. 553.—C. f. B. 1890. VIII. 300.—K. J. I. 95.

VII. Über einen neuen, in geblähtem Käse gefundenen Bacillus (B. Schafferi).—Landw. Jahrbuch d. Schweiz. 1890. IV. 17. —K. J. I. 96.

VIII. Über den Einfluss der bei dem Nachwärmen des Käses angewandten Temperatur auf die Bakterienzahl in der Milch und im Käse.—Landw. Jahrbuch d. Schweiz. 1895. IX. 100.—C. f. B. 2. Abt. 1895. I. 760.—K. J. VI. 246.

IX. Über einige Versuche die Blähung der Käse zu verhindern.—Landw. Jahrbuch d. Schweiz. 1893. VII. 81.—K. J. IV. 206.

X. Beitrag zur Kenntnis der Ursachen des bittern Käses und der bittern Milch.—Landw. Jahrbuch d. Schweiz. 1894. VIII. 135.—C. f. B. 2. Abt. 1895. I. 507.—K. J. V. 222.

XI. Bakteriologische Untersuchungen über den Kefir.—Landw. Jahrbuch d. Schweiz. 1896. X. 1.—C. f. B. 2. Abt. 1897. III. 47.—Ch. C. 1897. I. 872.—K. J. VII. 162.

Freudenreich, Ed. von, u. Jensen, Orla,

I. Über den Einfluss des Naturlabes auf die Reifung des Emmenthaler Käses.—C. f. B. 2. Abt. 1897. III. 545.—Ch. C. 1898. I. 73.

II. C. f. B. 2. Abt. 1897. III. 552.

Freudenreich, Ed. v., u. Schaffer, F.,

I. Über den Einfluss des Luftabschlusses auf die Reifung des Emmenthaler Käses. — Landw. Jahrbuch d. Schweiz. 1892. VI. —C. f. B. 1893. XIV. 139.—K. J. III. 186.

Freund, Aug.,

I. Die Produkte der sauren Gärung der Weizenkleie. — Journal f. prakt. Chemie. (2.) 1871. III. 224.—Ch. C. 1871. 306.

FREW,
I. Journ. Soc. Chem. Industry. 1898. XVII. 561.

FREW, William,
See FRANKLAND and FREW, *and* FRANKLAND, STANLEY, and FREW.

FREY, H.,
I. Über die Zersetzungsprodukte der im menschlichen Dünndarm vorkommenden Mikroben. — Schweiz. Wochenschrift f. Pharmacie. 1891. XXIX. 111.—Ch. C. 1891. I. 833. — K. J. II. 240.

DE FREYTAG, C. J.,
I. Über die Einwirkung konzentrierter Kochsalzlösungen auf das Leben von Bakterien.—A. f. Hygiene. 1890. XI. 60.—Ch. C. 1890. II. 449.

FRIEDEL,
I. Bull. Soc. Bot. de France. 1905. LII. 182.
II. *Ibid.* 1904. LI. 209.—C. R. 1904. CXXXVIII. 1118.

FRIEDENTHAL, H.,
I. Über den Einfluss von Induktions-Elektrizität auf Bakterien. —C. f. B. 1. Abt. 1896. XX. 505.—Ch. C. 1896. II. 939.—K. J. VII. 54.

FRIEDLÄNDER, P.,
I. Der Mikrokokkus der Pneumonie.—Fortschritte der Medizin. 1883. I. 715.

FRIES, Elias Magnus,
I. Systema mycologicum. 1821–29. Greifswald.
II. Systema orbis vegetabilis. 1825. Lund.

FRIIS, F., LUNDE, H. P., u. STORCH, V.,
I. Syrningsforsög.—32. Beretning fra den Kgl. Veterinär- og Landbohojskoles Laboratorium for Landökonomiske Forsög. Kjóbenhavn. 1895.—C. f. B. 2. Abt. 1895. I. 440.

FRISCH,
I. Über den Einfluss niederer Temperaturen auf die Lebensfähigkeit der Bakterien.—Sitzungsberichte der Wiener Akademie.—1877. 3. Abt. LXXV. 257. 1879. LXXX. 77.

FROMME, Arnold,
I. Über die Beziehungen des metallischen Eisens zu den Bakterien und über den Wert des Eisens zur Wasserreinigung. — Dissert. Marburg. 1891.—C. f. B. 1892. XII. 274.—Ch. C. 1891. II. 180.—K. J. II. 98.

FRY, George,
I. The Theory and Practice of Sweet Ensilage. London. 1885. —German transl.: Die Einsüssung der Futtermittel, Theorie und Praxis der süssen Ensilage nach George Fry.—Berlin. 1885. Parey. 1*s.*—Z. f. Spiritusind. 1885. VIII. 704.

FUCHS, C. J.,
I. Beiträge zur näheren Kenntnis der gesunden und fehlerhaften Milch der Haustiere.—Gurlt und Hertwig's Magazin f. d. gesamte Tierheilkunde. Berlin. 1841. 182.

FUNK, C., und BALOGH, N. von,
I. Verfahren zur Vergärung von Maischen, Teigen, Würzen, etc. German Patent No. 57865.—K. J. II. 170.

FÜISTING, W.,
I. Beiträge zur Entwicklungsgeschichte der Lichenen.—Bot. Ztg. 1868. XXVI. 641, 662.

FÜRBRINGER, P.,
I. Untersuchungen und Vorschriften über die Desinfektion der Hände des Arztes nebst Bemerkungen über den bakteriologischen Charakter des Nagelschmutzes. Wiesbaden. 1888. Bergmann.—C. f. B. 1888. III. 260.

GÄRTNER, A.,
I. Über die Fleischvergiftung in Frankenhausen am Kyffhäuser und den Erreger derselben.—Korrespondenzblatt des allgem. ärztl. Vereins von Thüringen. 1888. No. 9. 573. — Ch. C. 1889. I. 32.

GAILLARD, G.,
I. De l'influence de la lumière sur les Micro-organisms. Lyon. 1888.

GALEAZZI, J.,
I. Ricerche batteriologiche e chimiche sull' incerconimento del vino.—Staz. sper. agr. ital. 1895. XXVIII. 181.—C. f. B. 2. Abt. 1895. I. 892.

Galeotti, G.,
I. Ricerche biologiche sopra alcuni bacteri cromogeni.—Lo Sperimentale. 1892. XXXXVI. Fasc. III. p. 261.—C. f. B. 1893. XIV. 696.—K. J. IV. 103.

Galippe, M.,
I. Sur la présence de microorganismes dans les tissus végétaux. — La semaine médicale. 1887, p. 267.—C. f. B. 1888. III. 108.—Ch. C. 1888. 554.

Galli-Valerio, Bruno, und Strzyzowski, C.,
I. Über den biologischen Arsennachweis.—Pharmac. Post. 1900. XXXIII. 637.—Ch. C. 1901. I. 63.

Gantter, F.,
I. Über die Durchführung der Nachgärung bei unvollständig vergorenen Weinen.—Bericht ü. d. Verh. d. XV. Deutschen Weinbau - Kongresses in Heilbronn a. N. 1896. 98.

Garnier,
I. Abst. in Bot. Centralbl. 1904. XCV. 278.
II. Comptes rendus Soc. de Biologie. 1903. Dec. 4 and 8.

Garré, C.,
I. Über Antagonisten unter den Bakterien. — Korrespondenzblatt für Schweizer. Aerzte. 1887. XVII.—C. f. B. 1887. II. 312. —Ch. C. 1887. 1508.

Garros, F.,
I. Über die Filtration und Sterilisation des Wassers und anderer Flüssigkeiten durch Asbestporcellan.—Le Mercure scientifique. 1892. No. 610, p. 147.—K. J. III. 20.

Gasperini, G.,
I. Atti Soc. Toscana Sc. Nat. Pisa. 1887. VIII. 326.

Gasperini, Gustavo,
I. Recherches morphologiques et biologiques sur un microorganisme de l'atmosphère, le Streptothrix Foersteri Cohn.—Annales de micrographie. 1890. II. 449. —K. J. I. 19.
II. Il burro naturale come mezzo di trasmissione della tuberculosi. —Giornale della R. Soc. d'Igiene Milano. 1890.—C. f. B. 1890. VII. 641.

Gastine, G.,
I. Sur la fermentation alcoolique des miels et la préparation de l'hydromel.—C. R. 1889. CIX. 479.—Ch. C. 1889. II. 956.

Gauthier de Claubry,
I. Note sur une altération particulière observée sur le pain.— Annales d'hygiène publique et de médecine légale. 1er sér. 1843. XXIX. 347.—C. R. 1871. LXXIII. 725.
II. De l'altération du pain par diverses espèces de champignons. —Bulletin de l'Académie de médecine. 1871. XXXVI. 729.

Gautier, Arm.,
I. Sur une maladie non encore décrite des vins du midi de la France dits vins tournés.—C. R. 1878. LXXXVI. 1338.

Gautier, A., et Drouin, R.,
I. Sur la fixation de l'azote par le sol arable.—C. R. 1891. CXIII. 820.—Ch. C. 1888. 590. 1892. I. 171.—K. J. II. 205.

Gay, Fr.,
I. Die Schwefelwasserstoff - Hefe Crouzel's. — L'union pharmac. 1891. XXXIII. 117.—C. f. B. 1892. XI. 801.—K. J. II. 136.
II. L'Union pharm. 1892. XXXIII. 60.

Gay-Lussac, Louis Joseph,
I. Extrait d'un mémoire sur la fermentation. — Annales de Chimie. 1810. LXXVI. 245.
II. Ann. de chim. et de phys. 1815. XCV. 318.
See also Pelouze et Gay-Lussac.

Gayon, H. U.,
I. Etude sur les appareils de pasteurisation des vins.—Extrait de la Revue de viticulture. 1896. Paris.

Gayon, Ulysse,
I. Recherches sur la fermentation du fumier.—C. R. 1884. LXXXVIII. 528. — Ch. C. 1884. 378.
II. Sur les altérations des œufs.— Thèse pour le doctorat. 1875.— C. R. 1877. LXXXV. 1074.
III. Sur l'altération des vins dits "mildiousées."—Revue de viticulture. 1894. I. 33.

IV. Sur la constitution du glucose inactif des sucres bruts de cannes et des mélasses.—C. R. 1878. LXXXVII. 407.—Annales agronomiques. 1880.

V. Sur l'inversion et sur la fermentation alcoolique du sucre de canne par les moisissures.—C. R. 1878. LXXXVI. 52.—Z. g. Br. 1878. I. 113.

VI. De la fermentation alcoolique avec le Mucor circinelloides. —Annales de chimie et de physique. 5e série. 1878. XIV. 258.

Gayon, U., et Dubourg, E.,

I. Sur les vins mannités.—Ann. Inst. Past. 1894. VIII. 108.—Ch. C. 1894. I. 787.—K. J. V. 192.—I. (Chap. 64) Ann. Pasteur. 1901. XV. 527.

II. Sur la fermentation alcoolique de la dextrine et de l'amidon.—C. R. 1886. CIII. 885.—C. f. B. 1. Abt. 1887. I. 168.

III. De la fermentation de la dextrine et de l'amidon par les Mucors.—Ann. Inst. Past. 1887. I. 534.—C. f. B. 1. Abt. 1888. III. 620.

IV. Sur la sécrétion anormale des matières azotées des levures et des moisissures.—C. R. 1886. CII. 978.—Z. g. Br. 1886. IX. 244.

Gayon, U., et Dupetit, G.,

I. Sur un moyen nouveau d'empêcher les fermentations secondaires dans les fermentations alcooliques de l'industrie.—C. R. 1886. CIII. 883.—C. f. B. 1887. I. 232.—Z. g. Br. 1886. IX. 502.

II. Sur la fermentation des nitrates. —C. R. 1882. LXXXXV. 644, 1365.—Ch. C. 1882. 804. 1883. 73.

III. Recherches sur la réduction des nitrates par les infiniment petits.—Nancy. 1886.

IV. Ann. Pasteur. 1901. XV. 527.

Géduld, Robert,

I. Über ein neues Enzym: die Glucase.—Journal de la Distillerie française. 1891.—W. f. Br. 1891. VIII. 548.—Ch. C. 1891. II. 323.—K. J. II. 250.

II. W. f. Br. 1891. VIII. 545.

Geisler, Theodor,

I. Zur Frage über die Wirkung des Lichtes auf Bakterien.—C. f. B. 1892. XI. 161.—K. J. II. 94.

Gelis, Amédée,

See Pelouze et Gélis.

Gentil,

I. Z. f. Spiritusindustrie. 1898. XXI. 16.

Georgiewicz, Georg von,

I. Der Indigo vom praktischen and theoretischen Standpunkt dargestellt. Wien. 1892. 6*s.*, bound.

Georgiewski, Nikolai,

I. Über die Beziehungen des Kwass zum Biere und die diätetische Bedeutung der freien Säuren in diesen Getränken. Dissert. St. Petersburg. 1875.

Gérard, E.,

I. Fermentation de l'acide urique par les microorganismes.—C. R. 1896. CXXII. 1019; CXXIII. 185.—Ch. C. 1896. I. 1272.—K. J. VII. 218.

II. Sur une lipase végétale extraite du Penicillium glaucum.—C. R. 1897. CXXIV. 370.—Ch. C. 1897. I. 768.—K. J. VIII. 269.

III. Sur les cholésterines végétales. —C. R. 1892. CXIV. 1544.—Ch. C. 1892. II. 287.

IV. Sur les cholésterines des Cryptogames.—C. R. 1895. CXXI. 723.—Ch. C. 1896. I. 45.—K. J. VI. 62.

V. Sur les cholésterines des végétaux inférieurs.—C. R. 1898. CXXVI. 909.—Ch. C. 1898. I. 949.

VI. Comptes rendus Soc. de Biologie. 1893. 651.

VII. Journ. de pharm. et de chim. 1893. 5th Series. XXVIII. 11.

Gérard, E., et Darexy, P.,

I. Recherches sur la matière grasse de la levure de bière.—Bulletin de la Soc. mycol. de France. 1897. XIII. 183.—Journal de pharm. et de chimie. 1897. [6.] V. 275.—Ch. C. 1897. I. 780.—K. J. VIII. 89.

Gerber, C.,

I. Recherches sur la maturation des fruits charnus.—Annales des sciences naturelles. Botanique. 8e série. 1897. IV. 1.—Has a full antecedent bibliography.—

C. R. 1897. CXXIV. 1106, 1160.—Bot. Ztg. 1897. LV. 154, 243, 245.—Ch. C. 1897. II. 43.

Geret, L.,
I. Münch. med. Wochenschrift. 1901. XLVIII. 1836.

Geret, Ludwig, und Hahn, M.,
I. Zum Nachweis des im Hefepresssaft enthaltenen proteolytischen Enzyms.—Ber. d. D. Chem. Ges. 1898. XXXI. 202.—C. f. B. 2. Abt. 1898. IV. 491.—Ch. C. 1898. I. 680.—K. J. IX. 291.
II. Weitere Mitteilungen über das im Hefepresssaft enthaltene proteolytische Enzym.—Ber. d. D. Chem. Ges. 1898. XXXI. 2335.—C. f. B. 2. Abt. 1899. V. 41.—Ch. C. 1898. II. 1272.—K. J. IX. 292.
See also Hahn und Geret.

Geret, L., u. Martin, M.,
I. Ber. d. D. Chem. Ges. 1898. XXXI. 202, 2335.

Gerstmann, H.,
I. Über die Ursache der Gerinnung der Milch bei Gewittern.—Elektrotechnische Zeitschrift. 1896. III. 74.—Ch. C. 1896. II. 388.—K. J. VII. 165.

Gessard, C.,
I. Fonctions et races du bacille cyanogène (microbe du lait bleu).—Ann. Inst. Past. 1891. V. 737.—C. f. B. 1892. XI. 375.—K. J. II. 108.
II. De la pyocyanie et de son microbe. Thèse de Paris. 1882.
III. Nouvelles recherches sur le microbe pyocyanique. — Ann. Inst. Past. 1890. IV. 88.—C. f. B. 1890. VII. 740.—K. J. I. 33.
IV. Des races du bacille pyocyanique.—Ann. Inst. Past. 1891. V. 65.—C. f. B. 1891. IX. 541.—K. J. II. 107.
V. Sur la fonction fluorescigène des microbes.—Ann. Inst. Past. 1892. VI. 801.—C. f. B. 1893. XIV. 695.—K. J. III. 86.

Geuns, Ib. van,
I. Über die Einwirkung des sog. Pasteurisierens auf die Milch.—A. f. Hygiene. 1886. III. 464.—Ch. C. 1886. 30.
II. Über das Pasteurisieren von Bakterien.—A. f. Hygiene. 1889. IX. 369.—C. f. B. 1889. VI. 684.—Z. g. Br. 1889. XII. 353.

Geuther,
I. Liebig's Ann. 1863. CXXVI. 63.

Giard, A.,
I. Sur l'infection phosphorescente des Talitres et autres Crustacés.—C. f. B. 1889. VI. 645.
II. Nouvelles recherches sur les bactéries lumineuses pathogènes.—Comptes rendus de la Société de Biologie. Paris. 1890. No. 14.—C. f. B. 1890. VIII. 177.
III. Sur le Crenothrix Kühniana, cause de l'infection des eaux de Lille.—C. R. 1882. LXXXXV. 247.

Gibelli e Griffini,
I. Sul polimorfismo della Pleospora herbarum.—Archivio del Laborat. di Botanica crittogamica. Pavia. 1874. I. 53.

Gibson, Howard B.,
I. On the Liberation of Nitrogen during the Process of Putrefaction.—Dissert. Leipzig. 1893.—Wollny's Forschungen. 1895. VIII. 106.—Biedermann's Centralblatt. 1895. XXIV. 701.—Ch. C. 1896. I. 125.—K. J. IV. 237.

Gilbert, J. H.,
See Lawes and Gilbert.

Gilkinet, A.,
I. Mémoire sur le polymorphisme des Champignons. — Mémoires couronnés et autres mémoires publiés par l'Académie Royale des sciences, etc., de Belgique. 1878. XXVI. 1.

Gillot, Henri,
I. Sur la fermentation du raffinose par le Schizosaccharomyces Pombe.—Bulletin de la Société belge de Microscopie. 1899. XXV. 29.
II. Die Raffinose als Kohlenhydrat- Nahrungsmittel des Aspergillus niger.—Bulletin de l'Académie Roy. de Belgique. 1889, p. 211.—Ch. C. 1899. II. 129.
III. Bull. de l'Assoc. belge des Chimistes. 1900. 202.
IV. *Ibid.* 1899. 496.
V. *Ibid.* 1902. XVI. 240.
VI. Bull. de l'Acad. Royale de Belgique. 1899. 221.

Gilson, Eugène,
I. La cristallisation de la cellulose et la composition chimique de la membrane cellulaire végétale.—La Cellule. 1893. IX. 397.—Ch. C. 1893. II. 530.—Bot. Ztg. 1893. LI. 309.
II. Recherches chimiques sur la membrane cellulaire des Champignons.—La Cellule. 1894. XI. 7. —Ch. C. 1894. II. 874.
III. De la présence de la chitine dans la membrane cellulaire des Champignons. — C. R. 1895. CXX. 1000.—Ch. C. 1895. I. 1180.
IV. Das Chitin und die Membranen der Pilzzellen.—Ber. d. D. Chem. Ges. 1895. XXVIII. 821.—Ch. C. 1895. I. 1113.

Gilson, G.,
I. On the Affinity of Nuclein for Iron and other Substances.—Report of the British Association for the Advancement of Science. Edinburgh. 1892, p. 778.

Giltay, E.,
I. Pasteur und die alkoholische Gärung.—Jahrbücher f. wissenschaftl. Botanik. 1896. XXX. 71.

Giltay, E., und Aberson, J. H.,
I. Recherches sur un mode de dénitrification et sur le schizomycète qui la produit.—Archives néerlandaises. 1892. XXV. 341. —C. f. B. 1892. XII. 864.—K. J. III. 226.
II. Über den Einfluss des Sauerstoffzutritts auf Alkohol- und Kohlensäurebildung bei der alkoholischen Gärung.—Jahrbücher f. wissenschaftl. Botanik. 1894. XXVI. 543.—K. J. V. 119.

Gimel, G.,
I. Bull. de l'Assoc. de sucr. et de distill. 1905. XXIII. 669.

Girard, A. Ch.,
See Müntz et Girard.

Girard, Aimé,
I. Sur la fermentation panaire.—C. R. 1885. CI. 601.—Ch. C. 1885. 796.
II. Sur la température de cuisson du pain.—C. R. 1893. CXVII. 584.—Ch. C. 1894. I. 89.

Girardin,
I. Note pour servir à l'étude du lait.—C. R. 1853. XXXVI. 753.

Giunti, Michele,
I. Sull' azione della luce sulla fermentazione acetica. — Staz. sperim. agrar. ital. 1890. XVIII. 172.—Ch. C. 1890. II 65.—K. J. I. 139.

Glaser, Fritz,
I. Zur Gallertausscheidung in Rübensäften.—C. f. B. 2. Abt. 1895. I. 879.—Ch. C. 1896. I. 452.

Glasmacher,
I. Vergiftung durch Hühnereiweiss.—Berl. klin. Wochenschrift. 1886. 666.—C. f. B. 1887. I. 233.

Glaubitz, H.,
See Tollens und Glaubitz.

Globig,
I. Über Bakterienwachstum bei 50 bis 70°.—Z. f. Hygiene. 1888. III. 294.—C. f. B. III. 366.—Ch. C. 1888. 224.
II. Über einen Kartoffelbacillus mit ungewöhnlich widerstandsfähigen Sporen.—Z. f. Hygiene. 1887. III. 322.—Ch. C. 1888. 223.

Godlewski, Emil,
I. Zur Kenntnis der Nitrifikation. —Anzeiger d. Akad. d. Wiss. in Krakau. 1892. 408.—C. f. B. 1893. XIII. 559.—Ch. C. 1895. II. 87. 1896. I. 49; II. 637.—K. J. III. 219.

Godlewski, E., und Polzeniusz, F.,
I. Über Alkoholgärung bei der intramolekularen Atmung höherer Pflanzen.—Anzeiger der Krakauer Akademie. 1897. 267.—Bot. Ztg. 2. Abt. 1897. LV. 273.—Ch. C. 1898. I. 65.—K. J. VIII. 47.
II. Intramolekulare Atmung von Samen. — *Ibid.* 1901. 227.—Ch. C. 1901. II. 595.

Goethe, Rudolf,
I. Fäulniserscheinungen, welche durch Botrytis cinerea hervorgerufen werden.—Mitteilungen über Weinbau und Kellerwirtschaft. 1894. VI. 101.

Goldberg, A.,
I. Chemische Untersuchung von schleimigen Wässern, welche aus destilliertem Wasser entstanden sind. — Ber. d. naturw. Ges. Chemnitz. 1893. 56. — Chem. Ztg. 1894. Repert. XVIII. 3. —Ch. C. 1894. I. 390.

Golden, Kathr., and Ferris, G. C.,
I. The Botanic. Gazette. 1898. XXV. 39.—Abstr. in C. f. B. 2. Abt. 1898. IV. 647.
Goodfellow, John,
I. Engl. Patent No. 13722 of 1897. —French Patent No. 269939 of 1897.—Ch. C. 1898. I. 965. —K. J. IX. 103.
Goodsir, John,
I. History of a Case in which a Fluid periodically ejected from the Stomach contained Vegetable Organisms of an Undescribed Form. —Edinburgh Medical and Surg. Journal. 1842. LVII. 430.
Goppelsröder,
I. Beiträge zum Studium der Salpeterbildungen. — Poggendorff's Annalen d. Physik u. Chemie. 1862. CXV. 125.
Gorini, Constantin,
I. Studi sperimentali sul latte.—Rivista d'igiene e san. publ. 1892. No. 18.—C. f. B. 1892. XII. 666.—K. J. III. 182.
II. Il fermento coagulante del bacillo prodigioso.—*Ibid.* 1893, p. 549.—K. J. IV. 290.
Gorup-Besanez,
I. Weitere Beobachtungen über diastatische und peptonbildende Fermente im Pflanzenreiche.—Ber. d. D. Chem. Ges. 1875. VIII. 1510.
Gosio, B.,
I. Über Links Milchsäure bildende Vibrionen.—A. f. Hygiene. 1894. XXI. 114.—C. f. B. 2. Abt. 1895. I. 89.—Ch. C. 1894. II. 701.—K. J. V. 239.
II. Azione di alcune muffe sui composti fissi d'arsenico.—Rivista d'Igiene e Sanità pubblica. 1892, p. 201.—K. J. IV. 86.
III. Sul riconoscimento dell' arsenico per mezzo di alcune muffe.—Rivista d'Igiene e Sanità pubblica. 1892, p. 261.—Baumgarten's Jahresbericht. IX. 444.
IV. Zur Frage, wodurch die Giftigkeit arsenhaltiger Tapeten bedingt wird.—Ber. d. D. Chem. Ges. 1897. XXX. 1024.—Ch. C. 1897. I. 1218.
V. Die Arsenikatur der Felle in Hinsicht auf die Prophylaxis gegen Bubonenpest. — Hyg. Rundschau. 1897. VII. 1217.
VI. Archives ital. de Biologie. 1892. XVIII. 253.
VII. Abst. in Bot. Centralbl. 1901. LXXXVII. 131.
See also Sclavo und Gosio.
Gottstein, A.,
I. Über den Einfluss des elektrischen Stromes auf Bakterien. —C. f. B. 1. Abt. 1896. XIX. 602.—K. J. VII. 54.
II. Über die Zerlegung des Wasserstoffsuperoxyds durch die Zellen mit Bemerkungen über eine makroskopische Reaktion für Bakterien.—Virchow's Archiv für patholog. Anatomie. 1893. CXXXIII. 295.—Ch. C. 1894. I. 168.—K. J. IV. 118.
Gouirand, G.,
I. Sur la présence d'une diastase dans les vins cassés.—C. R. 1895. CXX. 887.
See also Ravaz et Gouirand.
Graf, F.,
I. Jahresb. d. Brauer-Akademie München pro 1899–1900, p. 28.
II. 6th Jahresber. d. Lehranstalt u. Versuchsstation München. Brauer-Akademie pro 1899–1900.
Grauaug, A., u. Kranz, J.,
I. Engl. Patent No. 14150 of 1899. —K. J. 1899. X. 138.
Green, J. Reynolds,
I. The Soluble Ferments and Fermentation. Cambridge. 1899. 12*s.* 4*d.* German translation by W. Windisch. Berlin. 1901. 16*s.*
II. The Supposed Alcoholic Enzyme in Yeast.—Annals of Botany. 1897. XI. 535.—K. J. VIII. 279.
III. The Alcohol-producing Enzyme of Yeast.—Annals of Botany. 1899. XII. 491.—K. J. X. 319.
IV. Die Enzyme. German translation by Windisch. Berlin. 1901.
Gréhant et Quinquand,
I. Dosage de solutions étendues de glucose par la fermentation.—C. R. 1888. CVI. 1249.—Z. g. Br. 1888. XI. 262.—C. f. B. 1. Abt. 1888. IV. 264.
II. Sur la respiration de la levure de grains à diverses températures. —C. R. 1888. CVI. 609.—Z. g. Br. 1888. XI. 182.—C. f. B. 1. Abt. 1888. IV. 264.

Griessmayer, Victor,
I. Über das Lupulin.—Dingler's polytechnisches Journal. 1874. CCXII. 67.
II. Die Peptone und das Dickmaischverfahren.—Z. g. Br. 1879, II. 137.
III. Die Proteide der Getreidearten, Hülsenfrüchte und Ölsamen, sowie einiger Steinfrüchte. Heidelberg. 1897.

Griffini,
See Gibelli e Griffini.

Griffith,
I. Chem. News. 1886. LIII. 28.

Griffiths, A. B.,
I. Sur la matière colorante du Micrococcus prodigiosus.—C. R. 1892. CXV. 321.—K. J. III. 86.

Grigoriew,
I. Vergleichende Studien über die Zersetzung des Hühnereiweisses durch Vibrionen.—C. f. B. 1. Abt. 1895. XVII. 885.

Grijns,
I. C. f. B. 2. Abt. 1903. XI. 330.

Grimbert, L.,
I. Fermentation anaérobie produite par le Bacillus orthobutylicus, ses variations sous certaines influences biologiques. — Ann. Inst. Past. 1893. VII. 353.—Ch. C. 1894. I. 871.—K. J. IV. 241.
II. Action du pneumobacille de Friedländer sur la xylose et l'arabinose.—Comptes rend. de la Société de Biologie de Paris. 1896, p. 191.—K. J. VII. 223.
III. Comptes rendus Soc. de Biologie. 1896. 191, 260.—Ann. Inst. Pasteur. 1896. X. 708.
IV. Ann. Inst. Pasteur. 1896. 192, 684.

Grimm,
I. Liebig's Ann. 1871. CLVII. 264.

Grimm, M.,
I. Landw. bakter. Laborat. an Ministerium d. Agricultur St. Petersburg. 1900, p. 18.

Grimmer, H.,
I. Betrachtungen und Analysen aus dem Grossbetriebe.—Z. g. Br. 1881. IV. 181.—Ber. d. D. Chem. Ges. 1881. XIV. 140.

Gromow, T., u. Grigoriew, O.,
I. Zeits. f. physiol. Chemie. 1904. XLII. 299.

Grönlund, Chr.,
I. Eine neue Torula-Art und zwei neue Saccharomyces-Arten.—Z. g. Br. 1892. XVII. 289.—C. f. B. 1. Abt. 1892. XII. 753.—K. J. III. 141.
II. Z. g. Br. 1892. XV. 281.

Grotenfelt, Gösta,
I. Über rote Milch.—Fortschritte d. Medicin. 1889. VII. 42.—C. f. B. 1889. V. 383.
II. Über die Virulenz einiger Milchsäure-Bakterien. And
III. Über die Spaltung von Milchzucker durch Sprosspilze und über schwarzen Käse. *Ibid.* 121.—C. f. B. 1889. V. 607.
IV. *Ibid.* VII. 131.
V. Milchztg. 1891. 759.

Gruber, Max,
I. Notiz über die Widerstandsfähigkeit der Sporen von Bacillus subtilis gegen Wasserdämpfe von 100° C.—C. f. B. 1888. III. 576.
II. Eine Methode der Kultur anaërobischer Bakterien.—C. f. B. 1887. I. 367.
III. Die Methoden des Nachweises von Mutterkorn in Mehl und Brot.—A. f. Hygiene. 1895. XXIV. 228.—C. f. B. 2. Abt. 1896. II. 132.—Ch. C. 1895. II. 908.
IV. Arch. f. Hygiene. 1893. XVI. 35.
See also Buchner und Gruber.

Gruber, Max, und Wiener, Emil,
I. Cholerastudien.—A. f. Hygiene. 1892. XV. 241.—C. f. B. 1893. XIV. 76. — Ch. C. 1893. I. 48.

Gruber, Th.,
I. Die Arten der Gattung Sarcina. —Arbeiten a. d. Bakteriolog. Institut der Techn. Hochschule zu Karlsruhe. 1895. I., Heft 2. 239.—C. f. B. 2. Abt. 1895. I. 588.

Grünfeld, Abraham,
I. Beiträge zur Kenntnis der Mutterkornwirkung.—Arbeiten des Pharmakologischen Institutes Dorpat. 1892. VIII. 108. 1895. XI. u. XII. 295.—Ch. C. 1892. II. 372. 1895. II. 175.

GRÜSS,
I. In Festschrift f. Schwendenen. 1899.
GRÜSS, J.,
I. Z. g. Br. 1904. XXVII. 686, 771.
II. W. f. Br. 1901. XVIII. 336.
III. Ber. d. D. Bot. Ges. 1902. XX. 36.
GUÉGUEN,
I. Bull Soc. Mycol. de France. 1899. XV. 171.
II. *Ibid.* 1898. XIV. 201. 1899. XV. 15.
III. Les Champignons parasites de l'homme et des Animaux. Paris. 1904.
IV. Bull. Soc. Mycol. de France. 1898. XIV. 88.
GUÉRARD,
I. Note sur une altération singulière du pain.—Annales d'Hygiène publique et de Médecine légale. 1843. XXIX. 35.
GUÉRIN, G.,
I. Sur la présence d'un alcaloide dans les vins naturels.—Journal de Pharmacie et de Chimie. 1898. [6.] VII. 323.—Ch. C. 1898. I. 1039.
GUÉRIN, P.,
I. Sur la présence d'un champignon dans l'ivraie (Lolium temulentum L.).—Journal de Botanique. 1898, 230, 384.
GUICHARD, P.,
I. Composition et analyse de la levure.—Bulletin de la Société chimique de Paris. Série III. 1894. XI. 230.—Ch. C. 1894. I. 687.—K. J. V. 115.
GUILLEBEAU, A.,
I. Beiträge zur Lehre von den Ursachen der fadenziehenden Milch.—Landw. Jahrbuch der Schweiz. 1891. V.—C. f. B. 1892. XI. 438.—K. J. II. 185.
GUILLIERMOND, A.,
I. Recherches sur la germination des spores dans le Saccharomyces Ludwigii (Hansen).—Bulletin de la Société mycologique de France. 1903. XIX. 18.
II. C. R. 1901. CXXXII. 175, 1194; CXXXIII. 242.—Ann. de la Soc. Bot. de Lyon. 1903.
IV. Recherches cytologiques s. les levures et quelques moisissures à formes levures. Lyon. 1902. 203.
V. *Ibid.*, p. 211.
VI. Revue génér. botan. 1903. XV. 49.
GUNNING,
I. Ber. d. D. Chem. Ges. 1875. V. 821.
GÜNTHER, Ernst,
I. Beitrag zur mineralischen Nahrung der Pilze.—Erlangener Dissert. 1897.—Bot. Ztg. 2. Abt. 1897. LV. 379.
GÜNTHER, Karl, und THIERFELDER, H.,
I. Bakteriologische und chemische Untersuchungen über die spontane Milchgerinnung.—A. f. Hygiene. 1895. XXV. 164.—C. f. B. 2. Abt. 1896. II. 118.—Ch. C. 1895. I. 295. 1896. I. 268.

HAAS, B.,
I. Über die Bildung von schwefliger Säure bei der Gärung.—Zeitschr. f. Nahrungsmittel-Untersuchung usw. 1890. III. 241.—Ch. C. 1890. I. 499.—K. J. I. 65.
II. Die Untersuchung des Weines.—Zeitschrift f. Nahrungs Mittel-Untersuchung usw. 1897. XI. 122, 124.—Ch. C. 1897. I. 1260.
HABERLANDT, Fr.,
I. Die Sojabohne. 1878.
HADDON,
See CAZENEUVE et HADDON.
HAENLEIN, F. H.,
I. Beitrag zur Kenntnis der Wirkung des Kochsalzes auf die Fäulnisbakterien der Haut.—Dingler's polyt. Journal. 1893. CCLXXXVIII. 214.
II. Bakterien auf unseren Gerberrinden und ihre Bedeutung.—Tharander forstliches Jahrbuch. 1893. XLIII. 56. — Deutsche Gerberzeitung. 1892. No. 48.—Ch. C. 1893. II. 700.—K. J. IV. 255.
III. Über die Ursache der sauren Gärung in Gerbebrühen.—Dingler's polytechn. Journal. 1894. CCLXXXXI. 186.—Ch. C. 1894. I. 801.—C. f. B. 2. Abt. 1895. I. 26.—K. J. V. 270.
HAGENMÜLLER,
I. Chem. Ztg. 1902. XXVI. 209.
HAHN, Martin,
I. Das proteolytische Enzym des Hefepresssaftes. — Ber. d. D Chem. Ges. 1898. XXXI. 200.—Ch. C. 1898. I. 680.—K. J. IX. 290.

II. Ber. d. D. Chem. Ges. 1900. XXXIII. 3555.
III. Z. f. Biologie. 1900. XC 172.
IV. Münch. med. Woehensehrift. 1902. 595.
See also Geret und Hahn.

Hahn, M., und Geret, L.,
I. Über das Hefe-Endotrypsin.—Zeitsehr. f. Biologie. 1900. XL. 117. — C. f. B. 2. Abt. 1901. VII. 394.—Ch. C. 1900. II. 641.

Haienke u. Kurtz,
I. Liebig's Ann. 1871. CLVII. 270.

Haitinger, Ludwig,
I. Über das Vorkommen organischer Basen im käuflichen Amylalkohol. — Sitzgsber. d. Wiener Akademie. Mathem.-naturh. Klasse. 1882. LXXXVI. 2. Abt. 608.

Halliburton, W. D.,
I. A Text-book of Chemieal Physiology and Pathology. 1891. London.

Hallier, Ernst,
I. Gärungserscheinungen. Leipzig. 1867.
II. Die Hefe der Alkoholgärung insbesondere der Biergärung. Weimar. 1896.—Z. g. Br. 1896. XIX. 609.—K. J. VII. 109.

Hammarsten, Olaf,
I. Über die Milchgerinnung und die dabei wirkenden Fermente der Magenschleimhaut.—Upsalas läkareförenings förhandlingar. 1872. VIII. 63.—Maly's Jahresber. f. Tierchemie. 1872. II. 118.
II. Untersuchungen von Kefir.—Upsalas läkareförenings förhandlingar. 1886. XXI.—Biedermann's Centralblatt f. Agrieulturehemie. 1888. XVII. 413.—Jahresber. f. Tierchemie. 1886. XVI. 163.
III. Zur Kenntnis der Nucleoproteide.—Z. f. physiolog. Chemie. 1894. XIX. 19.—Ch. C. 1894. I. 638.

Hammerl, Hans,
I. Über die in rohen Eiern durch das Wachstum von Cholera-vibrionen hervorgerufenen Veränderungen. — Z. f. Hygiene. 1894. XVIII. 153.—C. f. B. 1894. XVI. 787.—Ch. C. 1894. II. 701.

Hanausek, Thomas Franz,
I. Vorläufige Mitteilung über den von A. Vogl in der Frucht von Lolium temulentum entdeckten Pilz.—Ber. d. D. Bot. Ges. 1898. XVI. 203.—C. f. B. 2. Abt. 1899. V. 365.

Hankin, E. H.,
I. Immunity produced by an Albumose isolated from Anthrax Cultures.—British Med. Journal. 1889, p. 810.—C. f. B. 1889. VI. 617.

Hanriot,
I. Sur un nouveau ferment du sang.—C. R. 1896. CXXIII. 753.—Ch. C. 1897. I. 64.

Hansen, Adolf,
I. Die Verflüssigung der Gelatine durch Sehimmelpilze. — Flora. 1889. LXXII. 88.—Bot. Centralblatt. 1889. XL. 74. — Z. g. Br. 1889. XII. 488.
II. Über Fermente und Enzyme.—Arbeiten d. botan. Instituts zu Würzburg. 1885. III. 253.—Z. g. Br. 1885. VIII. 317.

Hansen, Emil Christian,
I. Hypothèse de Horváth.—Compte rendu des travaux du laboratoire de Carlsberg. 1879. I. 94.
II. Contributions à la connaissance des organismes qui peuvent se trouver dans la bière et le moût de bière et y vivre.—*Ibid.* 1879 and 1882. I. 49, 197. — Z. g. Br. 1880. III. 277. 1882. V. 208.
III. Untersuchungen aus der Praxis der Gärungsindustrie. Erstes Heft. 3. Ed. 1895; Zweites Heft. 1892. Münehen. Oldenbourg.—Z. g. Br. 1884. VII. 272.
IV. Methode zur Analyse des Brauwassers in Rüeksieht auf Mikroorganismen.—Z. g. Br. 1888. XI. 1.—C. f. B. 1888. III. 377.—Compare Untersuehungen a. d. Praxis der Gärungsindustrie. 2. Heft.
V. Ein paar für die Brauerei wiehtige Punkte.—Z. g. Br. 1890. XIII. 4.
VI. Mycoderma aceti et Myc. Pasteurianum. Compte rendu, etc., de Carlsberg. 1879. I. 96.

VII. Recherches sur les bactéries acétifiantes.—*Ibid.* 1894. III. 182. 1900. V. 39.—C. f. B. 2. Abt. 1896. I. 31. 1901. VII. 439.—K. J. V. 272; XI. 299.

VIII. Action des ferments alcooliques sur les diverses espèces de sucre.—Compte rendu, etc., de Carlsberg. 1887. II. 143.—Transl. in Z. g. Br. 1888. XI. 401.

IX. Sur le Saccharomyces apiculatus et sa circulation dans la nature.—Compte rendu, etc., de Carlsberg. 1881. I. 159; transl. in Z. g. Br. 1881. IV. 449.

X. Vorläufige Mitteilungen über Gärungspilze.—Botan. Centralblatt. 1885. XXI. 181.—Reproduced in Z. g. Br. 1885. VIII. 147.

XI. Nouvelles recherches sur la circulation du Saccharomyces apiculatus dans la nature.—Annales des sciences naturelles. Botanique. 7e sér. 1890. XI. 185.—Transl. in Z. g. Br. 1890. XIII. 413.—Ch. C. 1890. II. 757.—K. J. I. 41.

XII. Les ascospores chez le genre Saccharomyces.—Compte rendu, etc., de Carlsberg. 1883. II. 13.—Transl. in Z. g. Br. 1883. VI. 310.

XIII. Sur les Torulas de M. Pasteur.—Compte rendu, etc., de Carlsberg. 1883. II. 47.—Transl. in Z. g. Br. 1883. VI. 460.

XIV. Maladies provoquées dans la bière par des ferments alcooliques.—Compte rendu, etc., de Carlsberg. 1883. II. 52.—Transl. in Z. g. Br. 1883. VI. 477.

XV. Méthodes pour obtenir des cultures pures de Saccharomyces et de microorganismes analogues.—Compte rendu, etc., de Carlsberg. 1886. II. 92.—Transl. in Z. g. Br. 1886. IX. 270.

XVI. Les voiles chez le genre Saccharomyces.—Compte rendu, etc., de Carlsberg. 1886. II. 106.—Transl. in Z. g. Br. 1886. IX. 374.

XVII. Sur la germination des spores chez les Saccharomyces.—Compte rendu, etc., de Carlsberg. 1891. III. 44.—Transl. in Z. g. Br. 1891. XIV. 405.—C. f. B. 1891. IX. 663. 1893. XIII. 101.—K. J. II. 35.

XVIII. Kritische Untersuchungen über einige von Ludwig und Brefeld beschriebene Oïdium- und Hefenformen.—Bot. Ztg. 1892. L. 312.—C. f. B. 1. Abt. 1892. XII. 145.—K. J. III. 42.

XIX. Über die neuen Versuche, das Genus Saccharomyces zu streichen.—C. f. B. 1. Abt. 1893. XIII. 16.—K. J. IV. 44.

XX. Anlässlich Juhler's Mitteilung über einen saccharomycesbildenden Aspergillus.—C. f. B. 2. Abt. 1895. I. 65.—K. J. VI. 40.

XXI. Neue Untersuchungen über Alkoholgärungspilze.—Ber. d. D. Bot. Ges. 1884. II. S. XXXII.—Z. g. Br. 1884. VII. 471.

XXII. Nogle Undersögelser over Agaricineernes Biologi.—Hospitalstidende. 1897. 1109.—Botan. Centralblatt. 1898. LXXIV. 114.

XXIII. Über Saccharomyces apiculatus.—Hedwigia. 1880. 75.

XXIV. Sur la vitalité des ferments alcooliques et leur variation dans les milieux nutritifs et à l'état sec.—Compte rendu, etc., de Carlsberg. 1898. IV. 93.—Z. g. Br. 1898. XXI. 624.—C. f. B. 2. Abt. 1898. IV. 862.—K. J. IX. 89.

XXV. Observations sur les levures de bières.—Annales de micrographie. 1888. I. 11.

XXVI. Sur la production de variétés chez les Saccharomyces.—Ann. de micrographie. 1889. II. 214.—C. f. B. 1. Abt. 1890. VII. 795.

XXVII. Biologische Untersuchungen über Mist bewohnende Pilze.—Bot. Ztg. 1. Abt. 1897. LV. 111.

XXVIII. Neue Untersuchungen über die Sporenbildung bei den Saccharomyceten.—C. f. B. 2. Abt. 1899. V. 1.—K. J. X. 21.

XXIX. Über die in dem Schleimflusse lebender Bäume beobachteten Mikroorganismen.—C. f. B. 1. Abt. 1889. V. 632.—Z. g. Br. 1889. XII. 253.

XXX. Sur l'influence que l'introduction de l'air atmosphérique dans le moût qui fermente exerce sur la fermentation.—Compte rendu, etc., de Carlsberg. 1879. I. 88.
XXXI. Über die zymotechnische Analyse der Mikroorganismen der Luft.—Prager Brauer- u. Hopfenzeitung. 1888. 223.—Z. g. Br. 1888. XI. 471.
XXXII. Recherches comparatives sur les conditions de la croissance végétative et le développement des organes de reproduction des levures et des moisissures de la fermentation alcoolique.—Compte rendu, etc., de Carlsberg. 1902. V. 68.
XXXIII. Über Hefe und Hefereinzucht.—Allgem. Zeitschrift f. Bierbrauerei und Malzfabrikation. 1887. XVI. 518.—C. f. B. 1887. II. 118.
XXXIV. Qu'est-ce que la levure pure de M. Pasteur ?—Compte rendu, etc., de Carlsberg. 1891. III. 24.—Transl. in Z. g. Br. 1891. XIV. 305.—C. f. B. 1. Abt. 1891. X. 557.—K. J. II. 143.
XXXV. Unters. u. d. Praxis d. Gärungsindustrie. 3rd Ed. 1895.
XXXVI. W. f. Br. 1887. IV. 378.
XXXVII. Compte rendu, etc., de Carlsberg. 1883. II. 41.
XXXVIII. *Ibid.* 1886. II. 119.
XXXIX. *Ibid.* 1886. II. 130.
XL. *Ibid.* 1888. II. 189.
XLI. C. f. B. 1889. V. 664.
XLII. Medd. fra Carlsb. Laborat. 1892. III. 200; and Unters. aus d. Praxis d. Gärungsind. 1895. Heft I. 79.
XLIII. Annals of Botany. 1895. IX. 549.
XLIV. Compte rendu, etc., de Carlsberg. 1900. V. 1.
XLV. C. f. B. 2. Abt. 1905. XV. 353.
XLVI. Ann. de Micrographie. 1888. I. 49.—Compte rendu, etc., de Carlsberg. 1888 [? 1887]. II. 143.
XLVII. C. f. B. 1889. V. 638.
XLVIII. Compte rendu, etc., de Carlsberg. 1902. V. 68.
XLIX. C. f. B. 1904. 2. Abt. XII. 529.
L. Medd. fra Carlsberg Laborat. 1879. I. Pl. II.
LI. Compte rendu, etc., de Carlsberg. 1879. I. 72.
LII. *Ibid.*, p. 81.
LIII. *Ibid.* 1882 [1879]. I. 47.
LIV. Allgem. Brauer. u. Hopfen Ztg. 1887. XVII. 1109.
LV. Compte rendu, etc., de Carlsberg. 1902. V. 92.
LVI. C. f. B. 2. Abt. 1905. XIV. 545.
LVII. *Ibid.* 1905. XIV. 547.
LVIII. Compte rendu, etc., de Carslberg. 1902. V. 68.
LIX. Medd. fra Carlsberg Laborat. 1879. I. 185, 226.
LX. Compte rendu, etc., de Carlsberg. 1882. I. 203.
LXI. Medd. fra Carlsberg Laborat. 1881. I. 322.
LXII. A. des Sci. Nat. Bot. 1890. XI. 185.
LXIII. C. f. B. 2. Abt. 1903. X. 1. 1905. XIV. 545.
LXIV. Z. f. d. Ges. Br. 1888. XI. 417.
LXV. Untersuchungen a. d. Praxis d. Gärungsindustrie. München u. Leipzig. 1888. Heft I. 62.

Hansgirg, A.,
I. Über neue Süsswasser- und Meeres-Algen und Bakterien, etc. —Sitzgsber. d. Kgl. Böhm. Ges. d. Wissenschaften in Prag. 1890. —K. J. I. 19.

Hantke, E.,
I. Inwieweit stimmen die Gärversuche im kleinen (Laboratoriumsversuche) mit den Gärungen in der Praxis überein ?—Der amerik. Bierbrauer. 1896. XXIX. 213. —Z. g. Br. 1896. XIX. 458.—C. f. B. 2. Abt. 1896. II. 359. —Ch. C. 1896. II. 273.—K. J. VII. 112.
See also Wahl u. Hantke.

Hanuš, Jos., u. Stocký, Alb.,
I. Über die chemische Einwirkung der Schimmelpilze auf die Butter. —Zeitschrift f. Untersuchung der Nahrungs- u. Genussmittel. 1900. III. 606.—Ch. C. 1900. II. 922. —C. f. B. 2. Abt. 1901. VII. 29.—K. J. XI. 235.

Happ, C.,
I. Bakteriologische und chemische Untersuchungen über die schleimige Gärung. Basel. 1893.

This also gives the antecedent literature.—Z. f. B. 1893. XIV. 175.—Ch. C. 1894. I. 161.—Z. g. Br. 1893. XVI. 360.—K. J. IV. 247.

HARDEN, A.,
I. Ber. d. D. Chem. Ges. 1903. XXXVI. 715.

HARDEN, Arthur, and ROWLAND, Sydney,
I. Autofermentation and Liquefaction of Pressed Yeast.—Journal of the Chemical Society of London. 1901. LXXIX. 1227.—Ch. C. 1901. II. 1357.

HARDEN, A., u. YOUNG,
I. Ber. d. D. Chem. Ges. 1904. XXXVII. 1052.
II. Journ. of Physiology. 1905. XXXII. No. 1.
III. Proc. Chem. Soc. 1905. XXI. 189.

HARNACK, E.,
See SCHMIEDEBERG u. HARNACK.

HARRIOTT et CAMUS,
I. C. R. 1897. CXXIV. 235.

HARRISON, Francis C.,
I. Foul Brood of Bees.—Ontario Agricultural College. Bulletin 112. Toronto. 1900.—C. f. B. 2. Abt. 1900. VI. 421.—Gives the earlier literature.
II. C. f. B. 2. Abt. 1902. IX. 206.

HARTIG, Robert,
I. Der echte Haussehwamm. Berlin. 1885.—2nd Ed., edited by C. Freiherrn von Tubeuf. Berlin. 1902. Springer.

HARTMANN, M.,
I. W. f. Br. 1903. XX. 113.

HARZ, C. O.,
I. Grundzüge der alkoholisehen Gärung. München. 1877.—Z. g. Br. 1878. I. 52.
II. Über das Vorkommen von Lignin in Pilzen.—Botan. Centralblatt. 1885. XXIII. 371. 1886. XXV. 386.
III. Sitzungsb. d. Bot. Vereins in München. 1890.
IV. Bot. Centralbl. 1890. XLI. 378.
V. Sitz. d. Ges. f. Morphologie u. Biologie. München. 1901. XVI. 36.

HATCH and ROW,
I. The Lancet. 1900. Dec. 1.

HATTORI,
I. Journ. Coll. of Sci. Imp. Univ. Tokyo. XV. 371.

HAUBNER,
I. Über die fehlerhafte Beschaffenheit der Kuhmilch im Allgemeinen und die blaue Milch insbesondere. — Magazin f. d. gesamte Tierheilkunde. Berlin. 1852. 1, 129.

HAUSER, Gustav,
I. Über Fäulnisbakterien und deren Beziehungen zur Septikämie. Leipzig. 1885. F. C. Vogel.
II. Über Verwendung des Formalins zum Konservieren von Bakterienkulturen. — Münchner medizin. Wochenschrift. 1893. XL. 567, 655.—C. f. B. 1893. XIV. 290, 468.—Ch. C. 1893. II. 691.—K. J. IV. 113.
III. Über das Vorkommen von Mikroorganismen in den lebenden Geweben gesunder Tiere.—Archiv. f. experim. Pathologie und Pharm. 1885. XX. 162.—C. f. B. 1887. I. 230.

HAUTEFEUILLE, P., et PERREY, A.,
I. Contribution à l'étude des levures.—C. R. 1894. CXVIII. 589.—K. J. V. 184.

HAYDUCK, M.,
I. Über den Einfluss der Hopfenharze auf die Biergärung.—W. f. Br. 1892. IX. 617.—Z. g. Br. 1892. XV. 299.—C. f. B. XII. 663.—K. J. III. 117.
II. Über Milchsäuregärung.—W. f. Br. 1887. IV. 285.—C. f. B. 1887. II. 34.—Ch. C. 1887. 1042, 1165.
III. Über den Einfluss des Alkohols auf die Entwicklung der Hefe.—Z. f. Spiritusind. 1882. V. 183.—Reprinted in Z. g. Br. 1882. V. 287.
IV. Über die Entwicklung der Hefe in Nährlösungen von verschiedenem Stickstoff-Gehalt.—Z. f. Spiritusind. 1881. IV. 173.—Reprinted in Z. g. Br. 1881. IV. 287.
V. Über das Degenerieren der Hefe in der Brauerei. — W. f. Br. 1884. I. 345.
VI. Über die Regenerierung der Bierhefe —*Ibid.* 1884. I. 697.

VII. Z. f. Spiritusind. 1880. III. 174.
VIII. *Ibid.* 1881. IV. 341.
IX. W. f. Br. 1885. II. 267.
X. *Ibid.* 1887. IV. 397.
XI. *Ibid.* 1888. V. 937.

HAYDUCK, M., und DELBRÜCK, M.,
I. Bemerkung zu obiger Abhandlung (von Ad. Mayer) [VI.].—Z. f. Spiritusind. 1880. III. 214. —Z. g. Br. 1880. III. 514.

HEBEBRAND, A.,
I. Über die Veränderungen des Brotes beim Sehimmeln. — D. Landw. Versuehsstationen. 1893. XLII. 421.—Hygien. Rundsehau. 1892. II. 1057.—Ch. C. 1893. I. 223. 1896. I. 53.

HEIDEN, E.,
I. Über Stallmistversuehe. — Landwirtsehaftl. Vereinssehrift des Baltischen Centralvereins. 1887. 216, 235.—Ch. C. 1888. 633, 1122.

HEIDENHAIN, M.,
I. Kern und Protoplasma.—Festsehrift für Kölliker. 1892. 118. (Also in pamphlet form. Leipzig. Engelmann.)

HEIM, Carl,
I. Über das Vorkommen von Furfurol im Bier.—Z. g. Br. 1898. XXI. 155, 176.—Ch. C. 1898. I. 1082.—K. J. IX. 163.
II. Über das Vorkommen und den Nachweis des Furfurols im Bier.—Z. g. Br. 1898. XXI. 148.—Ch. C. 1898. II. 146.—K. J. IX. 163.

HEIM, L.,
I. Die Neuerungen auf dem Gebiete der bakteriologischen Untersuehungsmethoden seit dem Jahre 1887.—C. f. B. 1891. X. 260.
II. Zählebige Keime in Gelatine.—C. f. B. 1893. XIII. 649.—K. J. IV. 110.
III. Versuche über blaue Milch.—Arbeiten a. d. K. Gesundheitsamte. 1889. V. 518.—C. f. B. 1890. VIII. 46.—Ch. C. 1889. II. 1029.
IV. Über das Verhalten der Krankheitserreger der Cholera, des Unterleibstyphus und der Tuberkulose in Milch, Butter, Molken und Käse.—Arbeiten a. d. K. Gesundheitsamte. 1889. V. 294. —C. f. B. 1890. VII. 152.

HEIMANN, R.,
See ECKENROTH und HEIMANN.

HEINE, L.,
I. Die Mikrochemie der Mitose, zugleich eine Kritik mikrochemiseher Methoden. — Z. f. physiolog. Chemie. 1896. XXI. 494.—Ch. C. 1896. I. 1022.
II. Über die Molybdänsäure als mikroskopisches Reagens.—*Ibid.* 1897. XXII. 132.—Ch. C. 1896. II. 404.

HEINTZ, W.,
I. Über die Ursaehe der Koagulation des Milehkaseins durch Lab und über die sogen. amphotere Reaktion. — Journal f. prakt. Chemie. 1872. VI. 374.

HEINZE, B.,
I. C. f. B. 2. Abt. 1904. XII. 43. 1905. XIV. 9.
II. Ann. Myeologiei. 1903. I. 344. —C. f. B. 2. Abt. 1904. XII. 180. 1905. XIV. 9.
III. C. f. B. 2. Abt. 1902. VIII. 553. — Z. f. Hygiene. 1904. XLVI. 296.

HEINZE, B., u. COHN, E.,
I. Z. f. Hygiene. 1904. XLVI. 286.

HEINZELMANN, G.,
I. Ein Beitrag zur Sehaumgärungs-Frage bei Reinhefe Rasse II.—Z. f. Spiritusind. 1895. XVIII. 190, 207.—K. J. VI. 177, 178.
II. Beiträge zur Vergärung von Melassemaischen.—Z. f. Spiritusind. 1889. XII. 246.—Osterr.-ung. Zeitschrift f. Zuekerindustrie u. Landwirtsehaft. 1889. XVIII. 509.—K. J. V. 157.
III. Versuche mit einer Weissbierreinzuchthefe.—Z. f. Spiritusind. 1890. XIII. 133.—Ch. C. 1890. I. 1036.
IV. Verhalten verschieden ernährter Hefen in ihrer Gärwirkung. Diastase als Hefenahrungsmittel. —Z. f. Spiritusind. 1897. XX. 296.—Ch. C. 1898. II. 146.—K. J. VIII. 95.
V. Die Zerstörung der Diastase während der Gärung.—Z. f. Spiritusind. 1898. XXI. 357. —K. J. IX. 283.
VI. Z. f. Spiritusind. 1882. V. 458.

Hellriegel, Hermann,
I. Über die Beziehungen der Bakterien zu der Stickstoffernährung der Leguminosen.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. D. Reiches. 1886. 863.—C. f. B. 1887. I. 133.

Helm, Otto,
I. Über Monas prodigiosa und den von ihr erzeugten Farbstoff.—Archiv der Pharmacie. 1875. [3.] VI. 19.—Ch. C. 1875. 118.
II. Über die chemischen Bestandteile der Auswitterungen von Ziegelsteinmauern (Mauerfrass) und die damit verbundene Salpeterbildung. — Schriften der naturforschenden Ges. zu Danzig. 1894. [2.] Bd. 8. Heft 3. —Ch. C. 1894. I. 1084.—K. J. V. 264.

Helmholtz, H.,
I. Müller's Archiv. 1843. X. 453.

Hemmer, M.,
I. Experimentelle Studien über die Wirkung faulender Stoffe auf den tierischen Organismus.—Gekrönte Preisschrift. München. 1866.

Henius, L.,
I. Populäre Vorlesungen über Reinhefe. — American Brewer's Review. 1895, p. 449.—K. J. VI. 172.

Henius, Max,
See Wahl und Henius.

Henneberg, W.,
I. Die Brennereihefen Rasse II. und Rasse XII.—Z. f. Spiritusind. 1903. XXVI. 91.
II. Z. f. Spiritusind. 1902. XXV. 378.
III. W. f. Br. 1902. XIX. 781.
IV. *Ibid.* 1900. XVII. 633.
V. Z. f. Spiritusind. 1904. XXVII. 96.
VI. *Ibid.* 1902. XXV. 553.
VII. W. f. Br. 1903. XX. 178.
VIII. *Ibid.* 1900. XVII. 113.

Henninger,
I. C. R. 1882. XCV. 94.

Hennings, P.,
I. Der Hausschwamm und die durch ihn und andere Pilze verursachte Zerstörung des Holzes. Berlin. 1891. A. Seydel. 8*d.*—Zeitschr. f. Pflanzenkrankheiten. 1891. I. 125.
II. Hedwigia. 1895. XXXIV. 86.
III. Verhandl. d. Botan. Ver. d. Mark Brandenburg. 1898. XL. 173.

Henrici, H.,
I. Beiträge zur Bakteriologie des Käses.—Baseler Dissertat. 1894. —C. f. B. 2. Abt. 1896. II. 40, 245.—Ch. C. 1898. I. 790.—K. J. V. 244.

Henry, J.,
I. Ann. de brasserie et de distillerie. 1902. 129.—Abst. in W. f. Br. 1902. XIX. 325.

Heraeus, Wilhelm,
I. Über das Verhalten der Bakterien im Brunnenwasser, sowie über reduzierende und oxydierende Eigenschaften der Bakterien. Dissert. Berlin.—Z. f. Hygiene. 1886. I. 193.

Herfeldt, E.,
See Burri, Herfeldt und Stutzer.

Hérissey, H.,
I. Comptes rendus Soc. de Biologie, 1896. XLVIII. 915.
II. *Ibid.*, p. 640.
III. Bull. Soc. Mycol. de France. 1899. XV.
IV. C. R. 1901. CXXXIII. 49.
See Bourquelot et Hérissey.

Heron, J.,
I. Über die verschiedenen Methoden der Konservierung der Hefe.—Diary for the Brewing Room. 1896.—W. f. Br. 1896. XIII. 163.—Ch. C. 1896. I. 759.—K. J. VII. 108.

Herselin, M.,
See Rietsch et Herselin.

Héry, M.,
I. Sur une fermentation visqueuse de l'encre.—Annales de Micrographie. 1891. IV. 13.—C. f. B. 1892. XI. 690.—K. J. II. 223.

Herz, Fr. Josef,
I. Die Bedeutung der Bakteriologie für die Käsebereitung.—Mitteilungen des landw. Vereins im Allgäu. 1894. Heft 5.
See also Burstert u. Herz.

Herz, Josef,
I. Über schweflige Säure im Biere. — Repertorium der analyt. Chemie. 1885. V. 58.

HERZFELD, Alexander,
I. Das Auftreten rotfärbender Pilze im Rohzucker.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1891. 663.—C. f. B. 1892. XII. 661.—K. J. II. 225.
II. Über Hefe-Untersuchungen und Unterscheidung der Bierhefe und Presshefe. — Spirituszeitung. 1895. 239.—K. J. VI. 166.

HERZFELD, A., und PAETOW, U.,
I. Anwendbarkeit von Fluorverbindungen zur Verhinderung der Invertzuckerbildung. — Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1891. 678.—C. f. B. 1893. XIII. 191.—K. J. II. 225.

HERZOG, R. O.,
I. Z. f. physiolog. Chemie. 1903. XXXVII. 149.
II. *Ibid.*, p. 383.
III. *Ibid.*, p. 381.

HESS, Friedrich,
I. Vergärung von Saccharose durch die Hefen Saaz, Frohberg und Logos unter verschiedenen Ernährungsbedingungen. Dissert. Erlangen. 1897.—K. J. VIII. 105.

HESS, H.,
I. Über die Gärungsfähigkeit des Milchzuckers.—Bulletin scient. de l'Acad. de St.-Pétersbourg. 1837. II. 126.—Poggendorff's Annalen. 1837. XXXXI. 194.

HESS und BORGEAUD,
I. Eine kontagiöse Euterentzündung, gelber Galt genannt [Mastitis catarrhalis infectiosa.]—Schweizer Archiv f. Tierheilkunde. 1888. XXX. 157.—Baumgarten's Jahresbericht. 1888. IV. 33.

HESS, E., SCHAFFER, F., u. LANG, M.,
I. Über die Wirkungen des Glaubersalzes auf die Beschaffenheit des Euters und der Milch beim Rindvieh.—Landw. Jahrbuch d. Schweiz. 1894. VII. 210.—Ch. C. 1894. II. 486.

HESSE, W.,
I. Über die gasförmigen Stoffwechselprodukte beim Wachstum der Bakterien.—Z. f. Hygiene. 1893. XV. 17.—C. f. B. I. Abt. 1893. XIV. 730.—K. J. IV. 79.

HESSENLAND, Fritz,
I. Über die Zusammensetzung des Hefegummis.—Zeitschr. d. Vereins f. d. Rübenzucker-Industrie des Deutschen Reichs. 1892. XXXXII. 671.—Ch. C. 1892. II. 572.—K. J. III. 67.
See also TACKE, IMMENDORF, HESSENLAND, SCHÜTTE und MINSSEN.

HEST, VAN, J. J.,
I. W. f. Br. 1904. XXI. 1.
II. Z. g. Br. 1903. XXVI. 806, 808.

HEYROTH, Anton,
I. Über den Reinlichkeitszustand des künstlichen Eises.—Arbeiten a. d. K. Gesundheitsamte. 1888. IV. 1.—C. f. B. 1888. IV. 673.

HIEPE, W.,
I. Studien über die Isomaltose und die Amyloine. — The Country Brewer's Gazette. 1893.—W. f. Br. 1894. XI. 28 *et seq.*—Ch. C. 1894. I. 417.—K. J. V. 137.
II. J. Fed. Inst. Brewing. 1895. I. 288.

HIERONYMUS, Georg,
I. Über die Organisation der Hefezellen.—Ber. d. D. Bot. Ges. 1893. XI. 176.—Ch. C. 1893. II. 215. This has the illustrations.—K. J. IV. 50.

HILGER, A.,
I. Förschungsberichte u. Lebensmittel. 1894. I. 132.

HILL, C.,
I. Proc. Chem. Soc. 1898. 156.—Trans. Chem. Soc. LXXIII. 634.
II. Ber. d. D. Chem. Ges. 1901. XXXIV. 1380.
III. Chem. Ztg. 1903. XXVII. 391.

HILLER,
I. Proc. Indiana Acad. Sci. 1901. 272.—Abst. in Bot. Centralbl. 1903. XCII. 333.

HILL-JONES, Th., u. KRESSEL, E.,
I. Engl. Patents Nos. 15145 and 18714 of 1897.

HILTNER, Lorenz,
I. Vermögen auch Nichtleguminosen freien Stickstoff aufzunehmen ?—D. Landw. Versuchsstationen. 1894. XXXXV. 155.—C. f. B. 2. Abt. 1895. I. 198.—Ch. C. 1894. II. 707.—K. J. V. 259.
II. Über die Assimilation des freien atmosphärischen Stickstoffs durch in oberirdischen Pflanzen-

teilen lebende Mycelien.—C. f. B. 2. Abt. 1899. V. 831.—Ch. C. 1900. I. 212.

See also Nobbe und Hiltner, Nobbe, Hiltner u. Schmid, Nobbe, Schmid, Hiltner u. Hotter.

Hjort, Johann,

I. Neue eiweissverdauende Enzyme.—Centralblatt f. Physiologie. 1896. X. 192.—Ch. C. 1896. II. 390.

Höhnel, F. von,

I. Sitz. Kais. Akad. Wiss. Wien, Math.-naturw. Kl. 1902. CXI. 1036.

van 't Hoff, J. H.,

I. Die Lagerung der Atome im Raum. Braunschweig. Vieweg.

Hoffmann, F.,

I. Wie gross ist die Zahl der Mikroorganismen auf dem Getreide unter verschiedenen Bedingungen. —W. f. Br. 1896. XIII. 1153. —K. J. VII. 67.

Hoffmann, Hermann,

I. Mykologische Studien über die Gärung. — Bot. Ztg. 1860. XVIII. 49.

II. Untersuchungen über die Keimung der Pilzsporen. — Jahrbücher für wissenschaftl. Botanik. 1860. II. 267.

III. Über Bakterien. — Bot. Ztg. 1869. XXVII. 233.—Ann. des sciences naturelles. Botanique. 1869. XI. No. 1.—Compare C. R. 1870. LXXI. 150.

IV. Zur Naturgeschichte der Hefe. —Karsten's Botan. Untersuchungen. 1867. I. 341.

V. Mykologische Berichte. 1890. I. 30.

VI. Mitt. d. D. Ges. f. Natur. u. Völkerkunde Ostasiens. 1874. Heft 6, p. 8.

VII. Bot. Ztg. 1869. 309.

Hoffmann, Max,

I. Ein Beitrag zur Translokation des Kupfers beim Keltern gekupferter Trauben.—C. f. B. 2. Abt. 1898. V. 369.—Ch. C. 1898. II. 148.

Hoffmeister, W.,

I. Die Rohfaser und einige Formen der Cellulose. — Landwirtsch. Jahrbücher. 1888. XVII. 239. —Ch. C. 1888. 1211.

Hofmeister, Franz,

I. Die wirksamen Bestandteile des Taumellolchs.—Archiv f. experim. Pathologie u. Pharmakologie. 1892. XXX. 202.—Ch. C. 1892. II. 657.

Holm, J. C.,

I. Z. g. Br. 1889. XII. 301.

Holm, Justus Chr.,

I. Analyses biologiques et zymotechniques de l'eau destinée aux brasseries.—Compte rendu des travaux du laboratoire de Carlsberg. 1892. III. 107.—Z. g. Br. 1893. XVI. 78.—C. f. B. 1893. XIII. 193.—K. J. III. 131.

II. Die Vorrichtungen in der Brauerei zur Kühlung und Lüftung der Würze.—Z. g. Br. 1887. X. 449.

III. Über die Aufbewahrung der Hefe in Saccharoselösung.—C. f. B. 2. Abt. 1896. II. 313.—Ch. C. 1896. II. 251.—K. J. VII. 105.

IV. Sur les méthodes de culture pure et spécialement sur la culture sur plaques de M. Koch et la limite des erreurs de cette methode.—Compte rendu, etc., de Carlsberg. 1891. II. 1.—Z. g. Br. 1891. XIV. 458.—C. f. B. 1892. XI. 576.—K. J. II. 14.

Holm, J. Chr., und Poulsen, S. V.,

I. Jusqu'à quelle limite peut-on, par la méthode de M. Hansen, constater une infection de "levure sauvage" dans une masse de levure basse de Saccharomyces cerevisiæ.—Compte rendu des travaux du laboratoire de Carlsberg. 1886. II. 88.—Z. g. Br. 1886. IX. 241.—C. f. B. 1. Abt. 1887. I. 201.

II. *Ibid.* II.—*Ibid.* 1888. II. 137.—Z. g. Br. 1888. XI. 381.—C. f. B. 1. Abt. 1888. IV. 359.

Holschewnikoff,

I. Über die Bildung von Schwefelwasserstoff durch Bakterien.—Fortschritte der Medizin. 1889. VII. 201.—C. f. B. 1889. VI. 14.—Ch. C. 1889. I. 595.

Holtz, Wilhelm,

I. Beitrag zur Kenntnis der Baumflüsse und einiger ihrer Bewohner. —C. f. B. 2. Abt. 1901. VII. 113.

Holzner, Georg,
I. Dr. Elsner's Ansichten über Bier-Analysen.—Z. g. Br. 1878. I. 481.

Hoppe-Seyler, Felix,
I. Über Gärung der Cellulose mit Bildung von Methan und Kohlensäure.—Z. f. physiolog. Chemie. 1886. X. 201, 401.—Ch. C. 1886. 458, 775.
II. Über Chitin und Cellulose.—Ber. d. D. Chem. Ges. 1894. XXVII. 3329. 1895. XXVIII. 82.—Ch. C. 1895. I. 393 u. 435.
III. Über Huminsubstanzen, ihre Entstehung und ihre Eigenschaften. — Z. f. physiolog. Chemie. 1889. XIII. 66.
IV. Über die chemische Zusammensetzung des Eiters.—Medic.-chem. Untersuchungen. 1871. 486.
V. Über Lecithin und Nuclein in der Bierhefe.—Z. f. physiolog. Chemie. 1879. II. 427; III. 374.
VI. Pflüger's Archiv. 1876. XII. 7, 9.
VII. Physiolog. Chemie. 1877. I. 116. Archiv. Hyg. 1876. XII. 14.
VIII. Medic.-chem. Untersuchungen. 216.
IX. Ber. d. D. Chem. Ges. 1871. IV. 810.

Hoppe-Seyler, F., und Araki, Tr.,
I. Über die Einwirkung der bei Sauerstoffmangel im Harne ausgeschiedenen Milchsäuren auf polarisiertes Licht und die Rotationswerte aktiver Milchsäuren im Allgemeinen.—Z. f. physiolog. Chemie. 1895. XX. 365.—Ch. C. 1895. I. 703.

Horn, Franz Maximilian,
I. Benzoesäure in der Milch.—Zeitschrift für die chemische Industrie. 1887. 239. — Ch. C. 1888. 260.

Horváth, Alexis,
I. Über den Einfluss der Ruhe und der Bewegung auf das Leben.—Pflüger's Archiv für Physiologie. 1878. XVII. 125.

Hosaeus, Hans,
See Koch u. Hosaeus.

Hotter, Eduard,
I. Über den Gehalt an schwefeliger Säure der steirischen Obst- und Traubenweine. — IV. Jahresbericht d. Pomolog. Landes-Versuchs- und Samen-Kontroll-Station in Graz für 1895-96. 26.
II. Versuche und Untersuchungen über den Einfluss verschiedener Weinhefen auf die Vergärung des Apfelmostes.—I. Jahresber., etc. Graz. 1894. 28, 25.
III. Versuche über Beerenwein-Bereitung. — II. Jahresbericht, etc. Graz. 1894. 19.
See also Nobbe, Schmid, Hiltner u. Hotter.

Hotz,
I. Über Konservierung der Hefe.—American Brewer's Review. 1896. IX. 297.

Hoyer, D. P.,
I. Die Generationsdauer verschiedener Hefearten.—C. f. B. 2. Abt. 1899. V. 703.—K. J. X. 105.

Hubert, A.,
See Nivière et Hubert, Fernbach et Hubert.

Hüfner, G.,
I. Ausscheidung von freiem Stickgas bei der Verwesung stickstoffhaltiger organischer Materie.—Journal f. prakt. Chemie. 1876. XIII. 292.—Biedermann's Centralblatt. 1877. XI. 74.—Ch C. 1876. 423.

Hueppe, Ferdinand,
I. Die Methoden der Bakterien-Forschung. 5th Ed. 1891. Wiesbaden. C. W. Kreidel.
II. Die Formen der Bakterien und ihre Beziehungen zu den Gattungen und Arten. Wiesbaden. 1886. Kreidel. 4*s*.
III. Über einige Vorfragen zur Desinfektionslehre und über die Hitze als Desinfektionsmittel.—Deutsche militärärztliche Zeitschrift. 1882. No. 2.
IV. Untersuchungen über die Zersetzungen der Milch durch Mikroorganismen. — Mitteilung. aus dem K. Gesundheits-Amte. 1884. II. 309.—Ch. C. 1884. 315.
V. Über die Verwendung von Eiern zu Kulturzwecken.—C. f. B. 1888. IV. 80.

VI. Naturwissenschaftliche Einführung in die Bakteriologie. —Wiesbaden. 1896. C. W. Kreidel.

VII. Uber Milchsterilisierung und über bittere Milch mit besonderer Rücksicht auf die Kinder-Ernährung.—Berliner klinische Wochenschrift. 1891. 717.—Ch. C. 1891. II. 722.—K. J. II. 191.

VIII. Über Chlorophyllwirkung chlorophyllfreier Pflanzen. — Tageblatt der 60. Naturforscherversammlung zu Wiesbaden. 1887. 244.—C. f. B. 1888. III. 420.—Ch. C. 1887. 1512.

Hulle, L. van den, et Laer, H. van,

I. Nouvelles recherches sur les bières bruxelloises à fermentation dite spontanée.—Mém. cour. et autres mém. publ. par l'Acad. Royale de Belgique. 1891. XV. —Z. g. Br. 1891. XIV. 578.—W. f. Br. 1891. VIII. 952.—K. J. II. 131.

II. Mém. cour., etc., par l'Acad. Royale de Belgique. 1890.—Abst. in W. f. Br. 1891. VIII. 984.

Huppert, H.,

I. Anleitung zur qualitativen und quantitativen Analyse des Harns. 10th Ed. Wiesbaden. 1898.

Husemann, August u. Theodor,

I. Die Pflanzenstoffe in chemischer, physiologischer, pharmakologischer und toxikologischer Hinsicht. 2nd Ed. 1882.

Husemann, Th.,

I. Über Brot. — Pharmaceutische Zeitung. Berlin. 1892. XXXVII. 128.—Ch. C. 1892. I. 561.

II. Notices from the Pharmaceut. Zeitschrift f. Russland, 1866, 572, in Jahresbericht ü. d. Fortschritte d. Pharmakognosie, Pharmacie und Toxikologie. 1866. I. 428.

Huth, S. von,

1. Wie man im Brauereibetriebe der Sarcina Einhalt thut.—Allgem. Zeitschrift f. Brauerei und Malzfabrikation. 1888. 510.—Vierteljahrsschrift f. Nahrungsmittel - Untersuchung und Hygiene. 1888. III. 158.—Ch. C. 1888. 1539.

Hyde, Ch. F.,

I. On Fermentation.—Journal of the Federated Institutes of Brewing. 1895. I. 380.—Z. g. Br. 1896. XIX. 112.—W. f. Br. 1895. XII. 754.—K. J. VI. 141.

See also Miller and Hyde.

Ide, M.,

I. Anaérobiose du bacille commun de l'intestin et de quelques autres bactéries. — La Cellule. 1891. VI. 325.—K. J. II. 244.

Ikeno,

I. Ber. d. D. Bot. Ges. 1903. XXI. 259.

Iljenko, Paul,

I. Über Fäulnisprodukte des Tierkaseins.—Annalen d. Chem. u. Pharm. 1847. LXIII. 264.

Iljinsky, N. W.,

I. Materialien zur Lehre über den Hospitalkwass.—Wratsch. 1881. 85. Russian.

Ilkewitsch,

I. Neue Methode zur Entdeckung von Tuberkelbacillen in der Milch mit der Centrifuge.—Münch. med. Wochenschr. 1892. No. 5. —C. f. B. 1892. XII. 441.—K. J. III. 177.

Immendorff, H.,

I. Beiträge zur Lösung der Stickstoff - Frage. — Landw. Jahrbücher. 1892. XXI. 281.—Ch. C. 1892. I. 394.—K. J. III. 217.

II. Die Wirksamkeit der wichtigsten chemischen Konservierungsmittel des Stalldüngers.—Journal f. Landw. 1893. XXXXI. 1. —Ch. C. 1893. II. 115.

III. Zur Frage der Stickstoffkonservierung im Stalldünger.—Journal f. Landw. 1894. XXXXII. 69.—Ch. C. 1894. I. 1113.—K. J. V. 261.

See also Tacke, Immendorff, Hessenland, Schütte und Minssen.

Inouye, M.,

I. Die Bereitung und chemische Zusammensetzung von Tofu.—College of Agriculture. Tokio. Bulletin. 1895. II. 208.—Ch. C. 1896. I. 127.

II. Bemerkungen über Nukamiso. —College of Agriculture. Tokio. Bulletin. 1895. II. 216.—Ch. C. 1896. I. 128.

Inui, T.
I. Journ. College of Science, Univ. Tokyo. 1901. XV. 465.
II. *Ibid.* 1901. XV. 405.
III. W. f. Br. 1902. XIX. 218.

Irmisch, M.,
I. W. f. Br. 1889. VI. 201, 413.
II. *Ibid.* 1891. VIII. 1135.

Issaew,
I. Z. g. Br. 1900. XXIII. 796.

Istrati, C., und Proca, G.,
I. Zur Zusammensetzung der Braga. —Buletinul societăţii de seiinţe din Bucuresci. 1899. V. 78.—Ch. C. 1899. II. 317.

Istvánffi, Gyula von,
I. Über die Rolle der Zellkerne bei der Entwicklung der Pilze.—Ber. d. D. Bot. Ges. 1895. XIII. 452.—C. f. B. 2. Abt. 1896. II. 352.

Iwanoff, L.,
I. Beitr. z. chem. Physiologie u. Pathologie. 1902. I. 524.—C. f. B. 2. Abt. 1904. XIII. 139.
II. Z. f. physiolog. Chemie. 1904. XLII. 464.

Iwanowski, D.
I. Über die Wirkung des Sauerstoffes auf die alkoholische Gärung.—Mélanges biologiques tirées du Bulletin de l'Acad. imp. des sciences de St. Pétersbourg. 1894. XIII.—K. J. V. 116.
II. C. f. B. 2. Abt. 1903. X. 151.
III. Bull. de l'Acad. imp. des sciences de St. Pétersbourg. 1893. Noticed in Koch's Jahresber. 1894. V. 116.

Iwanowski, D., u. Obrastzow, S.,
I. Über die Wirkung des Sauerstoffes auf die Gärung.—C. f. B. 2. Abt. 1901. VII. 305.—Ch. C. 1901. I. 1380.

Jaccard, M.,
See Chuard u. Jaccard.

Jacobsen, J. C.,
I. Über das Ausarten der Hefe und über Mittel zur Beseitigung derselben.—Allgem. Zeitschrift f. Bierbrauerei u. Malzfabrikation. 1884. XIII. 496.
II. Die Reinzüchtung von Brauereihefe.—Z. g. Br. 1885. VIII. 117.

Jacobsthal, H.,
I. Die Fettbildung bei der Reifung des Käses.—Pflüger's Archiv. 1893. LIV. 484.—Ch. C. 1893. II. 218.

Jacoby, C.,
I. Das Sphacelotoxin, der spezifisch wirksame Bestandteil des Mutterkornes. — Archiv f. experim. Pathologie und Pharmakologie. 1897. XXXIX. 85.—Ch. C. 1897. I. 1059.

Jacquemart, F.,
I. Les Ptomaïnes. Histoire et caractères. Mémoire couronné.—Journal de médecine, de chirurgie et de pharmacologie. Bruxelles. 1890. No. 18.—C. f. B. 1891. IX. 107.

Jacquemin, Georges,
I. Fabrication industrielle de l'acide lactique.—Bulletin de la Société chimique de Paris. 1891. (3.) V. 294.—Journal de Pharmacie et de Chimie. 1891. XXIII. 229.—Ch. C. 1891. I. 698.—K. J. II. 178.
II. Les Fermentations rationelles. Nancy. 1900. 12*s.* 6*d.*
III. A New or Improved Method of an Apparatus for the Manufacture of Pure Yeast.—Engl. Patent No. 21011, Sept. 22, 1896.—K. J. IX. 100.

Jago, W.,
I. Fermentation in its Relation to Bread-making.—The Journal of the Society of Chemical Industry. 1887. VI. 164.—Ch. C. 1887. 570.

Jaksch, Rudolf von,
I. Studien über den Harnstoffpilz. —Z. f. physiolog. Chemie. 1881. V. 395.
II. Über die Entwicklungsbedingungen des Micrococcus ureæ. —Medic. Centralblatt. 1880. XVIII. 180. — Ch. C. 1880. 214.
III. Ber. d. D. Chem. Ges. 1886. XIX. 782.

Jalowetz, Eduard,
I. Über das Vorkommen der Glykase im Gersten-u. Mais-Darrmalz.—Mitteilgen. d. österr. Versuchsstation f. Brauerei u. Mälzerei. 1892. V. Heft.—K. J. III. 255.

II. Mitt. d. österr. Versuchsstation f. Brauerei u. Mälzerei in Wien. 1894. —Chem. Zeit. 1894. XVIII. 39.

Jamieson, J.,
I. The Influence of Light on the Development of Bacteria. — Nature. 1882. XXVI. 244.

Janczewski, Eduard von,
I. Les périthèces du Cladosporium herbarum.—Anzeiger der Akademie d. Wissenschaften in Krakau. 1893. 271.—Bot. Ztg. 1894. LII. 71.
II. Cladosporium herbarum i jego najpospolitsze na zbożu towarzysze. — Rozpravy Akademii Umiejetności. 1895. Ser. II. Tom. VII. p. 143. With four plates. Also gives the antecedent bibliography.—Résumé in Anzeiger, etc. 1894. 187.—Bot. Ztg. 1894. LII. 359.—Zeitschr. f. Pflanzenkrankheiten. 1894. IV. 247, 325.
III. Polymorphisme du Cladosporium herbarum.—Anzeiger, etc., pro 1892. 417.

Janowski, Th.,
I. Über den Bakteriengehalt des Schnees.—C. f. B. 1888. IV. 547.—Ch. C. 1889. I. 48.
II. Zur Biologie der Typhusbacillen. —C. f. B. 1890. VIII. 167.

Janssens, Fr. A.,
I. Beiträge zu der Frage über den Kern der Hefezelle.—C. f. B. 1893. XIII. 638.—Ch. C. 1893. II. 281.—K. J. IV. 46.

Janssens, Fr. A., et Leblanc, A.,
I. Recherches cytologiques sur la cellule de levure.—La Cellule. 1898. XIV. 203.—Annales de micrographie. 1898. X. 113.—C. f. B. 2. Abt. 1898. IV. 930.

Janssens, F. A., et Mertens, Ad.,
I. La Cellule. 1903. XX. 351.

Jégon,
I. Beiträge zur Kenntnis mannithaltiger Weine und Bestimmung des Mannits.—Journal de Pharm. et de Chimie. 1893. XXVIII. 103.—Ch. C. 1893. II. 500.—K. J. IV. 152.

Jegunow, Michael,
I. Sur les sulfo-bactéries des limans d'Odessa.—Archives des Sciences biologiques de St. Pétersbourg. 1895. III. 381.—Ch. C. 1895. I. 1123.
II. Bakteriengesellschaften.—C. f. B. 2. Abt. 1896. II. 11.—K. J. VII. 58.
III. *Ibid.*—*Ibid.* 441.

Jensen, C. O.,
I. Bakteriologiske Undersögelser over visse Mälke- og Smörfeil. 1891.—C. f. B. 1892. XI. 409. —K. J. II. 181.
II. C. f. B. 2. Abt. 1902. VIII. 251.
III. *Ibid.*, p. 11.

Jensen, Orla,
I. Studien über die Lochbildung in den Emmenthaler Käsen.—C. f. B. 2. Abt. 1898. IV. 217.
See also Freudenreich u. Jensen.

Jentys, Stefan,
I. Über den Einfluss hoher Sauerstoffspannungen auf das Wachstum der Pflanzen.—Unters. a. d. Botan. Inst. zu Tübingen. 1888. II. 419.

Jeserich, P., und Niemann, F.,
I. Über einige Fälle von Wurst- und Fleischvergiftung. — Hygien. Rundschau. 1893. III. 813.—Ch. C. 1894. I. 210.

Jesser, J.,
Über Gärungsprodukte der Raffinose. Österr.-ung. Zeitschr. f. Zuckerindustrie u. Landwirtschaft. 1889. XVIII. 598.—Ch. C. 1890. I. 451.

Jodlbauer, Max,
I. Über die Anwendbarkeit der alkoholischen Gärung zur Zuckerbestimmung. — Zeitschrift f. Rübenzucker-Industrie. 1888. —Z. g. Br. 1888. XI. 252.—C. f. B. 1. Abt. 1888. IV. 168.
II. Z. g. Br. 1888. II. 252.
III. Z. d. Vereins f. Rübenzuckerindustrie. 1888. XXV. 308.

Johan-Olsen, Olav,
I. Zur Pleomorphismusfrage.—C. f. B. 2. Abt. 1897. III. 273.
II. Die bei der Käsereifung wirksamen Pilze. I.—C. f. B. 2. Abt. 1898. IV. 161.—Ch. C. 1898. I. 952.
III. Medd. fra naturh. Forening in Kristiania. 1883. 50.

Johannsen, W.,
I. Sur le gluten et sa présence dans le grain de blé.—Compte rendu des travaux du laboratoire de Carlsberg. 1884. II. 199.—German transl. in Z. g. Br. 1888. XI. 437.

JOHNE, A.,
I. Der Trichinenschauer. With 125 figs. 6th Ed. Berlin. 1898. Parey.—3*s.* 6*d.*, bound.

JOHNSON, E.,
I. English Patent No. 29183 of 1897.

JONES,
I. The Analyst. 1889. XIV. 81.

JÖNSSON,
I. Der richtende Einfluss strömenden Wassers auf wachsende Pflanzen und Pflanzenteile (Rheotropismus).—Ber. d. D. Bot. Ges. 1883. I. 512.

JÖNSSON, B.,
I. Entstehung schwefelhaltiger Ölkörper in den Mycelfäden von Penicillium glaucum. — Botan. Centralblatt. 1889. XXXVII. 201.—Has antecedent bibliography.

JÖRGENSEN, Alfred,
I. Sarcina.—Allgem. Brauer- u. Hopfenzeitung. 1890. 1569.—Z. g. Br. 1890. XIII. 458.
II. Der Ursprung der Weinhefen.—C. f. B. 2. Abt. 1895. I. 321.—Z. g. Br. 1895. XVIII. 155.—Ch. C. 1895. I. 1006.—K. J. VI. 38.
III. Über den Ursprung der Alkoholhefen. — Ber. d. gärungsphysiol. Lab. v. A. Jörgensen. I. 1895.—Z. g. Br. 1895. XVIII. 367.—C. f. B. 2. Abt. 1895. I 823.—K. J. VI. 39.
IV. Über Pilze, welche Übergangsformen zwischen Schimmel und Saccharomyceshefe bilden und die in der Brauereiwürze auftreten.—C. f. B. 2. Abt. 1896. II. 41.—Ch. C. 1896. I. 1009.—K. J. VII. 35.
V. Die Hefenfrage.—C. f. B. 2. Abt. 1898. IV. 860.—K. J. IX. 35.
VI. Hansen's System of Pure Yeast Culture in English Top-Fermentation.—Transactions of the Institute of Brewing. 1894. VII. 227.—Z. g. Br. 1893. XVI. 299.—K. J. V. 184.
VII. Untersuchungen über das Ausarten der Brauereihefe.—Z. g. Br. 1898. XXI. 113.—C. f. B. 2. Abt. 1898. IV. 586.—K. J. IX. 91.
VIII. Zur Analyse der obergärigen Hefe in Brauereien und Brennereien nach Hansen's Methode. —Z. g. Br. 1891. XIV. 45.—C. f. B. 1. Abt. 1891. IX. 602. —K. J. II. 146.
IX. Die Aufbewahrung der ausgewählten Heferassen.—Allgem. Zeitschrift f. Bierbrauerei u. Malzfabrikation. 1890. XIX. 1215.—K. J. I. 70.
X. Die Behandlung der Würze mittelst der Centrifuge.—Z. g. Br. 1890. XIII. 49.
XI. Biological Studies on English Yeast-types, with Particular Regard to their Practical Use.—Journal of the Federated Institutes of Brewing. 1899. V. 257.—K. J. X. 120.
XII. Die Mikroorg. d. Gärungsindustrie. 1898. 276.
XIII. *Ibid.* 1898.

JÖRGENSEN, A., u. BERGH, A.,
I. Apparat zur kontinuierlichen Fortpflanzung von Mikroorganismen. German Patent No. 58075, Apr. 17, 1890.—W. f. Br. 1892. IX. 198.—K. J. III. 162.

JOST,
I. Vorlesungen u. Pflanzen-physiologie. Jena. 1904.

JOUBERT, J.,
See PASTEUR et JOUBERT, *and* PASTEUR, JOUBERT et CHAMBERLAND.

JOUSSET,
I. Comptes rendus de la Soc. de Biologie. Paris. 1903.—Abst. in Botan. Centralbl. 1904. XCV. 297.

JUBERT, P.,
1. Über das Gummi der Rübensäfte.—Sucrerie indigène. 1874. No. 9 et 10.—Zeitschrift d. Vereins f. d. Rübenzucker-Industrie d. Deutschen Reiches. 1875. 105, 110. 1876. 747.

JUHLER, John J.,
I. Umbildung eines Aspergillus in einen Saccharomyceten.—C. f. B. 2. Abt. 1895. I. 16, 326.—K. J. VI. 37.

JUMELLE, Henri,
I. Sur une espèce nouvelle de bactérie chromogène, le Spirillum luteum.—C. R. 1892. CXV. 843.—C f. B. 1893. XIII. 340.—K. J. III. 44.

KABRHEL, Gustav,
I. Über das Ferment der Milchsäuregärung in der Milch.—Allgem. Wiener mediz. Zeitung. 1889. No. 52 u. 53.—C. f. B. 1890. VII. 506.
II. Zur Frage der Stellung des Kaseins bei der Milchsäuregärung.—Archiv f. Hygiene. 1895. XXII. 392.—Ch. C. 1895. I. 1008.—C. f. B. 2. Abt. 1895. I. 439.

KABSCH, W.,
I. Untersuchungen über die chemische Beschaffenheit der Pflanzengewebe. — Jahrbücher f. wissenschaftl. Botanik. 1863. III. 357.

KAISER, A.,
I. Chemische Untersuchung des Agaricus muscarius L.—Göttinger Dissert. 1862.

KALANTHAR,
See KALANTHARIANTZ.

KALANTHARIANTZ, Anuschawan,
I. Über die Spaltung von Polysacchariden durch verschiedene Hefenenzyme. Dissert. Berlin. 1898.—Abstract in Z. f. physiolog. Chemie. 1898–99. XXVI. 88.—Ch. C. 1898. II. 1179.—C. f. B. 2. Abt. 1899. V. 43.

KALT, H. P.,
I. Über die Bestimmung von ungelösten stickstoffhaltigen Verbindungen in der Maische.—Z. g. Br. 1886. IX. 489.

KANTER,
I. Über die Wirkung einiger Salze d. Schwermetalle auf d. Wachsthum u. chemische Zusammensetzung v. Asp. niger. Dissn. (Russian). 1903.

KAPPES, Heinrich Constantin,
I. Analyse der Massenkulturen einiger Spaltpilze und der Soorhefe. Diss. 1890. Leipzig.—K. J. I. 28.

KARLIŃSKY, Justyn,
I. Zur Kenntnis der Bakterien der Thermalquellen.—Hygien. Rundschau. 1895. V. 685.—Ch. C. 1895. II. 873.
II. Zur Kenntnis des Bacillus enteritidis Gärtner.—C. f. B. 1889. VI. 289.

KASSNER, G.,
I. Apothekerzeitg. 1869. XI. 584.

KATZ, Julius,
I. Die regulatorische Bildung von Diastase durch Pilze.—Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1898. XXXI. 599.—C. f. B. 2. Abt. 1899. V. 288.—Z. g. Br. 1898. XXI. 434.—K. J. IX. 277.

KATZ, Oskar,
I. Zur Kenntnis der Leuchtbakterien.—C. f. B. 1891. IX. 157. Has the bibliography for 1887 to 1891.—K. J. II. 261.
II. Jahr. wiss. Bot. 1898. XXXIII. 599.

KAYSER, Edmund,
I. Etudes sur la fermentation lactique.—Ann. Inst. Past. 1894. VIII. 737.—C. f. B. 2. Abt. 1895. I. 436.—Ch. C. 1895. I. 92.—K. J. V. 235.
II. Les levures. Paris. 1896.
III. Etudes sur la fermentation du cidre.—Ann. Inst. Past. 1890. IV. 321.—C. f. B. 1890. VIII. 726.—Ch. C. 1891. I. 385.—K. J. I. 61.
IV. Contribution à l'étude physiologique des levures alcooliques du lactose.—Ann. Inst. Past. 1891. V. 395.—C. f. B. 1. Abt. 1891. X. 418.—K. J. II. 138.
V. Note sur les ferments de l'ananas.—Ann. Inst. Past. 1891. V. 456.—C. f. B. 1. Abt. 1891. X. 489.—K. J. II. 134.
VI. Über die Lebensdauer der Hefen.—La Bière. 1895, p. 14.—K. J. VI. 145.
VII. Contributions à l'étude des levures de vin.—Ann. Inst. Past. 1892. VI. 569.—C. f. B. 1. Abt. 1893. XIII. 389.—Ch. C. 1893 I. 838.—K. J. III. 114.
VIII. Action de la chaleur sur les levures.—Ann. Inst. Past. 1889. III. 513.—C. f. B. 1. Abt. 1890. VII. 201.
IX. Ann. Inst. Past. 1896 X. 51.
X. *Ibid.* 1900. XIV. 605.
XI. Le Cidre. 1903. XX. 351.
XII. *Ibid.* 1890. III. 371.
XIII. Ann. Inst. Past. 1893. VII. 41.

KAYSER, Ed., et BARBA, G.,
I. Contribution à l'étude des levures de vin.—Bulletin du Ministère de l'Agriculture. Paris. 1896.—K. J. VII. 126.

KAYSER, Edm., und BOULLANGER, E.,
I. Etude sur les ferments naturels de l'hydromel. Paris. 1897.—Z. g. Br. 1897. XX. 584.—K. J. VIII. 136.
II. Studien über die Bildung des Glycogens in der Hefe.—Annales de la Brasserie et de la Distillerie. 1898.—Ch. C. 1898. II. 440.—K. J. IX. 75.

KAYSER u. DIENERT,
I. Bull. de l'Assoc. d. Chimistes. 1901. XIX. 353.

KAYSER, R.,
I. Über den Gehalt der Weine an schwefliger Säure.—Zeitschr. f. öffentl. Chemie. 1897. III. 513.—Ch. C. 1897. II. 1121.

KEDROWSKI, W.,
I. Über zwei Buttersäure produzierende Bakterien-Arten.—Z. f. Hygiene. 1894. XVI. 444.—C. f. B. 1894. XVI. 124.—Ch. C. 1894. II. 48.—K. J. V. 266.

KELLER, C. C.,
I. Mitteilungen über die Wertbestimmung von Drogen.—Schweizer Woehenschrift f. Pharmacie. 1894. XXXII. 121, 133. 1896. No. 8.—Ch. C. 1894. I. 931, 932. 1896. I. 765.

KELLNER, MORI, u. NAGOAKA,
I. Z. f. physiolog. Chemie. 1889. XIV. 293.

KELLNER, O.,
I. Chem. Ztg. 1895. XIX. 120, 265.

KELLNER, Oskar,
I. Bildung von Fett aus Eiweisskörpern beim Reifen des Käses.—D. Landw. Versuchs-Stationen. 1880. XXV. 39.
II. Über die Bereitung von Saké, Shoyou und Miso.—Chemiker-Zeitung. 1895. XIX. 97.—Ch. C. 1895. I. 454.
III. Über das Verhalten des Harnstoffs im Ackerboden.—Landw. Jahrbücher. 1886. XV. 712.—C. f. B. 1887. I. 40.

KELLNER, O., und YOSHII, T.,
I. Über die Entbindung freien Stickstoffs bei der Fäulnis und Nitrifikation.—Z. f. physiolog. Chemie. 1887. XII. 95.—Biedermann's Centralblatt. 1887. XXI. 854.—Ch. C. 1887. 1552.—C. f. B. 1888. III. 301.

KERN, Eduard,
I. Über ein neues Milchferment aus dem Kaukasus.—Bulletin de la Société Impériale des Naturalistes de Moscou. 1881. No. 3.—Bot. Ztg. 1882. XL. 264.—Biolog. Centralbl. 1882. II. 137.

KERRY, R., und FRAENKEL, S.,
I. Über die Einwirkung der Bazillen des malignen Ödems auf Kohlehydrate.—Monatshefte f. Chemie. 1890. XI. 268. 1891. XII. 350.—K. J. I. 141; II. 239.
See also OBERMAYER und KERRY.

KHONDABACHIAN,
I. Ann. Inst. Past. 1892. VI. 600.

KIESENWALTER, A.,
I. Die Konservierung der Hefe.—Norddeutsche Brauerzeitung. 1886. 335.

KILIANI, H., und BAZLEN, M.,
I. Ber. d. D. Chem. Ges. 1894. XXVII. 3115.

KIRCHNER, Oskar,
I. Die Krankheiten und Beschädigungen unserer landw. Kulturpflanzen. Stuttgart. 1890. E. Ulmer. 9*s*.
II. Die Wurzelknöllchen der Sojabohne.—Cohn's Beiträge, etc. 1894. VII. 213.

KIRCHNER, O., u. BOLTSHAUSEN, H.,
I. Atlas der Krankheiten und Beschädigungen unserer landwirtschaftl. Kulturpflanzen. Stuttgart. E. Ulmer. 1896.—I. Reihe: Getreide.—II. R.: Hülsenfrüchte, Futtergräser u. Futterkräuter.—III. R.: Wurzelgewächse und Handelsgewächse.—IV. R.: Gemüse- und Küchenpflanzen.—V. R.: Obstbäume.—VI. R.: Weinstock und Beerenobst.

KISSLING, E.,
I. Zur Biologie der Botrytis cinerea.—Dissert. Bern. 1889.—C. f. B. 1890. VII. 543.

KITASATO, Shibasaburo, u. WEYL, Th.,
I. Zur Kenntnis der Anaëroben.—Z. f. Hygiene. 1890. VIII. 41. 1891. IX. 97.—C. f. B. 1890. VIII. 12.—K. J. I. 14; II. 73.

KITICSÁN, P.,
I. Ber. d. D. Chem. Ges. 1883. XVI. 1179.

KJELDAHL,
I. Medd. f. Carlsberg. Labor. 1881. III. 186.
KLADAKIS, Th. M.,
I. Über die Einwirkung des Leuchtgases auf die Lebenstätigkeit der Mikroorganismen. Dissert. Berlin. 1890.—C. f. B. 1890. VIII. 23.
KLARVERWEIDEN, Th. J.,
I. Eenige opmerkingen omtrent Blauwe Kaas. — Amsterdam. 1894. J. J. de Bussry.—Germ. transl. in Milchzeitung. 1894. 540.—Ch. C. 1894. II. 712.
KLASON,
I. Z. g. Br. 1898. XXI. 632.
KLAUDI, Josef, und SVOBODA, Anton,
I. Analyse von Flaschenbier.—Listy chemické. 1890.—Allgem. Brauer- und Hopfenzeitung. 1890. 2079.—K. J. I. 66.
KLEBS, Georg,
I. Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896. G. Fischer. 18*s*.—Bot. Ztg. 2. Abt. 1897. LV. 38.
II. Zur Physiologie der Fortpflanzung einiger Pilze.—Jahrbücher f. wissenschaftl. Botanik. 1898. XXXII. 1.—Bot. Ztg. 2. Abt. 1898. LVI. 278.
KLECKI, Valerian von,
I. Ein neuer Buttersäure-Gärungserreger (Bacillus [saccharobutyricus) und dessen Beziehungen zur Reifung und Lochung des Quargelkäses.—C. f. B. 2. Abt. 1896. II. 169.—Ch. C. 1896. II. 253.—K. J. VII. 189.
KLEIN, Carl,
I. Beitrag zur Kenntnis des roten Malzschimmels. — Mitteilungen der Österr. Versuchsstation f. Brauerei u. Mälzerei in Wien. 1892. V.—C. f. B. 1. Abt. 1893. XIII. 196.
KLEIN, E.,
I. Über ein neues Milchferment aus dem Kaukasus.—Bulletin de la Soc. imp. des naturalistes de Moscou. 1881, p. 141.
KLEIN, E., u. GORDON, M.,
I. C. f. B. 1. Abt. 1903. XXXV. 138.
KLEIN, Ludwig,
I. Botanische Bakterienstudien. I.—C. f. B. 1889. VI. 313.
II. Über einen neuen Typus der Sporenbildung bei den endosporen Bakterien.—Ber. d. D. Bot. Ges. 1889. VII. 57.
III. Über die Ursachen der ausschliesslich nächtlichen Sporenbildung bei Botrytis cinerea.—Bot. Ztg. 1885. XXXXIII. 6.
KLEINSCHMIDT, K., jr.,
I. German Patent No. 105573, Nov. 26, 1898.—Ch. C. 1900. I. 160.
KLÖCKER, Albert,
I. Recherches sur le Saccharomyces Marxianus, Sacch. apiculatus et Sacch. anomalus.—Compte rendu des travaux du laborat. de Carlsberg. 1895. IV. 20.—Annales de Micrographie. 1895. VII. 313.—C. f. B. 2. Abt. 1895. I. 446.—K. J. VI. 34.
II. Die Gärungsorganismen in der Theorie und Praxis der Alkoholgärungsgewerbe. Stuttgart. 1900. 8*s*.
III. Comptes rendus, etc., de Carlsberg. 1895. IV. 22.
IV. *Ibid.* 1900. V. 58, 59.
V. C. f. B. 2. Abt. 1902. VIII. 129.—Comptes rendus, etc., de Carlsberg. 1903. VI. 84.
VI. Comptes rendus, etc., de Carlsberg. 1903. VI. 92. C. f. B. 2. Abt. 1904. XII. 501.
VII. Die Gärungsorganismen. Stuttgart. 1900, p. 242.
KLÖCKER, Alb., und SCHIÖNNING, H.,
I. Experimentelle Untersuchungen über die vermeintliche Umbildung des Aspergillus oryzæ in einen Saccharomyceten.—C. f. B. 2. Abt. 1895. I. 777.—K. J. VI. 40.
II. Experimentelle Untersuchungen über die vermeintliche Umbildung verschiedener Schimmelpilze in Saccharomyceten.—C. f. B. 2. Abt. 1896. II. 185.—K. J. VII. 33.
III. Que savons-nous de l'origine des Saccharomyces ?—Compte rendu des travaux du Laboratoire de Carlsberg. 1896. III. 36.—Annales de Micrographie. 1897. IX. 233.—C. f. B. 2. Abt. 1897. III. 193.—K. J. VII. 34.
IV. Noch einmal Saccharomyces und Schimmelpilze.—C. f. B. 2. Abt. 1898. IV. 460.

V. Über Durchwachsungen und abnorme Konidienbildungen bei Dematium pullulans de Bary und bei anderen Pilzen.—C. f. B. 2. Abt. 1899. V. 505. 1901. VII. 152.
VI. Medd. fra Carlsberg Labor. 1896. IV. 120.
VII. Comptes rendus, etc., de Carlsberg. 1900. V. 47.
VIII. *Ibid.* 1896. IV. 36.

Knecht, W.,
I. C. f. B. 2. Abt. 1901. VII. 161.

Knieriem, Woldemar von, u. Mayer, Adolf,
I. Über die Ursache der Essiggärung.—D. Landw. Versuchsstationen. 1873. XVI. 305.—Ch. C. 1873. 666.

Knoesel, C.,
I. C. f. B. 2. Abt. 1902. VIII. 241.

Kny, L.,
I. Die Beziehungen des Lichtes zur Zellteilung bei Saccharomyces cerevisiæ.—Ber. d. D. Bot. Ges. 1884. II. 129.—Reproduced in Z. g. Br. 1884. VII. 210.
II. Unterrichtsblatt Math. u. Naturw. 1902. No. 1.

Kobert, Rudolf,
I. Über die Bestandteile und Wirkungen des Mutterkorns.—Archiv für experim. Pathologie. 1884. XVIII. 316.—Ch. C. 1885. 66. 1895. II. 175.
II. Über den Kwass und dessen Bereitung.—Histor. Studien a. d. pharmakolog. Inst. d. K. Universität Dorpat. 1896. V. —C. f. B. 2. Abt. 1897. III. 253.—Therapeut. Monatshefte. 1895. IX. 273.—Ch. C. 1895. II. 240.—K. J. VI. 155; VII. 119.
III. Lehrbuch d. Intoxicationen. 1904. II. 186.
IV. Pflüger's Archiv. 1903. XCIX. 116.

Koch, Alfred,
I. Über Morphologie und Entwicklungsgeschichte einiger endosporer Bakterienformen. Habilitationsschrift. Göttingen.—Bot. Ztg. 1888. XXXXVI. 277.—C. f. B. 1888. IV. 358.
II. Apparat zum Filtrieren bakterienhaltiger Flüssigkeiten.—Zeitschrift f. wissenschaftl. Mikroskopie. 1891. VIII. 186.—K. J. II. 21.
III. Vergleichende bakteriologische Untersuchung über die Haltbarkeit der Norweger und Nordsee-Schellfische. — Mitteilungen der Sektion für Küsten- und Hochsee-Fischerei. 1894. No. 8.—C. f. B. 1894. XVI. 967.
IV. Zur Kenntnis der Fäden in den Wurzelknöllchen der Leguminosen. — Bot. Ztg. 1890. XXXXVIII. 607.—C. f. B. 1890. VIII. 709.—K. J. I. 129.
V. Weinbau u. Weinhandel. 1898, p. 236.

Koch, Alfred, u. Hosaeus, Hans,
I. Über einen neuen Froschlaich der Zuckerfabriken.—C. f. B. 1894. XVI. 225.—Ch. C. 1894. II. 703.—K. J. V. 58.
II. Das Verhalten der Hefen gegen Glycogen.—C. f. B. 1894. XVI. 145.—Ch. C. 1894. II. 869.—K. J. V. 110.

Koch, Friedrich,
I. Experimentelle Prüfung des Holzgummi und dessen Verbreitung im Pflanzenreiche.—Pharmac. Zeitschrift f. Russland. 1886. XXV. 619.—Ber. d. D. Chem. Ges. 1887. XX. Ref. 145.

Koch, Robert,
I. Über Desinfektion. — Mitteilungen a. d. Kaiserl. Gesundheits-Amte. 1881. I. 252.—Ch. C. 1882. 509.

Koch, R., und Wolffhügel, G.,
I. Untersuchungen über Desinfektion mit heisser Luft.

Kochs, W.,
I. Gibt es ein Zellleben ohne Mikroorganismen ?—Biolog. Centralblatt. 1894. XIV. 481.—Botan. Centralblatt. 1895. LXII. 112.

Koernicke,
I. Ber. d. D. Bot. Ges. 1904. XXII. 163.

Kogelmann, Franz,
I. Über Milchwein (Kefir). — Deutsche Med. Ztg. 1886.—Pharmaceut. Centralhalle. 1886. XXVII. 42.

Kohl, F. G.,
I. Über den Polymorphismus von Pleospora herbarum Tulasne.—Botan. Centralblatt. 1883. XVI. 26.

Köhler, Julius,
I. Saccharomyces membranæfaciens Hansen.—Mitteilungen d. Österr. Versuchs-Station f. Brauerei, etc. 1892. V.—C. f. B. 1. Abt. 1893. XIII. 131.—K. J. III. 140.

Kohlrausch, Otto,
I. Über die Zusammensetzung einiger essbaren Pilze mit besonderer Berücksichtigung ihres Nahrungswertes. Dissert. Göttingen. 1867.

Kohnstamm, P.,
I. Bot. Centralbl. 1901. X. Beiheft 2, p. 91.

Kohnstein, B.,
I. Beitrag zur Kenntnis der säurebildenden Stoffe in den Gerberbrühen.—Der Gerber. 1886. No. 293.

Kokosinski, Ed.,
I. Application industrielle de la méthode Hansen à la fermentation haute (dans le Nord de la France).—Comptes rendus de la Société des brasseries belges. Station scientifique de Brasserie. Gand. 1890. I. 13.—Z. g. Br. 1891. XIV. 56.—K. J. I. 70.

Kolbe,
I. Liebig's Ann. 1860. CXIII. 244.

Kolkwitz, R.,
I. Über den Einfluss des Lichtes auf die Atmung der niederen Pilze.—Pringsheim's Jahrbücher f. w. Bot. 1898. XXXIII. 128. —C. f. B. 2. Abt. 1899. V. 222. —Ch. C. 1899. I. 1250.

Komeros, K., u. Haunalter, E. von,
I. Z. f. d. landw. Versuchswesen in Österreich. 1902. V. 1225.

König, J.,
I. Chemie der menschlichen Nahrungs- und Genussmittel. 3rd Ed. Berlin. 1889–93.

Korff, Gustav,
I. Einfluss des Sauerstoffs auf Gärung, Gärungsenergie und Vermehrungsvermögen verschiedener Heferassen unter verschiedenen Ernährungsbedingungen. Dissert. Erlangen. 1898.—C. f. B. 2. Abt. 1898. IV. 465.—Ch. C. 1898. II. 441.—K. J. IX. 79.

Korschelt, O.,
I. Über Sake, das alkoholische Getränk der Japaner.—Dingler's polytechn. Journal. 1878. CCXXX. 76.
II. Mitteil. d. D. Ges. f. Natur-und, Völkerkunde Ostasiens. 1876. Heft 16. 240.

Kosai, J., und Yabe, K.,
I. Über die bei der Sakebereitung beteiligten Pilze.—C. f. B. 2. Abt. 1895. I. 619.—K. J. VI. 152.

Kosinski,
I. Jahr. wiss. Bot. 1901. XXXVII. 137.

Kosjatschenskow,
I. Journ. f. experim. Landw. 1903. 439. (Russian.)—Bot. Centralbl. 1904. XCV. 591.

Kossel, Albrecht,
I. Zur Chemie des Zellkerns.—Z. f. physiolog. Chemie. 1883. VII. 7.
II. Über die chemische Zusammensetzung der Zelle.—Archiv d. Anatomie u. Physiologie. 1891. Physiolog. Abt. 181.—Ch. C. 1891. II. 37.
III. Über das Nuclein der Hefe.—Z. f. physiol. Chemie. 1879. III. 284. 1880. IV. 290.—Ch. C. 1879. 583.
IV. Über die Nucleine.—Centralbl. f. die mediz. Wissenschaften. 1889. XXVII. 417, 593.—Ch. C. 1889. II. 143, 504.
V. Über die Nucleinsäure.—Archiv d. Anatomie und Physiologie. Physiolog. Abt. 1893. 157.—Ch. C. 1893. I. 787.
VI. Nucleinsäure.—Centralbl. f. d. mediz. Wissenschaften. 1893. 497.—Ch. C. 1893. II. 649.
VII. Über die Herkunft des Hypoxanthins in den Organismen.—Z. f. physiolog. Chemie. 1881. V. 152.
VIII. Über die Verbreitung des Hypoxanthins im Tier- und Pflanzenreich. *Ibid.* 1881. V. 267.
IX. Über Xanthin und Hypoxanthin. — *Ibid.* 1882. VI. 422.
X. Über Guanin. — *Ibid.* 1884. VIII. 404.
XI. Weitere Beiträge zur Chemie des Zellkernes.—*Ibid.* 1886. X. 248.—Ber. d. D. Chem. Ges. 1885. XVIII. 79. 1928.

Kossel, A., und Dakin, H. D.,
I. Z. f. physiolog. Chemie. 1904. XLI. 321; XLII. 181.

Kossel, A., und Neumann, A.,
I. Über das Thymin, ein Spaltungsprodukt der Nucleinsäure.—Ber. d. D. Chem. Ges. 1893. XXVI. 2753.—Ch. C. 1894. I. 40.
II. Darstellung und Spaltungsprodukte der Nucleinsäure (Adenylsäure).—Ber. d. D. Chem. Ges. 1894. XXVII. 2215.—Ch. C. 1894. I. 1158; II. 708.

Kossowicz, Alexander,
I. Untersuchungen über das Verhalten der Hefen in mineralischen Nährlösungen. I.—Z. f. d. landw. Versuchswesen in Österreich. 1903. VI. 27.—Ch. C. 1903. I. 475.
II. Z. f. d. landw. Versuchswesen in Österreich. 1903. VI. 731.
III. *Ibid.* 1906. IX. 688.

Kossowitsch, P.,
I. Durch welche Organe nehmen die Leguminosen den freien Stickstoff auf?—Bot. Ztg. 1892. L. 697.—Ch. C. 1893. I. 613.—K. J. III. 193.
II. Fixieren die Algen freien Stickstoff?—Bot. Ztg. 1. Abt. 1894. LII. 97.—Ch. C. 1894. II. 244.—K. J. V. 257.

Kostytschew,
I. Ber. d. D. Bot. Ges. 1902. XX. 327. 1904. XXII. 207.

Kosutány,
I. Landw. Versuchs-Stationen. 1898. XLIX. 174.
II. *Ibid.* 1892. XL. 217.

Kotljar, E.,
I. Zur Frage über den Einfluss des Lichtes auf Bakterien.—Wratsch. 1892. No. 39–40.—C. f. B. 1892. XII. 836.—K. J. III. 77.

Kozai, Y.,
I. Chemische und biologische Untersuchungen über Sake-Bereitung.—C. f. B. 2. Abt. 1900. VI. 385.—Ch. C. 1900. II. 220.
II. C. f. B. 2. Abt. 1900. VI. 385.

Krafkoff,
I. Zur Frage vom Glycogen der Pilze.—Scripta botanica horti universitatis Imperialis Petropolitanæ. III. fasc. 1. p. 17.

Král, Franz,
I. Weitere Vorschläge und Anleitungen zur Anlegung von bakteriologischen Museen.—Z. f. Hygiene. 1889. V. Heft 3.—C. f. B. 1889. V. 392.
See also Soyka und Král.

Kramer, Ernst,
I. Bakteriologische Untersuchungen über das Umschlagen des Weines.—D. landw. Vers.-Stat. 1890. XXXVII. 325.—C. f. B. 1891. IX. 268.—Ch. C. 1890. II. 180.—K. J. I. 141.
II Studien über die schleimige Gärung.—Sitzungsber. d. k. Akad. d. Wiss. in Wien. Monatshefte für Chemie. 1889. X. 467.—C. f. B. 1890. VIII. 77.
III. Österr. landw. Centralbl. 1891. I. 30.—Abst. in Chem. Centralbl. 1891. II. 707.

Krämer, G., und Pinner, A.,
I. Über die Destillationsprodukte des Rohspiritus.—Ber. d. D. Chem. Ges. 1869. II. 401. 1870. III. 75.

Krannhals, H.,
I. Über das kumysähnliche Getränk Kefir und über den Kefir-Pilz.—Deutsches Archiv f. klin. Medicin. 1884. XXXV. 18.

Krasnosselsky, T.,
I. C. f. B. 2. Abt. 1904. XIII. 673.
II. Ber. d. D. Bot. Ges. 1905. XXIII. 142.

Krasser, Fridolin,
I. Über das angebliche Vorkommen eines Zellkerns in den Hefezellen.—Österr. Bot. Zeitschrift. 1885. XXXV. 373.—Bot. Ztg. 1886. XXXXIV. 389.
II. Über den Zellkern der Hefe.—Österr. Bot. Zeitschrift. 1893. XLIII. 14.—Bot. Ztg. 1893 LI. 49.—K. J. IV. 45.

Kratschmer, Florian, und Niemilowicz,
I. Über eine eigentümliche Brotkrankheit. — Wiener klinische Wochenschrift. 1889. No. 30.—C. f. B. 1889. VI. 501.—Ch. C. 1889. II. 981.

Krauch, C.,
I. Beiträge zur Kenntnis der ungeformten Fermente im Pflanzenreich.—D. landw. Vers.-Stat. 1879. XXIII. 77.
II. Über peptonbildende Fermente in den Pflanzen.—*Ibid.* 1882. XXVII. 383.

KRAUS,
I. Versuche mit Pflanzen im farbigen Licht.—Sitzgsber. d. Naturforschenden Gesellschaft in Halle. 1876.—Bot. Ztg. 1876. XXXIV. 505.

KRESSEL, Edward,
I. German Patent No. 89819 of 1896.
See also HILL-JONES und KRESSEL.

KRIEGER,
I. Über die Veränderungen, welche die Bestandteile der Lagerbiere während der Lagerung erleiden. —Amerikan. Bierbrauer. 1894. —K. J. V. 141.

KRÖBER, E.,
I. Z. g. Br. 1895. XVIII. 337.

KRÖNIG, Bernhard, und PAUL, Theodor,
I. Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfektion. — Z. f. Hygiene. 1897. XXV. 1. A bibliography of the subject is given.—Ch. C. 1897. II. 369.—K. J. VIII. 54.
See also PAUL und KRÖNIG.

KRUEGER, R.,
I. Bakteriologisch-chemische Untersuchungen käsiger Butter.— C. f. B. 1890. VII. 425.—K. J. I. 87.
II. Über bittere Milch.—Molkereizeitung. 1890. No. 30.
III. Molkereizeitung. Hildesheim. 1892. Nos. 34–38.

KRUEGER, S.,
I. Über den Einfluss des konstanten elektrischen Stromes auf Wachstum und Virulenz der Bakterien.—Zeitschrift für klinische Medizin. 1893. XXII. 190.—Ch. C. 1893. I. 484.—K. J. IV. 115.

KRÜGER, Friedrich,
I. Über den Einfluss von Kupfervitriol auf die Vergärung von Traubenmost durch Saccharomyces ellipsoideus.—C. f. B. 2. Abt. 1895. I. 10.—Ch. C. 1895. I. 696.

KRÜGER, Wilhelm,
I. Über zwei aus Saftflüssen reingezüchtete Algen.—Zopf's Beiträge zur Physiologie und Morphologie niederer Organismen. 4. Heft. 91. — Leipzig. 1894. Engelmann.

KRÜCKMANN, Emil,
I. Eine Methode zur Herstellung bakteriologischer Museen und Konservierung von Bakterien.— C. f. B. 1894. XV. 851.—K. J. V. 30.

KRUIS, K., und RAÝMAN, B.,
I. Etudes chimique et biologique. II^e partie.—Bulletin de l'Académie des sciences de l'Empereur François Joseph I. Classe des sciences mathematiques et naturelles. Prag. 1894. I.—Z. g. Br. 1895. XVIII. 190.—C. f. B. 2. Abt. 1895. I. 637.—Ch. C. 1895. II. 311.—K. J. V. 143.
II. Z. f. Spiritusind. 1896. XIX. 131.
III. *Ibid.* 1904. XXVII. 311.
See also RAÝMAN und KRUIS.

KRUTZSCH,
I. J. f. pr. Chem. 1844. XXXI. 1.
II. Liebig's Ann. 1844. LII. 311.

KRYSIŃSKI, S.,
I. Pathologische und kritische Beiträge zur Mutterkornfrage. Jena. 1888. G. Fischer.—Has the antecedent bibliography relating to toxicology.

KUBAREW, G. W., u. TERESCHTSCHENKO, N. A.,
I Über Soldatenkwass.—Wratsch. 1897. No. 47.—Pharm. Z. f. Russland. 1897. XXXVI. 733. —Ch. C. 1898. I. 644.

KÜHN, Julius,
I. Das Einsäuern (Einmachen) der Futtermittel. Reprinted from Mentzel u. v. Lengerke's landwirt. Kalender für 1885. II. Teil. Berlin. Parey.

KÜHN, M.,
I. Versuche über Konservierung der Milch für analytische Zwecke. —Der Landwirt. 1894. XXX. 239.—Ch. C. 1894. II. 460.

KÜHNE, W.,
I*a*. Lehrbuch der physiologischen Chemie. 1868. 334.

KÜHNE, W.,
I. Kieselsäure als Nährboden für Organismen. — Z. f. Biologie. 1890. XXVII. 172.—C. f. B. VIII. 410.—K. J. I. 11.

KÜSTER, E.,
I. Zur Kenntnis der Bierhefe.— Biolog. Centralblatt. 1898. XVIII. 305.—C. f. B. 2. Abt. 1899. V. 196.

Kützing, Friedrich Traugott,
I. Mikroskopische Untersuchungen über die Hefe und Essigmutter. —Journal f. prakt. Chemie. 1837. XI. 385.
II. Sphærotilus natans.—Linnea. 1833. VIII. 385.
III. Phycologia generalis. 1843.
IV. Species algarum. 1849. 145.
V. Phycologia generalis. 1843, p. 148.

Kukla, Anton,
I. Die Reinhefe in Böhmen.—Ber. d. Versuchsanstalt für Brauindustrie in Böhmen für 1890.—K. J. II. 147.

Kulisch, Paul,
I. Über die Abnahme der Säure in Obst und Traubenweinen während der Gärung und Lagerung. — Weinbau und Weinhandel. 1889. Nos. 42–44. — Landw. Jahrbücher. 1890. XIX. 83.—Chemikerzeitung. 1889. XIII. 1407.—Ch. C. 1889 II. 954.
II. Die deutschen Ausleseweine, ihr Werden und Wesen. Vortrag. —Weinbau und Weinhandel. 1895. XIII. 389.—Ch. C. 1896. I. 72.—K. J. VI. 149.
III. Untersuchungen über das Böcksern der Weine.—Weinbau und Weinhandel. 1895. XIII. 2.—K. J. VI. 147.
IV. Z. f. angew. Chem. 1896. 418.

Kupfer u. Voit,
I. Münch. med. Wochenschrift. 1897. XLIV. 321.

Kuprianow, J.,
I. Beiträge zur Biologie der Vibrionen. — A. f. Hygiene. 1893. XIX. 282, 291.—C. f. B. 1894. XV. 489.—Ch. C. 1894. I. 161, 280.—K. J. IV. 84.

Kurzwelly,
I. Jahr. wiss. Bot. 1900. XXXVIII. 291.

Kusserow, Reinhold,
I. Die Bedeutung mineralischer und stickstoffhaltiger Nährsubstanzen für die Hefen und deren Gärfähigkeit. — Brennerei-Zeitg. 1897. XIV. No. 318.—C. f. B. 2. Abt. 1898. IV. 154.—K. J. VIII. 84.
II. Die Haltbarkeit der Hefe.—Brennerei-Zeitg. 1898. XV. 1998.—C. f. B. 2. Abt. 1899. V. 39.
III. Die quantitative Bestimmung der Hefe bei Gärversuchen.—Z. f. Spiritusind. 1897. XX. 106.—Ch. C. 1897. II. 767.—K. J. VIII. 94.
IV. Abkürzung der Gärzeit.—Z. f. Spiritusind. 1897. XX. 97.—K. J. VIII. 129.

Kutscher, Fr.,
I. Die Vibrionen- und Spirillen-Flora der Düngerjauche.—Z. f. Hygiene. 1895. XX. 46.—C. f. B. 2. Abt. 1895. I. 645.
II. Z. f. physiolog. Chemie. 1901. XXXII. 59.
III. *Ibid.* 1901. XXXII. 59, 419. 1902. XXXIV. 517, 520.

Kutscher, Fr., u. Lohmann,
I. Z. f. physiolog. Chemie. 1903. XXXIX. 159, 313.

Kuyper,
I. Ann. Mycologici. 1905. III. 52.

Laborde, J.,
I. Sur la casse des vins.—C. R. 1896. CXXIII. 1074.—Ch. C. 1897. I. 447.—K. J. VII. 145.
II. Sur l'absorption d'oxygène dans la casse du vin.—C. R. 1897. CXXV. 248.—Ch. C. 1897. II. 649.—K. J. VIII. 274.
III. De la glycérine dans les vins provenant de raisins atteints de pourriture noble.—Revue de viticulture. 1897, p. 524.—Ch. C. 1898. I. 643.
IV. Sur l'oxydase du Botrytis cinerea.—C. R. 1898. CXXVI. 536.—Ch. C. 1898. I. 746.—K. J. IX. 302.
V. Contribution à l'étude de l'azote contenu dans le vin.—Ann. Inst. Past. 1898. XII. 517.—Ch. C. 1898. II. 734.—K. J. IX. 158.
VI. Ann. Inst. Past. 1897. XI. 1.—Comptes rendus de la Soc. Biologie. 1895. 472.
VII. C. R. 1899. CXXIX. 344.
VIII. Ann. Inst. Past. 1897 [? 1887]. I. 1.

Labourasse, G.,
See Petit et Labourasse.

Lachmann, Johannes,
I. Über Knollen an den Wurzeln der Leguminosen.—Zeitschrift d. Kgl. Lehranstalt und Versuchs-Station Poppelsdorf. 1858. Heft I.—Reproduced later in Biedermann's Centralblatt. 1891. XX. 837.—C. f. B. 1891. X. 90.

Laer, Henri van,
I. Contributions à l'histoire des ferments des hydrates de carbone (Bacille des bières tournées).—Mém. cour. de l'Acad. Royale de Belgique. 1892. XLVII.—C. f. B. 1893. XIII. 129.—Z. g. Br. 1892. XV. 340.—K. J. III. 97.
II. Note sur les fermentations visqueuses. — Extrait des Mémoires publiés par l'Académie Royale de Belgique. 1889. XXXXIII. 36.—German in Z. g. Br. 1890. XIII. 11.—C. f. B. 1890. VII. 308.
III. Studies on Secondary Fermentations and Frets.—Transactions of the Federated Institutes of Brewing. 1894. VII.—K. J. V. 179.
IV. A Few Words on the Occurrence of Furfurol in Alcoholic Liquids.—Journal of the Federated Institutes of Brewing. 1898. IV. 2.—K. J. IX. 161.
V. La question des rapports de l'oxygène avec la levure.—Bulletin de l'association belge des chimistes. 1893. VII. No. 3.—W. f. Br. 1894. XI. 353.—Ch. C. 1894. I. 1083.—K. J. IV. 137.
VI. Recherches sur la composition d'une levure mixte de fermentation haute.—Bulletin de l'Association belge des Chimistes. 1895. IX. 216.—C. f. B. 2. Abt. 1896. II. 91.—K. J. VI. 158.
VII. C. f. B. 2. Abt. 1905. XIV. 550.
VIII. Journ. Federated Inst. Brewing. 1901. VII. 337.
IX. German Patent No. 117,303, Dec. 29, 1898.—Abst. in Chem. Centralbl. 1901. I. 352.
X. Bull. de l'Association belge des Chimistes. 1890. XVI. 177.
XI. W. f. Br. 1894. XI. 353.
XII. Moniteur Scient. 1895. 499.

Laer, H. van, u. Denamur,
I. Moniteur Scient. July 1, 1895.

Lafar, F.,
I. Z. g. Br. 1894. XVII. 10. 1897. XX. 679.
II. Landw. Jahrbücher. 1895. XXIV. 445.
III. C. f. B. 1893. XIII. 684.

Lagerheim, G. von,
I. Svensk Farmac. Tidskrift. 1903. No. 18.

Lamanna, Paolo Antonio,
I. Über die Giftigkeit der Alkohole. — Bollettino chimico farmaceutico. 1899. XXXVIII. 473. —Ch. C. 1899. II. 446.

Landolt, H.,
I. Über die Ursache der Nitrifikation der Ammoniaksalze im Erdboden. — Deutsche landw. Presse. 1888. XV. 185.

Landwehr, Herm. Ad.,
I. Über Mucin, Metalbumin und Paralbumin.—Z. f. physiolog. Chemie. 1884. VIII. 114.

Lang, M., u. Freudenreich, Ed. von,
I. Über Oïdium lactis.—Landw. Jahrbuch d. Schweiz. 1893. VII. 229.—C. f. B. 1894. XVI. 119.—K. J. IV. 184.
See also Hess, Schaffer u. Lang.

Lange, H.,
I. Über den Einfluss verschiedenartiger Stickstoffernährung auf die Hefe.—W. f. Br. 1899. XVI. 49.—C. f. B. 2. Abt. 1899. V. 226.—Ch. C. 1899. I. 699.—K. J. X. 108.
II. Beitrag zur alkoholischen Gärung ohne Hefezellen.—W. f. Br. 1898. XV. 877.—C. f. B. 2. Abt. 1898. IV. 861.—Ch. C. 1898. II. 548.—K. J. IX. 317.
III. Jahrb. d. Versuchs. u. Lehranstalt f. Brauerei in Berlin. 1904. VII. 43.
IV. Chem. Ztg. 1902. XXVI. 200.

Laquerrière,
See Apostoli et Laquerrière.

Laroque,
I. C. R. 1852. XXXV. 221.

Lasché, A.,
I. Saccharomyces Joergensenii.—Der Braumeister. Chicago. 1892. —Z. g. Br. 1892. XV. 113.—C. f. B. 1. Abt. 1892. XII. 558. —K. J. III. 144.
II. Zwei rote Mycoderma-Arten.—Der Braumeister. Chicago. 1892. 278.—C. f. B. 1. Abt. 1893. XIII. 485.—K. J. III. 144.
III. Die Mycoderma und die Praxis. —Der Braumeister. 1891. No. 10.—C. f. B. 1. Abt. 1891. X. 192.—K. J. II. 152.

IV. Die amerikanische Brauereihefe.—Der Braumeister. Chicago. 1891. 180.—K. J. III. 159.

V. Einfluss verschiedener Wärmegrade auf gewisse Hefearten, sowie deren Verhalten in säurehaltigen und alkalischen Nährsubstanzen.—American Brewer's Review. 1893. VI. 237.—K. J. IV. 174.

VI. Der Braumeister. Feb. 20, 1892.—K. J. III. 144.

Laser, Hugo,

I. Die makroskopische Wasseruntersuchung durch Anwendung von Wasserstoffsuperoxyd.—C. f. B. 1894. XVI. 180.—Ch. C. 1894. II. 854.

II. Z. f. Hygiene. 1891. X. 513.

Laurent, Emile,

I. Etudes sur la variabilité du bacille rouge de Kiel.—Ann. Inst. Past. 1890. IV. 465.—C. f. B. 1891. IX. 105.—K. J. I. 38.

II. La bactérie de la fermentation panaire.—Bulletins de l'Acad. Royale des Sciences de Belgique. 1885. 3ème série. X. 765.—C. f. B. 1887. I. 504.—Ch. C. 1887. 118.

III. Über den angeblichen Bakterienursprung der Diastase.—Bullet. de l'Acad. Belg. 1886. X. 38.—Ch. C. 1886. 460.

IV. Sur le microbe des nodosités des Legumineuses.—C. R. 1890. CXI. 754.—C. f. B. 1. Abt. 1891. IX. 703.—K. J. I. 129.

V. Note sur les formes-levures chromogènes.—Bulletin de la Soc. Royale de Botanique de Belgique. 1890. XXIX. II. 76.—K. J. I. 39.

VI. Etudes biologiques. Première partie. Recherches physiologiques sur les levures.—Annales de la Soc. belge de Microscopie. 1890. XIV. 29.—K. J. I. 54.

VII. Recherches sur le polymorphisme du Cladosporium herbarum.—Ann. Inst. Past. 1888. II. 603.

VIII. Nutrition hydrocarbonée et formation de glycogène chez la levure de bière.—Ann. Inst. Past. 1888. II. 113.—C. f. B. 1. Abt. 1889. V. 794.

IX. Recherches sur la valeur comparée des nitrates et des sels ammoniacaux comme aliment de la levure de bière et de quelques autres plantes.—Ann. Inst. Past. 1889. III. 362.—C. f. B. 1. Abt. 1889. VI. 411.

X. Ann. Soc. belge de Microscopie. 1890. XXIV. 54.

See also Schlösing et Laurent.

Lavoisier, Antoine Laurent,

I. Traité de Chimie. 2ème édition. 1793. I. 159.

II. Œuvres. III. 780.—System der antiphlogistischen Chemie. 1792. 161.

Lawes, J. B., and Gilbert, J. H.,

I. The Sources of the Nitrogen of our Leguminous Crops.—Journal of the Royal Agricultural Society of England. Third Series. Vol. II. Part IV. 1892, p. 657.—C. f. B. 1892. XII. 298.—K. J. III. 191.

Lawrow, D.,

I. Z. f. physiolog. Chemie. 1901. XXXIII. 312.

Laxa, Ottokar,

I. Über einen thermophilen Bacillus aus Zuckerfabriksprodukten.—C. f. B. 2. Abt. 1898. IV. 362.—Ch. C. 1898. II. 53.

II. A. f. Hygiene. 1902. XLI. 119.

Lea, A. S., and Dickinson, W. S.,

I. Action of Rennin and Fibrin Ferment.—Journal of Physiology. 1890. XI. 307.—K. J. I. 175.

Lebbin,

I. Über die Verwendbarkeit von Wasserstoffsuperoxyd in der Nahrungsmittel - Analyse. — Pharm. Ztg. 1897. XXXXII. 148.—Ch. C. 1897. I. 670.

II. Über Ovos, ein neues, aus Hefe hergestelltes Fleischextrakt-Ersatzmittel.—Die mediz. Woche. 1901. 195.—Ch. C. 1901. I. 1381.

Lebrasseur, A.,

See Cathelinau u. Lebrasseur.

Lechartier, G., et Bellamy, F.,

I. Etude sur les gaz produits par les fruits.—C. R. 1869. LXIX. 356, 466. 1872. LXXV. 1203. 1874. LXXIX. 949, 1006. 1875. LXXXI. 1127.

Lecomte, Henri,
I. Les tubercules radicaux de l'Arachis hypogæa L.—C. R. 1894. CXIX. 302.—C. f. B. 2. Abt. 1895. I. 520.

Ledderhose, Georg,
I. Über salzsaures Glycosamin.—Ber. d. D. Chem. Ges. 1876. IX. 1200.
II. Über Chitin und seine Spaltungsprodukte. — Z. f. physiolog. Chemie. 1878. II. 213.

Lederer, L.,
I. Über Buttersäure und den Baeillus subtilis.—Chemiker-Zeitung. 1892. XVI. 252.—C. f. B. 1892. III. 242.

Legros,
See Rechter et Legros.

Lehmann, C. G.,
I. Lehrbuch der physiologischen Chemie. Leipzig. 1842.

Lehmann, Jul.,
I. Über die zur Ernährung der Pflanzen geeignetste Form des Stickstoffes.—Biedermann's Centralblatt für Agrieulturehemie. 1875. VII. 403.

Lehmann, Karl Bernhard,
I. Vorläufige Mitteilung über die Desinfektion von Kleidern, Lederwaren, Bürsten und Büchern mit Formaldehyd.—Münchner medizin. Wochensehrift. 1893. No. 30 u. f.—Ch. C. 1894. I. 292.—K. J. IV. 113.
II. Über die Sauerteiggärungen und die Beziehungen des Bacillus levans zum Baeillus eoli eommunis.—C. f. B. 1894. XV. 350.—Ch. C. 1894. II. 102.
III. Qualitative und quantitative Untersuehungen über den Säuregehalt des Brotes.—A. f. Hygiene. 1893. XIX. 363.—C. f. B. 1894. XV. 556.—Ch. C. 1894. I. 518.
IV. Hygienische Studien über Kupfer. VI.—A. f. Hygiene. 1898. XXXI. 279.—Ch. C. 1898. I. 580.

Lehmann, K. B., u. Neumann, R.,
I. Atlas und Grundriss der Bakteriologie. Zwei Bände. With 63 coloured plates. Münehen. 1896. J. F. Lehmann. 15*s.*, bound.—K. J. VII. 1.
Engl. Ed. 1897. London. Baillière, Tindall & Cox. 12*s.* 6*d.*

Lehmann, Victor,
I. Über das Verhalten des Guanins. Xanthins und Hypoxanthins bei der Selbstgärung der Hefe.—Z. f. physiolog. Chemie. 1885. IX. 563.

Leichmann, G.,
I. Über die freiwillige Säuerung der Mileh.—Milehzeitung. 1894. XXIII. 523. 1896. XXV. 67.—C. f. B. 1. Abt. 1894. XVI. 826.—K. J. V. 239.
II. Über die im Brennereiprozess bei der Bereitung der Kunsthefe auftretende spontane Milehsäuregärung.—C. f. B. 2. Abt. 1896. II. 281.—K. J. VII. 139.
III. Über eine sehleimige Gärung der Mileh. — Vers.-Stat. 1894. XLIII. 375.—C. f. B. 1894. XVI. 122.—Ch. C. 1894. I. 827.—K. J. V. 216.

Lemaire, J.,
I. Rôle des infusoires et des matières albuminoides dans la fermentation, la germination et la fécondation.—C. R. 1860. LI. 536.
II. De l'aeide phénique, de son action sur les végétaux, les animaux, les ferments, les venins, les virus, les miasmes et de ses applications à l'industrie, à l'hygiène, aux seiences anatomiques et à la thérapeutique. 2nd edition. Paris. 1865.

Lendner, A.,
I. The Combined Influence of Light and Medium on the Development of Fungi.—U. S. Department of Agrieulture. Experiment Station Record. 1896. VIII.—K. J. VII. 43.
II. Bull. de l'Herbier Boissier. 1903. 3rd Series. III. 362.

Lenz, Wilhelm,
I. Über die Beurteilung von Cognae auf Grund der chemisehen Analyse.—Zeitsehrift f. öffentl. Chemie. 1899. V. 258.—Ch. C. 1899. II. 539.

Lepeschkin, W. W.,
I. C. f. B. 2. Abt. 1903. X. 105.

Lermer, J. C.,
I. Notiz über das Alkaloid des Bieres.—Dingler's polyt. Journal. 1867. CLXXXIV. 159.

Lesage, Pierre,
I. Recherches expérimentales sur la germination des spores du Penicillium glaucum.—Annales des sciences naturelles. Botanique. 8e série. 1895. I. 309.—K. J. VI. 91.
II. Action de l'alcool sur la germination des spores de champignons.—*Ibid.* 1897. III. 151.—K. J. VII. 45.
III. Recherches physiologiques sur les champignons.—C. R. 1894. CXVIII. 607.—Bot. Ztg. 2. Abt. 1895. LIII. 159.—K. J. V. 78.
IV. C. R. 1902.—Bot. Centralbl. 1902. LXXXIX. 87. 1903. XCII. 94.

Leube, W.,
I. Über die ammoniakalische Harngärung.—Virchow's Archiv f. patholog. Anatomie. 1885. C.540.
II. Über Harnstoff-Ferment. — Deutsch. mediz. Zeitg. VII. 379.—Ch. C. 1886. 459.

Leuchs,
I. Weinkunde. 1847.

Leudet, R.,
See Wurtz et Leudet.

Leufvén, Gust. J.,
I. Einfluss der Melkung auf den Bakteriengehalt der Milch. — Redogörelse för verksamheten vid Ultuna landtbruksinstitut år 1894. 38.—Upsala. 1895.—C. f. B. 2. Abt. 1895. I. 824.

Levy, A.,
I. Über spontane Milchgerinnung und die biologische Bedeutung des Gerinnungsprozesses.—C. f. B. 1888. III. 421.
II. Die wahre Natur des Kefirs.—Deutsche Med. Ztg. 1886. 783.

Lewandowski, A.,
I. Über Indol- und Phenolbildung durch Bakterien. — Deutsche mediz. Wochenschrift. 1890. 1186.—K. J. I. 36.

Lewith,
I. Über die Ursache der Widerstandsfähigkeit der Sporen gegen hohe Temperaturen.—Archiv f. experim. Pathologie u. Pharmakologie. 1889. XXVI. 341.

Lewkowitsch, J.,
I. Darstellung rechtsdrehender Mandelsäure aus inaktiver Mandelsäure.—Ber. d. D. Chem. Ges. 1882. XV. 1505.
II. Spaltung der inaktiven Mandelsäure in ihre beiden optisch aktiven Isomeren.—Ber. d. D. Chem. Ges. 1883. XVI. 1568.

Liborius, Paul,
I. Beiträge zur Kenntnis des Sauerstoffbedürfnisses der Bakterien.—Z. f. Hygiene. 1886. I. 115.—Ch. C. 1886. 579.—Bot. Centralbl. 1886. XXVII. 198.

Liebermann, Leo,
I. Über das Nuclein der Hefe und künstliche Darstellung eines Nucleines aus Eiweiss und Metaphosphorsäure. — Ber. d. D. Chem. Ges. 1888. XXI. 598.—Ch. C. 1888. S. 447.
II. Über Nucleine.—Centralblatt f. d. medizin. Wissenschaften. 1889. 210, 497.—Ch. C. 1889. I. 540, 590; II. 335.
III. Nachweis der Metaphosphorsäure im Nuclein der Hefe. — Pflüger's Archiv. 1890. XXXXVII. 155.—Ch. C. 1890. I. 1028.—K. J. I. 32.
IV. Ber. d. D. Chem. Ges. 1882. XV. 437, 2553.

Liebermann, L., u. Bittó, B. v.,
I. Ein Beitrag zur Chemie der Hefezellen.—Centralblatt f. Physiologie. 1894. VII. 857.—Ch C. 1894. I. 824.—K. J. V. 115.
II. Nucleinsäure.—Centralblatt f. d. mediz. Wissenschaften. 1893. 465.—Ch. C. 1893. II. 649.

Liebig, Johannes,
I. Über die Ursachen des raschen Gerinnens der Milch und die Mittel dasselbe zu verhindern. Dissertation. Heidelberg. 1890.—K. J. I. 84.

Liebig, Justus von,
I. Das enträtselte Geheimnis der geistigen Gärung.—Annalen d. Chemie und Pharmacie. 1839. XXIX. 100.
II. Über die Gärung und die Quelle der Muskelkraft.—Sitzungsber. d. bayr. Akademie. 1868 u. 1869.—Annalen d. Chemie und Pharmacie. 1870. CLIII. 1, 137.
III. Liebig's Ann. 1870. CLIII. 8.

Liebscher,
I. Beitrag zur Stickstoff-Frage.—Journal f. Landwirtschaft. 1893. XXXXI. 139. — Ch. C. 1893. II. 94.—K. J. IV. 228.

Liesenberg, C., und Zopf, W.,
I. Über den sogenannten Froschlaichpilz (Leuconostoc) der europäischen Rübenzucker- und der javanischen Rohrzuckerfabriken. —Beiträge zur Physiologie und Morphologie niederer Organismen. Edited by W. Zopf. Heft 1. Leipzig. 1892. 1. Continuation in Heft 2, 1.—C. f. B. 1892. XII. 659. 1893. XIII. 339.—K. J. III. 89.

Lilienfeld, Leo,
I. Über die Wahlverwandtschaft der Zellelemente zu gewissen Farbstoffen.—Archiv f. Anatomie und Physiologie. 1893. Physiol. Abt. S. 391.

Lilienfeld, Leon, und Monti, A.,
I. Über die mikrochemische Lokalisation des Phosphors in den Geweben. — Z. f. physiolog. Chemie. 1893. XVII. 410.—K. J. IV. 74.—Rendiconti della Accademia dei Lincei. Roma. 1893. II. 310.—Ch. C. 1893. I. 50.—Bot. Ztg. 1893. 2. Abt. LI. 245.

Lind, K.,
I. Über das Eindringen von Pilzen in Kalkgesteine und Knochen.—Pringsheim's Jahrbücher. 1898. XXXII. 603. Gives the antecedent bibliography.—C. f. B. 2. Abt. 1899. V. 192.

Lindau, G.,
I. Hedwigia. 1904. XLIII. 306.
II. Hyphomycetes in Rabenhorst's Kryptogamenflora Deutschlands. 2nd Ed. 1904. I. Abt. 8.
III. In Engler-Prantl., Natürl. Pflanzenfamilien. 1900. Teil. 1. Abt. 1. 383, 416.

Lindet, L.,
I. Sur l'oxydation du tannin de la pomme à cidre.—Le Cidre. 1893, p. 150.—C. R. 1895. CXX. 370. Ch. C. 1895. I. 656.
II. Sur le dosage des bases dans les flegmes industriels.—C. R. 1888. CVI. 280.
III. Bull. Soc. Chim. 1889. 3e Série. II. 195.
IV. C. R. 1891. CXII. 102, 663.
V. *Ibid.* 1888. CVII. 182.
VI. *Ibid.* 1890. CXI. 236.

Lindet et Marsais,
I. Ann. de la Brasserie. 1905. 3.

Lindner, G.,
I. Studien über die Biologie u. hygienische Bedeutung der im Essig lebenden Nematoden.—C. f. B. 1889. VI. 633.

Lindner, Paul,
I. Über ein neues, in Malzmaischen vorkommendes, Milchsäure bildendes Ferment.—W. f. Br. 1887. IV. 437.—C. f. B. 1. Abt. 1887. II. 340.—Ch. C. 1887. 1507.
II. Die Sarcina-Organismen der Gärungsgewerbe. Dissert. Berlin. 1888.—C. f. B. 1. Abt. 1888. IV. 427.
III. Die Ursache des langen Weissbieres.—W. f. Br. 1889. VI. 181.
IV. Die Weinsäurekur für sarcinahältige Zeuge.—W. f. Br. 1895. XII. 316.—C. f. B. 2. Abt. 1896. II. 36.
V. Das Langwerden der Würze durch Dematium pullulans.—W. f. Br. 1888. V. 290.—Z. g. Br. 1888. XI. 225.—C. f. B. 1. Abt. 1888. III. 750.
VI. Mikroskopische Betriebskontrolle in den Gärungsgewerben. 2nd Ed. Berlin. 1898. Parey.—3rd Ed. 1901. 17*s.*—C. f. B. 2. Abt. 1902. IX. 936.
VII. Fruchtäther-Bildung durch Hefen in Grünmalz und in Würzen.—W. f. Br. 1896. XIII. 552.—Z. g. Br. 1896. XIX. 675. —Ch. C. 1896. II. 273.—K. J. VII. 100.
VIII. Beobachtungen über die Sporen- und Glycogenbildung einiger Hefen auf Würzegelatine. Die Blaufärbung der Sporen von Schizosaccharomyces octosporus durch Jodlösung.—C. f. B. 2. Abt. 1896. II. 537.—Ch. C. 1896. II. 938.—K. J. VII. 99.
IX. Über Durchwachsungen an Pilzmycelien.—Ber. d. D. Bot. Ges. 1887. V. 153. Gives the antecedent bibliography.
X. Hefezellen als Amöbennahrung und amöbenförmige Hefezellen. —W. f. Br. 1889. VI. 1069.—Z. g. Br. 1890. XIII. 45.—Ch. C. 1890. I. 45.
XI. Das Vorkommen von Amöben im Gärungsbetriebe.—W. f. Br. 1896. XIII. 1231.—K. J. VII. 117.

XII. Monilia variabilis, eine formenreiehe und rassenspaltige neue Pilzart.—W. f. Br. 1898. XV. 209.—C. f. B. 2. Abt. 1898. IV. 931.

XIII. Die Vegetationsverhältnisse im untergärigen Bier während der Vergärung.—W. f. Br. 1895. XII. 477.—C. f. B. 2. Abt. 1895. I. 890.—K. J. VI. 163.

XIV. Saeeharomyees farinosus nnd Saeeharomyees Bailii. Zwei neue Hefenarten aus Danziger Jopenbier.—W. f. Br. 1894. XI. 153.—Ch. C. 1894. I. 609.—K. J. V. 37.

XV. Die Tröpfehenkultur und die Bedeutung des Mikroskopes in der Brauerei.—W. f. Br. 1894. XI. 697. 1896. XIII. 80.—K. J. V. 10.

XVI. Empfiehlt es sieh an Stelle von Kräusen in gewissen Fällen das Bier im Lagerfass nur mit Hefe zu behandeln ?—W. f. Br. 1898. XV. 692.—K. J. IX. 123.

XVII. Bemerkungen zum Moeller'sehen Filter.—W. f. Br. 1893. X. 775.—K. J. IV. 33; III. 157.

XVIII. Ergebnisse einiger Luftuntersuehungen von Brauereien nebst Bemerkungen zu Hansen's Methode der Luftanalyse.—W. f. Br. 1888. V. 877.—Z. g. Br. 1888. XI. 490.

XIX. Welehes sind die besten Hefenrassen zur Vergärung von Diekmaisehen und welehe eignen sieh hervorragend zur Erzielung hoher Hefenausbeuten in der Presshefefabrikation. Mitteilung von Züehtungsresultaten von 37 Reinhefen.—Z. f. Spiritusind. 1890. XIII. Ergänzungsheft. 29.—K. J. I. 60.

XX. Die Resultate von 99 Gärversuehen in tabellariseher Darstellung mit dazugehörigen Erläuterungen.—W. f. Br. 1894. XI. 381.—K. J. V. 109.

XXI. Die Einzellkultur im hängenden Tropfen. Gealterte Zellen in friseher Würze. Abnormale Zellformen und Querwandbildungen bei Betriebshefen.—W. f. Br. 1893. X. 1354.—K. J. IV. 13.

XXII. Über die Erkennung der Heferassen und ihre photographisehe Darstellung.—W. f. Br. 1891. VIII. 815.—C. f. B. 1892. XII. 250.—K. J. II. 149.

XXIII. Das Waehstum der Hefen auf festen Nährböden.—W. f. Br. 1893. X. 690.—C. f. B. 1893. XIV. 372.—K. J. IV. 44.

XXIV. Atlas der mikroskopisehen Grundlagen der Gärungskunde. 111 Tafeln. Berlin. 1903. Parey.

XXV. Wie konserviert man obergärige Hefe am besten ?—Jahrbueh d. Versuehs- u. Lehranstalt f. Brauerei in Berlin. 1899. II. 235.—K. J. X. 173.

XXVI. Der Hefereinzuehtapparat unseres Vereinslaboratoriums.—W. f. Br. 1888. V. 917. 1892. IX. No. 20. Beilage.

XXVII. Hat sieh Reinzuehthefe für Weissbierbrauereien bewährt. In weleher Weise wäre die Einführung zu organisieren ?—W. f. Br. 1898. XV. 717.—K. J. IX. 102.

XXVIII. W. f. Br. 1901. XVIII. 130.

XXIX. Mikroskopisehe Betriebskontrolle, ete. 3rd Ed. 1901. 327.

XXX. W. f. Br. 1893. X. 1298.

XXXI. Mikroskopisehe Betriebskontrolle. 1895, and 3rd Ed., 1901.

XXXII. Jahrb. d. Versuehs- und Lehranstalt, ete., in Berlin. 1904. VII. 448.

XXXIII. Mikroskopische Betriebskontrolle, ete. 3rd Ed. 1901. 314, 317-319.—Atlas der Mikroskopisehen Grundlagen der Gärungskunde. Berlin. Plates 33 and 35.

XXXIV. W. f. Br. 1887. IV. 853.

XXXV. *Ibid.* 1900. XVII. 713 *et seq.*

XXXVI. Mikroskopisehe Betriebskontrolle. 4th Ed. Berlin. 1905. 421.

XXXVI*a*. *Ibid.*, p. 424.

XXXVI*b*. *Ibid.*, p. 427.

XXXVII. *Ibid.*, p. 422.

XXXVIII. W. f. Br. 1903. XX. 505.

XXXIX. C. f. B. 2. Abt. 1895. I. 782.

XL. Mikroskopische Betriebskontrolle. 4th Ed. Berlin. 1905.
XLI. W. f. Br. 1894. XI. 1312.
XLII. Mikroskopische Betriebskontrolle. 3rd Ed. 1901. 109, 380.
XLIII. W. f. Br. 1897. XIV. 546.
XLIV. Mikroskopische Betriebskontrolle. 3rd Ed. 1901. 385.
XLV. W. f. Br. 1900. XVII. 713.
XLVI. *Ibid.* 1893. X. 1298.
XLVII. Mikroskopische Betriebskontrolle, etc. 3rd Ed. 1901. 358.
See also Fischer und Lindner.

Linossier, G.,
I. Action de l'acide sulfureux sur quelques champignons inférieurs et en particulier sur les levures alcooliques.—Ann. Inst. Past. 1891. V. 170.—K. J. II. 93.
II. C. R. 1891. CXII. 489, 807.

Lintner, Karl, der ältere,
I. Zymotechnische Rückblicke auf das Jahr 1877.—Z. g. Br. 1878. I. 17.
II. Desgl. auf das Jahr 1878.—Z. g. Br. 1879. II. 2.
III. Altes und Neues über Bierbrauerei.—Z. g. Br. 1880. III. 2 *et seq.* 1881. IV. 11 *et seq.*
IV. Zymotechnische Rückblicke auf das Jahr 1882.—Z. g. Br. 1883. VI. 397.

Lintner, C. J.,
I. Handbuch der landwirtschaftlichen Gewerbe.—Berlin. 1893. Parey.
II. Über die Entstehung von Dextrose aus der Stärke durch fermentative Prozesse.—Z. g. Br. 1892. XV. 123.—Ch. C. 1892. I. 740.—K. J. III. 255.
III. Studien über die Selbstgärung der Hefe.—C. f. B. 2. Abt. 1899. V. 793.—Ch. C. 1900. I. 54.—K. J. X. 106.
IV. Zur Kenntnis der sogen. stickstofffreien Extraktstoffe in der Gerste, bez. im Malze und Biere. — Zeitschrift f. angewandte Chemie. 1890. 519.—Z. g. Br. 1890. XIII. 472. 1891. XIV. 81.—Ch. C. 1890. II. 804.
V. Zur Kenntnis der Stickstoffaufnahme durch die Hefe bei der Gärung.—W. f. Br. 1884. I. 3.—Z. g. Br. 1884. VII. 143.
VI. Z. g. Br. 1892. XV. 213.
VII. Chem. Ztg. 1899. XXIII. 851.
VIII. Z. f. angew. Chem. 1896. 538.
IX. Forschungsberichte über Lebensmittel, etc. 1894. I. 275.
X. Z. g. Br. 1892. XV. 106. 1894. XVII. 414.
XI. *Ibid.* 1892. XV. 6
XII. Z. f. angew. Chem. 1892. 328.

Lintner, C. J., und Düll, G.,
I. Über die chemische Natur des Gerstengummi. — Zeitschrift f. angew. Chemie. 1891. 538.—Ch. C. 1891. II. 799.
II. Ber. d. D. Chem. Ges. 1893. XXVI. 2533.

Lintner, C. J., und Kröber, E.,
I. Ber. d. D. Chem. Ges. 1895. XXVIII. 1050.

Lippmann, Edmund O. von,
I. Die Chemie der Zuckerarten. 2nd Ed. 1895. Braunschweig. Fr. Vieweg u. Sohn.
II. *Ibid.* 1904.
III. Ber. d. D. Chem. Ges. 1887. XX. 1001.
IV. *Ibid.* 1903. XXXVI. 331.
V. Die Chemie der Zuckerarten. 1904. 1623.

Lister, Joseph,
I. A Further Contribution to the Natural History of Bacteria and the Germ Theory of Fermentative Changes.—Quarterly Journal of Microscopical Science. 1873. XIII. 380.
II. On the Lactic Fermentation and its Bearing on Pathology.—Transactions of the Pathological Society of London. 1878. XXIX.

Lloyd, E. J.,
I. Observations on Cheddar Cheese-making. — Reports for 1891, 1892 and 1893.

Loder,
I. A. f. Hygiene. 1902. XLII. 107.
II. *Ibid.*, p. 152.

Loé, W.,
I. Enthält das Malz ein peptonisierendes Enzym ?—Z. g. Br. 1899. XXII. 212.—Ch. C. 1899. I. 1248.

Loeffler, Friedrich,
I. Eine neue Methode zum Färben der Mikroorganismen im besonderen ihrer Wimperhaare und Geisseln.—C. f. B. 1889. VI. 209.
II. Weitere Untersuchungen über die Beizung und Färbung der Geisseln bei den Bakterien.—C. f. B. 1890. VII. 625.—K. J. I. 9.
III. Über Bakterien in der Milch.—Berliner klin. Wochenschrift. 1887. 607.—C. f. B. 1887. II. 524.

Loew, E.,
I. Über Dematium pullulans.—Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1867. VI. 464.
II. Zur Entwicklungsgeschichte von Penicillium. — *Ibid.* 1870. VII. 472.
III. Zur Physiologie niederer Pilze.—Verh. der K. K. zoolog.-botan. Ges. in Wien. 1867. XVII. 643.
IV. Jahr. wiss. Bot. 1869–70. VII. 472.
V. *Ibid.* 1867. VI. 467.

Loew, Oskar,
I. Physiologische Notizen über Formaldehyd.—Ch. C. 1889. I. 90.
II. Ein natürliches System der Giftwirkung. München. 1893.—C. f. B. 1893. XIV. 234.
III. Katalytische Bildung von Ammoniak aus Nitraten.—Ber. d. D. Chem. Ges. 1890. XXIII. 675.—K. J. I. 35.
IV. Miso und Natto.—Mitteilungen der D. Ges. f. Natur- und Völkerkunde Ostasiens in Tokio. Heft 57.—Ch. C. 1896. II. 186.
V. Über die Ernährungsweise des nitrifizierenden Spaltpilzes Nitromonas.—Botan. Centralblatt. 1891. No. 20.—C. f. B. 1891. IX. 691.—Ch. C. 1891. II. 549. K. J. II. 209.
VI. Bemerkung zu einer Abhandlung von K. Yoshimura.—Ch. C. 1896. I. 57.
VII. Zur Frage der Vertretbarkeit von Kaliumsalzen durch Rubidiumsalze bei niederen Pilzen.—Bot. Centralblatt. 1898. LXXIV. 202.—Ch. C. 1898. II. 41.—K. J. IX. 55.
VIII. Über den Nachweis des Lecithins.—Pflüger's Archiv f. d. gesamte Physiologie. XIX. 342.
IX. Münchn. medic. Wochenschr. 1892. XXXIX. 370.
X. Chem. Ztg. 1900. XXIV. 1137.

Lohmann, W.,
I. Über den Einfluss des intensiven Lichtes auf die Zellteilung bei Saccharomyces cerevisiæ und anderen Hefen. Dissert. Rostock. 1896.—C. f. B. 2. Abt. 1897. III. 369.—K. J. VII. 90.

Loiseau, D.,
I. Sur une nouvelle substance organique cristallisée. — C. R. 1876. LXXXII. 1058.
II. Sur la fermentation de la raffinose, en présence des diverses espèces de levure de bière.—C. R. 1889. CIX. 614.—Z. g. Br. 1889. XII. 483.—Ch. C. 1889. II. 869.

Longard, K.,
See Buchner, Longard und Riedlin.

Lookeren-Campagne, C. J. van,
I. Bericht über Indigo-Untersuchungen, ausgeführt an der Versuchs-Station zu Klatten auf Java. — D. landw. Vers.-Stat. 1894. XXXXIII. 401.—Ch. C. 1894. I. 1000.
II. Über Indigobildung aus Pflanzen der Gattung Indigofera.—D. landw. Vers.-Stat. 1895. XXXXVI. 249.—Ch. C. 1896. I. 113.—K. J. V. 289.

Lopriore, G.,
I. Über einen neuen Pilz, welcher die Weizensaaten verdirbt.—Deutsche landw. Presse. 1891. 321.—Ber. d. D. Bot. Ges. 1892. X. 72.—Zeitschr. f. Pflanzenkrankheiten. 1891. I. 1892. II. 172. 1895. V. 242.—Bot. Ztg. 1895. II. 322.
II. Über die Einwirkung der Kohlensäure auf das Protoplasma der lebenden Pflanzenzelle.—Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1895. XXVIII. 531.—Gives the antecedent bibliography.—Bot. Ztg. 2. Abt. 1896. LIV. 132.

Lubavin, N.,
I. Über die künstliche Pepsin-Verdauung des Caseins und die Einwirkung von Wasser auf Eiweisssubstanz. — Hoppe-Seyler's Mediz.-chem. Untersuchungen. 1871. Heft 4. 463.
II. Über das Nuclein aus dem Casein der Kuhmilch.—Ber. d. D. Chem. Ges. 1877. X. 2237. 1879. XII. 1021.

Lubawin,
See Lubavin.

Lucas, Fr.,
I. Das Obst und seine Verwertung. Stuttgart. Ulmer. 6*s.*, bound.

Lucet,
I. De l'Aspergillus fumigatus chez les animaux. Paris. 1899.

Ludwig, Ernst,
I. Über das Vorkommen des Trimethylamins im Wein.—Sitzgsber. der Kais. Akademie d. Wiss. zu Wien. Mathem.-naturw. Klasse. 1867. LVI. 2. Abt. 287.—Ch. C. 1867. II. 911.

Ludwig, Friedrich,
I. Die bisherigen Untersuchungen über photogene Bakterien.—C. f. B. 1887. II. 372. Gives all the bibliography prior to 1887.—Ch. C. 1887. 1510.
II. Die Genossenschaften der Baumflussorganismen. Sammelreferat.—C. f. B. 2. Abt. 1896. II. 337. Gives the bibliography up to 1896.—K. J. VII. 25.

Lübbert, A.,
I. Über die Natur der Giftwirkung peptonisierender Bakterien der Milch.—Z. f. Hygiene. 1896. XXII. 1.—Ch. C. 1896. II. 47.—K. J. VII. 194.

Lüdersdorff,
I. Pogg. Ann. 1846. LXVII. 408.

Luksch, Ludwig,
I. Zur Differentialdiagnose des Bacillus typhi abdominalis (Eberth) und des Bacterium coli commune (Escherich).—C. f. B. 1892. XII. 427.—K. J. III. 79.

Lumsden, J. S.,
See Frankland and Lumsden.

Lunde, H. P.,
See Friis, Lunde u. Storch.

Lundström, C.,
I. Die Zersetzung von Harnstoff durch Mikroben. Helsingfors. 1890.—K. J. II. 260.

Lutz, L.,
I. Bull. de la Soc. Mycol. de France. 1899. XV. 68, 157.
II. *Ibid.* 1905. XXI. 131.

Maas, H.,
I. Über Fäulnisalkaloide. — Fortschritte der Medizin. 1883. I. 373. 1884. II. 729.—Ch. C. 1883. S. 712. 1884. 975.

Maassen, Alb.,
I. Arb. Kais. Ges.-Amt. 1901. XXIII. 21.

Maassen, Albert,
I. Beiträge zur Ernährungsphysiologie der Spaltpilze. Die organischen Säuren als Nährstoffe und ihre Zersetzbarkeit durch die Bakterien.—Arbeiten a. d. K. Gesundheits-Amte. 1895. XII. 390.—Ch. C. 1896. I. 655.

Macagno, J.,
I. Über die Wirkung des in den umgeschlagenen Weinen enthaltenen Fermentes auf gesunde Weine.—Biedermann's Centralbl. 1879. VIII. 310 nach Le Stazioni sperimentali agrarie italiane. 1877. VI. 79.

Macallum, A. B.,
I. On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells. — Quarterly Journal of Microscopical Science. 1895. XXXVIII. II. 175.
II. On the Demonstration of Iron in Chromatin by Microchemical Methods.—Proceedings of the Royal Society. London. 1892. L. 277.

MacAlpine,
I. Agricult. Gazette of N.S. Wales. May, 1896.

Macé,
I. Traité pratique de Bactériologie. First edition, 1889; second edition, 1892. Paris. Baillière et fils.—C. f. B. 1893. XIII. 484.
II. Archiv de Parasitologie. 1903. VII. 313. Also issued as a thesis, Paris, 1903, "Etudes sur les Mycoses experimentales."

Macfadyen, A.,
I. Chemisch - bakteriolog. Untersuchungen über einen Euterentzündung und Käseblähung bewirkenden Bacillus (Bacillus Guillebeau c.).—Landw.Jahrbüch d. Schweiz. 1890. IV. 64.—K. J. II. 196.

Macfadyen, Allan, Morris, G. Harris, und Rowland, Sidney,
I. Über ausgepresstes Hefezellplasma. I.—Ber. d. D. Chem. Ges. 1900. XXXIII. 2764.—C. f. B. 2. Abt. 1901. VII. 25.—Ch. C. 1900. II. 1028.

Macfadyen, A., Nencki, M., und Sieber, N.,
I. Untersuchungen über die chemischen Vorgänge im menschlichen Dünndarm.—Archiv f. experim. Pathol. u. Pharmakologie. 1891. XXVIII.—Centralbl. f. Physiologie. 1891. V. 199.—C. f. B. 1891. X. 82.—Ch. C. 1891. II. 387.

MacGregor, J.,
See Frankland and MacGregor.

Mach, Edmund,
I. Botrytis cinerea und der Rauchgeschmack des Weines.—Weinlaube. 1872. IV. 215.

Mach, Edmund, und Portele, K.,
I. Über die schwere Vergärbarkeit und die Zusammensetzung des Preisselbeersaftes. — D. landw. Vers.-Stat. 1890. XXXVIII. 69.—Ch. C. 1890. II. 633.—K. J. I. 63.
II. Nachweis und quantitative Bestimmung von Milchsäure und Buttersäure in Weinen, die aus verschlämmten Trauben in verschiedener Weise hergestellt wurden.—D. landw. Vers.-Stat. 1890. XXXVII. 305.
III. Über die Gärung von Trauben- und Apfelmost mit verschiedenen rein gezüchteten Hefearten.—D. landw. Vers.-Stat. 1892. XLI. 233.—Ch. C. 1893. I. 218.—K. J. III. 147.

Macheleidt,
I. W. f. Br. 1898. XV. 365.

Maercker, Max,
I. Handbuch der Spiritusfabrikation. 7. Ed. Berlin. 1898. 22*s.*, bound.
II. *Ibid.* 8th Ed. 1903. 71.
III. *Ibid.* 392.
IV. *Ibid.* 762.
V. *Ibid.* 496.

Maggiora, A.,
I. Ricerche quantitative sui microorganismi del suolo con speciale riguardo all' inquinazione del medesimo.—Estrad. dal Giorn. d. R. Accad. di medicina. Torino. 1887. No. 3.—C. f. B. 1887. I. 677.—Ch. C. 1887. 1116.
II. Über die Zusammensetzung des überreifen Käses.—A. f. Hygiene. 1892. XIV. 217.—K. J. III. 188.
See also Perroncito e Maggiora.

Majstorović, R.,
See Zega und Majstorović.

Malerba, P.,
I. Untersuchungen über die Natur der von dem Gliscrobacterium gebildeten schleimigen Substanz.—Z. f. physiolog. Chemie. 1891. XV. 539.—K. J. II. 223.

Malerba, P., e Sanna-Salaris, G.,
I. Ricerche sul Gliscrobatterio.—Rendiconto della R. Accademia delle Scienze fis. e mat. di Napoli. 1888. Fasc. 6.—C. f. B. 1888. IV. 486.

Malfatti, Hans,
I. Zur Chemie des Zellkerns.—Berichte d. naturwissenschaftlich-medizinisehen Vereines in Innsbruck. XX. Jahrgang. 1891.—92. pag. IX.

Malfitano, G.,
I. La protéolyse chez l'Aspergillus niger.—Ann. Inst. Past. 1900. XIV. 60, 420.—C. f. B. 2. Abt. 1900. VI. 472.—Ch. C. 1900. II. 391.

Mallèvre, A.,
See Bertrand et Mallèvre.

Malpighi, Marcello,
I. Opera omnia. Leiden. 1687. II. 126.

Malvezin, F.,
I. Manuel de Pasteurisation des Vins et traitement de leur maladies. Bordeaux. 1899. 6*s.* 6*d.*

Man, C. de,
See Forster u. de Man.

Manassein, Marie von,
I. Beiträge zur Kenntnis der Hefe und zur Lehre von der alkoholischen Gärung.—Wiesner's Mikroskopische Untersuchungen. 1872. Stuttgart. 116.
II. Zur Frage von der alkoholischen Gärung ohne lebende Hefezellen.—Ber. d. D. Chem. Ges. 1897. XXX. 3061.—Ch. C. 1898. I. 395.—K. J. VIII. 284.

Manetti, L., und Musso, G.,
I. Über die Zusammensetzung und die Reife des Parmesankäses.—D. Landw. Versuchsstationen. 1878. XXI. 211.

Mangin, Louis,
I. Sur la constitution de la membrane des végétaux.—C. R. 1888. CVII. 144.—Ch. C. 1888. 1100. — Bot. Ztg. 1889. XXXXVII. 450.
II. Observations sur la constitution de la membrane chez les Champignons. — C. R. 1893. CXVII. 816.
III. Sur l'emploi du rouge de ruthénium en Anatomie végétale.—C. R. 1893. CXVI. 653.—Ch. C. 1893. I. 789.
IV. Recherches anatomiques sur la distribution des composés pectiques chez les végétaux.—Journal de Botanique. 1893.
See also Bonnier et Mangin.

Mann, Harold,
I. Action de certaines substances antiseptiques sur la levure.—Ann. Inst. Past. 1894. VIII. 785.—C. f. B. 2. Abt. 1895. I. 521.—Ch. C. 1895. I. 93.—K. J. V. 91.

Marcano, V.,
I. Fermentation de la fécule.—C. R. 1882. LXXXXV. 345, 856.—Ch. C. 1882. 641, 806.
II. Sur la panification.—C. R. 1883. LXXXXVI. 1733.—Ch. C. 1883. 548.
III. Sur la formation de quantités notables d'alcool dans la fermentation panaire.—C. R. 1883. LXXXXVII. 1070. — Ch. C. 1883. 808.
See also Müntz u. Marcano.

Marchal, Emile,
I. Sur la production de l'ammoniaque dans le sol par les microbes. — Bulletin de l'Acad. Royale de Belgique. 1893. XXV. 727.—Naturw. Rundschau. 1893. VIII. 601. — Ch. C. 1894. I. 97.—K. J. IV. 238, 239.
II. The Production of Ammonia in the Soil.—Agricult. Science. 1894. VIII. 574.—C. f. B. 2. Abt. 1895. I. 753. — Biedermann's Centralbl. 1895. XXIV. 519.—Ch. C. 1895. II. 839.
III. Contribution à l'étude microbiologique de la maturation des fromages mous.—Annales de la Société belge de microscopie. 1895. XIX.—C. f. B. 2. Abt. 1895. I. 506.

Marchand, Richard Felix,
I. Über die Zusammensetzung der Milch des Kuhbaumes (Palo de vaca). — Journal für prakt. Chemie. 1840. XXI. 45.

Marckwald,
I. Ber. d. D. Chem. Ges 1902. XXXV. 1595.

Mariani, Giovanni,
I. Über die Gegenwart und die Menge des Kupfers in dem Parmesan-Käse (formaggio di grana lombardo).—Stazioni sperimentali agrarie italiane. 1889. XVII. — Milchzeitung. 1889. XVIII. 1033.

Marischler, Julius,
I. Klinische Untersuchungen über die Wirkung der an Aldehyd gebundenen schwefligen Säure im Weine. — Wiener klinische Wochenschrift. 1896. IX. 711.—Ch. C. 1896. II. 548.—Vergl. Österr. Chemiker-Zeitg. 1899. II. 33.—Ch. C. 1899. I. 571.

Marpmann, G.,
I. Über die Erreger der Milchsäuregärung. — Ergänzungsheft z. Centralblatt f. allgem. Gesundheitspflege. 1886. II. 117.—Ch. C. 1886. 344.
II. Bakteriologische Mitteilungen.—C. f. B. 1. Abt. 1897. XXII. 122.
III. Über schwarze Pilzwucherungen in offizinellen Flüssigkeiten und über eine neue Hefe-Spezies: Saccharomyces niger.—Archiv der Pharmacie. 1886. CCXXIV. 705.

IV. Käsegärung und Käsepilze.—Pharmac. Centralhalle. 1893. XXXIV. 76.
V. Über die biochemische Arsenreaktion. *Ibid.* 1900. XXXXI. 666.—Ch. C. 1900. II. 1187.
VI. Centralbl. f. allgem. Gesundsheitspflege. 1886. II. 422.
VII. Z. f. angewandte Mikroskopie. 1896. II. 68.
VIII. Pharmac. Ztg. 1903. XLVII. 1010.

Marschall,
I. Über die Zusammensetzung des Schimmelpilzmycels.—A. f. Hygiene. 1897. XXVIII. 16.—C. f. B. 2. Abt. 1897. III. 154.—Ch. C. 1897. I. 115.—K. J. VIII. 42.

Martin, C. J.,
I. Journ. of Physiology. 1896. XX. 364.

Martin, C. J., and Chapman,
I. Proc. Physiological Soc. June 11, 1898.

Martinand, V.,
I. Action de l'air sur le moût de raisin et sur le vin.—C. R. 1895. CXX. 1426; CXXI. 502.—Ch. C. 1895. II. 324, 1100.
II. Influence des rayons solaires sur les levures que l'on rencontre à la surface des raisins.—C. R. 1891. CXIII. 782.—Ch. C. 1892. I. 212.—K. J. II. 127.

Martinand, V., et Rietsch, M.,
I. Des microorganismes que l'on rencontre sur les raisins mûrs et de leur développement pendant la fermentation.—C. R. 1891. CXII. 736.—K. J. II. 128.
II. C. R. 1891. LXII. 763. [*Sic* in the German; LXII. is a misprint for CXII., and 763 should perhaps be 736.]

Martiny, Benno,
I. Die Milch, ihr Wesen und ihre Verwertung. Zwei Bände. Danzig. 1871. Kafemann. With about 1300 bibliographical references.
II. Das Verarbeiten erhitzter Milch.—Zeitschrift für Fleisch- und Milch-Hygiene. 1893. III. 9.—K. J. IV. 201.

Marx, Louis,
I. Les levures des vins.—Moniteur scientifique. 1888. No. 563.—Z. g. Br. 1888. XI. 522.—C. f. B. 1. Abt. 1889. V. 313.—Ch. C. 1889. I. 47.

Massart, Jean,
I. Sensibilité et adaption des organismes à la concentration des solutions salines.—Archives de biologie. Liège. 1889. IX. 515.
II. Recherches sur les organismes inférieurs.—Bulletin de l'Académie Royale de Belgique. 1888, 11. 1891. (3.) XXII.—C. f. B. 1. Abt. 1892. XI. 566.—K. J. III. 60.
III. La loi de Weber vérifiée pour l'héliotropisme d'un Champignon.—Bulletin de l'Acad. Roy. de Belgique. 1888. (3.) XVI. No. 12.
IV. La sensibilité tactile chez les organismes inférieurs.—Journal de Médecine de Bruxelles. 1891.—C. f. B. 1. Abt. 1892. XI. 566.

Masson,
See Balland et Masson.

Mastbaum, H.,
I. Z. f. Unter. d. Nahrungs- u. Genussmittel. 1903. VI. 49.

Mathew, W. de Vere,
See Salomon und Mathew.

Mathieu, L.,
I. Rev. de Viticulture. 1898. X. 155.
II. W. f. Br. 1896. XXIII. 334.

Mattern, J.,
I. Ber. ü. d. 7 Vers. d. freien Verunigung bayr. Vertreter d. angew. Chemie in Speyer. Berlin. 1889.—Abst. in Z. g. Br. 1889. XII. 188.

Matthews, Charles George,
I. On the Red Mould of Barley.—Journal of the Royal Microscop. Society. London. 1883. Ser. 2. Vol. III., p. 321.
II. The Nutrition of Yeast.—Journal of the Federated Institutes of Brewing. 1897. III. 369.—K. J. VIII. 84.
III. The Brewer's Guardian. 1887.—Abst. in W. f. Br. 1887. IV. 380.

Maurea, G.,
I. Über eine bewegliche Sarcine.—C. f. B. 1892. XI. 228.—K. J. III. 44.
Maximow,
I. Ber. d. D. Bot. Ges. 1904. XXII. 225.
Mayer, Adolf,
I. Untersuchungen über die alkoholische Gärung, den Stoffbedarf und den Stoffwechsel der Hefepflanze. Heidelberg. 1869. —Poggendorff's Annalen. 1871. CXXXXII.293.—Dingler's polyt. Journal. 1871. CCI. 69.
II. Studien über die Milchsäure-Gärung.—Z. f. Spiritusind. 1891. XIV. 183.—C. f. B. 1892 XII. 99.—K. J. II. 173.
III. Neue Beiträge zur Kenntnis der Wirkung des Labfermentes. —D. Landw. Versuchs-Stationen. 1882. XXVII. 247.
IV. Über die Nägeli'sche Theorie der Gärung ausserhalb der Hefezellen.—Zeitschrift für Biologie. 1882. XVIII. 522.—Z. g. Br. 1882. V. 473.
V. Über den Bedarf des Hefepilzes (Saccharomyces cerevisiæ) an Aschebestandteilen.—D. Landw. Versuchs-Stationen. 1869. XI. 443.
VI. Über den Einfluss der Sauerstoffzufuhr auf die Gärung.—Ber. d. D. Chem. Ges. 1880. XIII. 1163.
VII. Studien über die alkoholische Gärung.—D. Landw. Versuchs-Stationen. 1873. XVI. 277.
VIII. Über den Einfluss des Sauerstoffzutritts auf die alkoholische Gärung.—D. Landw. Versuchs-Stationen. 1880. XXV. 301.—Z. g. Br. 1880. III. 592.
IX. Saccharomyces cerevisiæ und der freie Sauerstoff.—Ber. d. D. Chem. Ges. 1874. VII. 579.
X. Untersuchungen ü. d. alkoholische Gärung. Heidelberg. 1869. 47–51.
XI. Die Lehre v. d. chemischen Fermenten oder Enzymologie. 1882. 64.
XII. *Ibid.* 20.
XIII. *Ibid.* 30.
XIV. *Ibid.* 41.
XV. *Ibid.* 70.
XVI. *Ibid.* 100.
Mazé, P.,
I. Ann. Inst. Past. 1903. XVII. 11.
II. C. R. 1902. CXXXIV. 191; CXXXV. 113.
III. *Ibid.* 1904. CXXXVIII. 1514.
Mazé et Perrier,
I. Ann. Inst. Past. 1904. XVIII. 553.
II. C. R. 1904. CXXXIX. 311.
Medicus u. Immerheiser,
I. Z. f. analyt. Chem. 1891. XXX. 665.
Mège-Mouriès,
I. Recherches chimiques sur le froment, sa farine et sa panification.—C. R. 1856. XXXXII. 1122. 1857. XXXXIV. 40, 449. 1858. XXXXVI. 126.
Mehring,
I. Z. f. physiolog. Chemie. 1881. V. 196.
Meisenheimer,
I. Z. f. physiolog. Chemie. 1903. XXXVII. 518.
Meissl,
I. J. f. prak. Chem. 1880. XXII. 100.
Meissner, E.,
I. Akkomodationsfahigkeit einiger Schimmelpilze. Dissert. Leipzig. 1903.
Meissner, Richard,
I. Über eine neue Spezies von Eurotium Aspergillus.—Bot. Ztg. 2. Abt. 1897. LV. 337.
II. Studien über das Zähewerden von Most und Wein.—Landwirtschaftl. Jahrbücher. 1898. XXVII. 715.—C. f. B. 2. Abt. 1899. V. 232. 1900. VI. 344. —Ch. C. 1899. I. 71. 1900. II. 218.
III. Über das Auftreten und Verschwinden des Glycogens in der Hefenzelle.—C. f. B. 2. Abt. 1900. VI. 517.—Ch. C. 1900. II. 771.
IV. Weinlaube. 1903. 521.
V. Landw. Jahrbücher. 1901, p. 72.
VI. Bericht d. Kgl. Württ. Weinbau-Versuchsanstalt Weinsberg. 1904, p. 72.
VII. Württ. Wochenbl. f. Landwirtschaft. 1901. 755.
VIII. Bericht d. Kgl. Württ. Weinbau-Versuchsanstalt Weinsberg. 1901–1903, p. 49.

IX. Anleitung z. mikrosk. Untersuchung u. Reinzüchtung d. häufigsten im Most u. Wein vorkommenden Pilze. Stuttgart. 1891, p. 41.
X. Bericht d. Kgl. Weinbau-Versuchsanstalt Weinsberg. 1904, pp. 53 and 69.
XI. Landw. Jahrbücher. 1901. XXX. 497.

Meissner, Roderich,
I. Studien ü. d. Einfluss d. Essigsäure u. Milchsäure auf die Hefen Saaz, Frohberg u. Logos in Saccharoselösung. Dis. Berlin. 1897.

Melsens,
I. Note sur la vitalité de la levure de bière.—C. R. 1870. LXX. 629.

Meltzer, S. J.,
I. Über die fundamentale Bedeutung der Erschütterung für die lebende Materie.—Zeitschrift für Biologie. 1894. XXX. 464.—C. f. B. 1894. XVI. 743.

Mendelsohn, Benno,
See Cohn u. Mendelsohn.

Mendoza, Anton,
I. Über einen neuen Micrococcus.—C. f. B. 1889. VI. 566.

Menge, Karl,
I. Über einen Micrococcus mit Eigenbewegung.—C. f. B. 1892. XII. 49.—K. J. III. 44.
II. Über rote Milch.—C. f. B. 1889. VI. 596.

Messea, Al.,
I. Contribuzione allo studio delle ciglia dei batterii e proposito di una classificazione. — Rivista d'Igiene e Sanità publica. 1890. I. No. 14.—C. f. B. 1891. IX. 106.

Metschnikoff, Elias,
I. Pasteuria ramosa, un représentant des bactéries à division longitudinales.—Ann. Inst. Past. 1888. II. 165.—C. f. B. 1888. IV. 17.
II. Contributions à l'étude du pléomorphisme des bactéries.—Ann. Inst. Past. 1889. III. 61.—C. f. B. 1889. V. 511.
III. Virchow's Archiv. 1884. XCVI. 177.

Meulemeester, Emile de,
I. Germ. Patent No. 105626, April 30, 1898.—Ch. C. 1900. I. 492.

Meusel, E.,
I. Über Nitritbildung durch Bakterien.—Tageblatt der Naturforscher-Versammlung zu Graz. 1875. 55.—Ch. C. 1875. 678.

Meyen,
I. Wiegmann's Archiv. 1838. IV. 100.

Meyer, Lothar,
I. Chemische Untersuchung der Thermen zu Landeck in der Grafschaft Glatz. — Journal f. prakt. Chemie. 1864. LXXXXI. 1.

Miciol,
I. Note sur les végétations qui se développent pendant la fermentation du tabac.—Mémorial des Manufactures de l'Etat. Tabacs. 1891. II. 182.—K. J. II. 221.

Miescher, F.,
I. Über die chemische Zusammensetzung der Eiterzellen.—Hoppe-Seyler's Mediz.-chem. Untersuchungen. 1871. Heft 4. 441.

Migula, W.,
I. Über den Zellinhalt von Bacillus oxalaticus Zopf.—Arbeiten a. d. bakteriolog. Inst. der Techn. Hochschule zu Karlsruhe. 1894. I. 139.—C. f. B. 2. Abt. 1895. I. 242.—K. J. V. 54.
II. Über ein neues System der Bakterien.—Arbeiten a. d. bakteriolog. Inst. d. Techn. Hochschule zu Karlsruhe. 1895. I. 235.—C. f. B. 2. Abt. 1895. I. 406. 1896. II. 307.
III. Die Artzahl der Bakterien bei der Beurteilung des Trinkwassers.—C. f. B. 1890. VIII. 353.
IV. Über sogenannte Kapselbildung bei Bakterien.—Deutsche tierärztliche Wochenschrift. 1896. No. 4 u. 5.—C. f. B. 2. Abt. 1896. II. 583.—K. J. VII. 24.

Milburn,
I. C. f. B. 2. Abt. 1904. XIII. 268.

Miller, A., and Hyde, F.,
I. The Application of Pure Yeast (Hansen) to High Fermentation.—Transactions of the North of England Institute of Technical Brewing. 1894. III.—K. J. V. 183.

Millon, E.,
I. Théorie chimique de la nitrification.—C. R. 1860. LI. 548.

II. Faits nouveaux concernant les métamorphoses alcooliques.—C. R. 1863. LVII. 235. 1864. LIX. 144.

Minck, F.,

I. Zur Frage über die Einwirkung der Röntgen'schen Strahlen auf Bakterien und ihre eventuelle therapeutische Verwendbarkeit. —Münchener mediz. Wochenschrift. 1896. XXXXIII. 202. —C. f. B. 2. Abt. 1896. XIX. 631.—K. J. VII. 53.

Minssen,

See Tacke, Immendorf, Hessenland, Schütte und Minssen.

Miquel, P.,

I. Manuel pratique d'Analyse bactériologique des eaux. Paris. 1891.

II. Monographie d'un bacille vivant au delà de 70 centigrades.— Annales de Micrographie. 1888. I. 3.—C. f. B. 1889. V. 282.

III. Les organismes vivants de l'atmosphère. 1883.

IV. Annuaire de l'observatoire de Montsouris. 1879.

V. De la présence dans l'air du ferment de l'urée.—Bulletin de la Soc. chim. de Paris. 1878. XXIX. 387. 1879. XXXI. 391. —Ch. C. 1879. 633.

VI. Etudes sur la fermentation ammoniacale et sur les ferments de l'urée.—Annales de Micrographie. 1889. I. 414. 1889–1890. II. 53, 145. 1890–1891. III. 275, 305. 1893. V. 161 *et seq.* 1895. VII. 49. 1896. VIII. 55. 1897. IX. 302 *et seq.*—K. J. I. 176; II. 259; IV. 285.

VII. Sur le ferment soluble de l'urée.—C. R. 1890. CXI. 397. —K. J. I. 176.

VIII. De la présence dans l'air du ferment alcoolique.—C. R. 1878. LXXXVII. 759.—Z. g. Br. 1879. II. 57.

IX. Dixième mémoire sur les poussières organisées de l'air et des eaux.—Mémoire de l'Observatoire de Montsouris pour 1888.— C. f. B. 1888. IV. 276.

Mirsch u. Eberhard,

I. German Patent No. 149432.

Mirsky,

I. Sur quelques causes d'erreur dans la determination des Aspergillées parasites de l'homme. Nancy. 1903.

Mitchell und Reichert,

I. Über Schlangengifte.—Ch. C. 1887. 1152.

Mitscherlich, Eilhard,

I. Über die Zusammensetzung der Wand der Pflanzenzelle.—Monatsberichte d. K. Akad. der Wiss. zu Berlin. 1840. 102.

II. Über die Mykose den Zucker des Mutterkornes. — Monatsberichte, etc. 1857. 469.—Ann. d. Chemie u. Pharmacie. 1858. CVI. 15.

III. Über die Asche der Hefe.— Monatsber., etc. 1845. 236.

IV. Monatsber. d. Kgl. Akad. Wiss. Berlin. 1841, p. 392.

Mittelmeier, H.,

See Scheibler und Mittelmeier.

Mix, Charles L.,

I. On a Kephir-like Yeast found in the United States.—Contr. from the Cryptog. Laborat. of Harvard University. Vol. XVI. —Proceedings of the American Acad. of Arts and Sciences. 1891. XXVI. 102.

Miyoshi, Manabu,

I. Über Chemotropismus der Pilze. —Bot. Ztg. 1. Abt. 1894. LII. 1. —C. f. B. 1. Abt. 1894. XVI. 193.

II. Die Durchbohrung von Membranen durch Pilzfäden.—Jahrbuch f. wissensch. Botanik. 1895. XXVIII. 269.—C. f. B. 2. Abt. 1895. I. 824.

III. Jahrb. wiss. Bot. 1895. XXVIII. 227.

Moeller, Hermann,

I. Bemerkungen zu Franks Mitteilungen über den Dimorphismus der Wurzelknöllchen der Erbse. —Ber. d. D. Bot. Ges. 1892. X. 242.—C. f. B. 1893. XIII. 500.—K. J. III. 201, 205.

II. Über den Zellkern und die Sporen der Hefe.—C. f. B. 1892. XII. 537.—Ch. C. 1893. I. 43. —K. J. III. 37.

III. Weitere Mitteilungen über den Zellkern und die Sporen der Hefe.—C. f. B. 1893. XIV. 358.—K. J. IV. 47.

IV. Neue Untersuchungen über den Zellkern und die Sporen der Hefen.—Ber. d. D. Bot. Ges. 1893. XI. 402.—K. J. IV. 48.

Mohl, Hugo von,
I. Blaue Färbung der vegetabilischen Zellmembran durch Jod. Flora. 1840.

Mohr, P.,
I. Über das Vorkommen von Pentosanen, resp. Pentosen im Biere.—W. f. Br. 1895. XII. 769.—Ch. C. 1895. II. 549.—K. J. VI. 169.
II. W. f. Br. 1886. III. 200.

Molisch, Hans,
I. Die Pflanze in ihren Beziehungen zum Eisen. Jena. 1892. G. Fischer.—Ch. C. 1892. II. 332.—K. J. III. 53.
II. Die mineralische Nahrung der niederen Pilze.—Sitzgsber. d. K. Akad. d. Wiss. zu Wien; math.-nat. Kl. 1894. CIII. 1. Abt. 554.—Ch. C. 1895. I. 494.—K. J. V. 72.
III. Bemerkung über den Nachweis von maskiertem Eisen.—Ber. d. D. Bot. Ges. 1893. XI. 73.—Ch. C. 1893. I. 857.
IV. Die Ernährung der Algen. I. u. II.—Sitzgsber. d. K. Akad. d. Wiss. zu Wien; math.-nat. Kl. 1895. CIV. 1. Abt. 783. 1896. CV. 1. Abt. 633.—Ch. C. 1896. II. 496.
V. Untersuchungen über das Erfrieren der Pflanzen. Jena. 1897.—Bot. Ztg. 2. Abt. 1897. LV. 281.

Moller, F. J.,
I. Über die Einwirkung des elektrischen Stromes auf Bakterien.—C. f. B. 2. Abt. 1897. III. 110.—Ch. C. 1897. I. 768.—K. J. VIII. 68.

Mollerau,
See Nocard et Mollerau.

Molliard et Coupin,
I. C. R. 1903. CXXXVI. 1695.
II. Rev. génér. de Botanique. 1903. XV. 401.

Montesano, Giuseppe,
See Fermi und Montesano.

Monti, Achille,
See Lilienfeld und Monti.

Morck, D.,
I. Über die Formen der Bakteroiden bei den einzelnen Species der Leguminosen.—Dissertation. Leipzig. 1892.—K. J. II. 207.

Moreau,
I. Ann. Soc. Roy. Sci. Med. et Nat. de Bruxelles. 1904. XII.—W. f. Br. 1905. XXII. 37.

Morgen, August,
See Behrend und Morgen.

Morin et Claudon,
I. C. R. 1887. CIV. 1109.

Morin, Edouard Charles,
I. Sur les bases extraites des liquides ayant subi la fermentation alcoolique.—C. R. 1888. CVI. 360. — Bot. Ztg. 1889. XXXXVII. 120.
II. Sur la composition chimique d'une eau-de-vie de vin de la Charente-Inférieure.—C. R. 1887. CV. 1019.
See also Claudon et Morin.

Morini,
I. Malpighia. 1888–1889. II. 224.

Moritz, E. R.,
I. Z. g. Br. 1891. XIV. 199.

Moritz, J.,
I. Zur Gärungsfrage.—Ber. d. D. Chem. Ges. 1874. VII. 156, 434.
II. Chem. Ztg. 1886. X. 322.
III. Landw. Jahrbücher. 1884. 929.

Moritz und Morris,
I. Handbuch der Brauwissenschaft (A Text-book of the Science of Brewing).—German translation by W. Windisch. Berlin. 1893. Parey. 12*s.*

Morpurgo, Giulio, u. Brunner, Alfred,
I. Über die Anwendung der mikrobiologischen Reaktion zum Nachweise des Arsens in Teerfarbstoffen. — Österr. Chemiker-Zeitg. 1898. I. 167.—Ch. C. 98. II. 505.

Morren, Charles,
I. Recherches sur la rubéfaction des eaux.—Mém. de l'Académie de Bruxelles. 1841, p. 70.

Morris, G. Harris,
I. Tötung der Keime in der Würze während des Sudprozesses.—Z. g. Br. 1890. XIII. 124.—Ch. C. 1890. I. 844.
II. Bemerkungen über die Glukase. —Transactions of the Institute of Brewing. 1893. VI. 132.—Z. g. Br. 1893. XVI. 203.—Ch. C. 1893. I. 837.—K. J. IV. 282.
III. Chem. News. 1895. LXXI. 196.

Morris, G. H. (2),
I. Brewing Trade Review. 1895. 91.
II. Journ. Fed. Inst. Brewing. 1893. 350.
See also Macfadyen, Morris u. Rowland, Moritz u. Morris, Brown u. Morris.

Morris, G. H., and Wells, J. G.,
I. Fractional Fermentation: A Contribution to the Study of the Amyloines (Maltodextrines). — Transactions of the Institute of Brewing. 1892. V. 133.—K. J. III. 120.

Mosler, F.,
I. Über blaue Milch und durch deren Genuss herbeigeführte Krankheiten.—Virchow's Archiv f. pathol. Anatomie. 1868. XXXXIII.
II. Mykologische Studien am Hühnerei. — *Ibid.* 1864. XXIX. 510.
III. Virchow's Archiv. 1864. XXIX. 510.

Mosso, A.,
I. Ein Gift, welches sich im Blute der Mureniden vorfindet.—Rendiconti della Acad. dei Lincei. Roma. 1887. IV. 665, 673.—Ch. C. 1888. 1004.

Mouginet, Charles,
I. Quelques bactéries des putréfactions. Thèse. Nancy. 1894.—C. f. B. 2. Abt. 1895. I. 186.

Moussette,
I. Observations sur la fermentation panaire. — C. R. 1883. LXXXXVI. 1865. — Ch. C. 1883. 549.

Müdie,
I. Journ. f. Pharmacie. 1832. XVIII. 705.

Mühlhäuser,
I. Über Spirillen.—Virchow's Archiv. 1884. LXXXXVII. 84.

Müller, Alexander,
I. Über den gegenwärtigen Stand der Städtereinigungs- und Wasserbeschaffungsfrage für Berlin. —D. Landw. Vers.-Stat. 1873. XVI. 241.

Müller, C.,
I. Chemisch-physikalische Beschreibung der Thermen von Baden in der Schweiz. Baden. 1870.

Müller, Carl,
I. Kritische Untersuchungen über den Nachweis maskierten Eisens in der Pflanze und den angeblichen Eisengehalt des Kaliumhydroxydes.—Ber. d. D. Bot. Ges. 1893. XI. 252.—Ch. C. 1893. II. 155.

Müller-Thurgau, Hermann,
I. Der Milchsäurestich in Trauben- und Obstweinen.—III. Jahresbericht der Deutsch-schweizerischen Versuchs-Station und Schule für Obst-, Wein- und Gartenbau in Wädensweil für 1892–93. Zürich. 1894. 90.—K. J. V. 195.
II. Neue Forschungsresultate auf dem Gebiet der Weingärung und deren Bedeutung für die Praxis. —Bericht über die Verhandlungen des XI. Deutschen Weinbau-Kongresses in Trier. Mainz. 1889. 80.
III. Über die Ergebnisse neuer Untersuchungen auf dem Gebiete der Weinbereitung.—Bericht des XII. D. Weinbau-Kongresses in Worms. Mainz. 1891. 128.—K. J. II. 148.
IV. Weitere Untersuchungen über die Physiologie der Hefe und die Bedeutung ausgewählter und reingezüchteter Heferassen für die Weingärung.—III. Jahresbericht, etc., in Wädensweil. 1892–93. 73. Weinbau- u. Weinhandel. 1894. No. 24.—K. J. V. 169.
V. Neuere Erfahrungen bei Anwendung der Reinhefen in der Weinbereitung. — Ber., etc., d. XIV. D. Weinbau-Kongresses in Neustadt a. d. Hardt. 1895. 30.

VI. Das Braun- oder Rahnwerden der Weine.—Schweizerische Zeitschrift f. Obst- und Weinbau. 1894. III. 8.

VII. Über das Verhalten von Stärke und Zucker in reifenden und trocknenden Tabaksblättern.—Landw. Jahrbücher. 1885. XIV. 485.

VIII. Veränderungen, welche das Obst beim Faulen erleidet.—III. Jahresbericht, etc., in Wädensweil. 61.—Zeitschrift f. Pflanzenkrankheiten. 1895. V. 249. —K. J. V. 75.

IX. Die Edelfäule der Trauben.—Landw. Jahrbücher. 1888. XVII. 83.—C. f. B. 1888. IV. 179.—Ch. C. 1888. S. 957.—Bericht, etc., d. X. D. Weinbau-Kongresses zu Freiburg i. Br. 1887. 50.

X. Züchtung von Heferassen für bestimmte Zwecke.—V. Jahresbericht, etc., in Wädensweil pro 1894–95. 72.

XI. Das Zusammenwirken verschiedener Hefcrassen bei der Weingärung.—*Ibid.* S. 76.

XII. Die Herstellung unvergorener und alkoholfreier Obst- u. Traubenweine. 4th Ed. 1897. Frauenfeld. J. Huber.

XIII. Die Hefe als Kulturpflanze in den Weinbergen.—Weinbau und Weinhandel. 1894. 428. —IV. Jahresbericht, etc., in Wädensweil pro 1893–94. 68.

XIV. Über den Einfluss der Temperatur auf Verlauf und Produkt der Weingärung.—Ber. ü. d. Gen.-Vers. d. D. Weinbau-Vereines. 1884. 50.

XV. Botrytis und Peronospora als Schädiger der Gescheine und jungen Früchte des Weinstockes. — Weinbau und Weinhandel. 1888. VI. 256.

XVI. Einfluss der zugespitzten Hefe (Saccharomyces apiculatus) auf die Gärung der Obst- und Traubenweine. — VII. Jahresbericht, etc., in Wädensweil pro 1898. VII. 50.—C. f. B. 2. Abt. 1899. V. 684.—Ch. C. 1899. II. 916. —K. J. X. 148.

XVII. Einfluss der schwefligen Säure auf die Gärung.—VII. Jahresbericht, etc., in Wädensweil pro 1898. VII. 56.—C. f. B. 2. Abt. 1899. V. 788.—K. J. X. 128.

XVIII. In welcher Weise lässt sich die Weingärung günstig beeinflussen ?—Bericht, etc., des IX. D. Weinbau-Kongresses zu Rüdesheim. 1886. 66.

XIX. Welches ist die geeignetste Temperatur für die Weingärung. —Bericht, etc., des X. D. Weinbau-Kongresses zu Freiburg i. B. 1888. 94.

XX. Gewinnung und Vermehrung vonWeinheferassen.—IV. Jahresbericht, etc., in Wädensweil pro 1893–94. S. 64.—K. J. VI. 182.

XXI. Ber. ü. d. Verhandl. d. VIII. D. Weinbau-Kongresses in Colmar, 1885. Mainz. 1886. 126.

XXII. Ber. ü. d. Verhandl. d. XI. D. Weinbau-Kongresses in Trier. Mainz. 1889, 87.

XXIII. Ber. ü. d. Verhandl. d. XII. D. Weinbau - Kongresses in Worms. Mainz. 1891, 129.

XXIV. Jahresbericht, etc., in Wädensweil pro 1896–97. VII. 56.—IX. Jahresber., etc., pro 1898–99, 74.

XXV. Die Verstellung unvergorener u. alkoholfreier Obst- u. Traubenweine. 3rd Ed. Frauenfeld. 1896.

XXVI. Ber. ü. d. Verhandl. d. XI. D. Weinbau-Kongresses in Trier. 1889, 88.

XXIX. Weinbau u. Weinhandel. 1889. Nos. 40 and 41.

XXX. V. Jahresber. d. Schweiz. Versuchsanstalt in Wädensweil f. 1894–95. 76.—Weinbau u. Weinhandel. 1897. No. 17.

See also Czéh u. Müller-Thurgau.

Müntz, A.,

I. Sur la décomposition des roches et la formation de la terre arable. —C. R. 1890. CXI. 1370.—K. J. I. 109.

See also Schlösing et Müntz.

Müntz, A., et Girard, A. Ch.,

I. Les pertes d'azote dans les fumiers. — C. R. 1893. CXV. 1318 ; CXVI. 108.—Ch. C. 1893. I. 270, 577.

Müntz, A., et Marcano, V.,
I. Sur la proportion de nitrates contenus dans les pluies des régions tropicales.—C. R. 1889. CVIII. 1062.—Ch. C. 1889. II. 156.

Mulder, Gerardus Johannes,
I. Sur quelques combinaisons de la protéine.—Bulletin des sciences physiques et naturelles en Néerlande. Rotterdam. 1838. — Journal f. prakt. Chemie. 1839. XVI. 297.
II. Die Chemie des Weines.—German trans. by Karl Arenz. 1856. Leipzig. J. J. Weber.
III. Untersuchung der Essigmutter.—Annalen d. Chemie und Pharmacie. 1843. XLVI. 207.
IV. Versuch einer allgemeinen physiologischen Chemie. German Ed. 1844–51. I. 203.
V. Het bier scheikundig beschouwd. Rotterdam. 1857.

Munro,
I. Über die Bildung und Zerstörung von Nitraten in künstlichen Lösungen und im Fluss- und Quellwasser.—Journal of the Chemical Society. 1886. XLIX. 632.—Ch. C. 1886. 700. —Biedermann's Centralblatt f. Agrikulturchemie. 1888. XVII. 649.

Münzer,
I. Münch. med. Wochenschrift. 1903. L. 1949.

Murphy, J.,
I. Some Aspects of the Purc Yeast Question.—Journal of the Federated Institutes of Brewing. 1899. V. 432.—K. J. X. 118.

Musculus,
I. Sur le ferment de l'urée.—C. R. 1876. LXXXII. 333.—Ch. C. 1876. 247.

Musset, Franz,
I. Zum Nachweis von Mutterkorn im Mehl.—Pharmac. Centralhalle. 1899. XL. 353.—Ch. C. 1899. II. 149.

Musso, Giov.,
See Manetti und Musso.

Nägeli, Carl von,
I. Mechanisch - physiologische Theorie der Abstammungslehre. 1884. München.
II. Theorie der Gärung. 1879. München.
III. Über Gärung ausserhalb der Hefezellen.—Zeitschrift f. Biologie. 1882. XVIII. 543.—Z. g. Br. 1882. V. 485.
IV. Die Ernährung der niederen Pilze. — Sitzungsberichte der Bayr. Akademie. Mathem.-physik. Kl. 1879. IX. 395.—Ch. C. 1881. 45.
V. Verhandlungen d. 33 Versammlung D. Naturforscher und Ärzte zu Bonn. 1857. — Bot. Ztg. 1857. XV. 760.
VI. Die niederen Pilze in ihren Beziehungen zu den Infektionskrankheiten und der Gesundheitspflege. München. 1877.
VII. Verhalten der Zellhaut zum Jod.—Sitzgsber. d. Bayr. Akad. d. Wiss. 1863. 383.

Nägeli, C. von, u. Loew, O.,
I. Über die Fettbildung bei den niederen Pilzen. — Journal f. prakt. Chemie. 1880. XXI. 97.—Reprinted in Z. g. Br. 1880. III. 499.—Ch. C. 1880. 231.
II. Über die chemische Zusammensetzung der Hefe.—Sitzgsber. d. Kgl. Akademie d. Wiss. in München. 1878. VIII. 161.—Reproduced in Z. g. Br. 1878. I. 337; also in Journal. f. prakt. Chemie. 1878. CXXV. 403.
III. Sitz. d. Kgl. Akad. d. Wiss. München. 1878. VIII. 173.

Nasse,
I. A. f. Hygiene. 1875. XI. 138.
II. *Ibid.* 1877. XV. 471.

Nastukoff, A.,
I. Essais sur le pouvoir réducteur des levures pures; moyen de le mesurer.—C. R. 1895. CXXI. 535.—Ch. C. 1895. II. 1049.—K. J. VI. 59.
II. Über die Sporenbildung der russischen Weinhefen.—C. f. B. 2. Abt. 1898. IV. 420.—K. J. IX. 34.

Nathan, L.,
I. Fortschritte auf d. Gebiete der Fruchtweinbereitung. Stuttgart. 1893.—Abst. in K. J. 1893. IV. 160.
II. W. f. Br. 1903. XX. 397.

NAUMANN, O.,
I. Über den Gerbstoff der Pilze. Dissert. Erlangen. 1895.—Bot. Ztg. 2. Abt. 1896. LIV. 157.
NEEDHAM, John Tuberville,
I. New Mieroscopieal Diseoveries. London. 1745.
NEELSEN, F.,
I. Studium über die blaue Milch.—Cohn's Beiträge zur Biologie der Pflanzen. III. 187.
NEISSER,
I. Versuehe über die Sporenbildung bei Xerosebaeillen, Streptokokken und Choleraspirillen.—Z. f. Hygiene. 1888. IV. 268.—C. f. B. 1888. IV. 139.
NENCKI, Leon,
I. Recherches ehimiques sur les mierobes produisant l'inflammation des glandes mammaires des vaches et des chèvres laitières.—Archives de sciences biologiques publ. p. l'Institut imp. de médeeine expérim. St. Pétersbourg. 1892. I. 25.—K. J. III. 179.
NENCKI, L., und FABIAN, A.,
I. O przetworach fermentowanych z mleka, a mianowicie o kumysie i kefirze. — Gazeta Lekarska. 1887. No. 3.—C. f. B. 1. Abt. 1887. II. 523.
NENCKI, Mareel von,
I. Über Misehkulturen.—C. f. B. 1892. XI. 225.—K. J. III. 55.
II. Die isomeren Milchsäuren als Erkennungsmittel einzelner Spaltpilzarten.—C. f. B. 1891. IX. 304.—Ch. C. 1891. I. 884.—K. J. II. 17.
III. Der ehemisehe Meehanismus der Fäulnis.—Journal f. prakt. Chemie. 1878. CXXV. 105.—Ch. C. 1878. 294.
IV. Untersuchungen über die Zersetzung des Eiweisses durch anaerobe Spaltpilze.—Sitzungsber. d. k. Akademie d. Wiss. zu Wien. Monatshefte für Chemie. 1889. X. 506.—C. f. B. 1890. VII. 129.—Ch. C. 1889. II. 847.
V. Über das Verhalten der Gelatine, des Eiweisses, des Leueins, Tyrosins und Glykokolls bei der Fäulnis mit Pankreas. Bern. 1876. — Medizin. Centralblatt. 1876. XV. 297.—Ch. C. 1877. 374.
VI. Journ. f. prakt. Chem. 1883. XVII. 105.
NENCKI, M. v., u. SCHAFFER, F.,
I. Über die chemisehe Zusammensetzung der Fäulnisbakterien.—Journal für prakt. Chemie. 1879. CXXVIII. 443.
See also MACFADYEN, NENCKI u. SIEBER.
NENCKI, M. v., u. SIEBER, N.,
I. Über die Bildung der Paramilchsäure durch Gärung.—Monatshefte f. Chemie. 1889. X. 532.—C. f. B. 1890. VII. 130.—Ch. C. 1889. II. 849.
II. Zur Kenntnis der bei der Eiweissgärung auftretenden Gase.—Sitzungsber. d. K. Akademie d. Wiss. zu Wien. Monatshefte f. Chemie. 1889. X. 526.—C. f. B. 1890. VII. 131.—Ch. C. 1889. II. 848.
NESSLER, Julius,
I. Der Tabak. Mannheim. 1867.
II. Die Bereitung, Pflege und Untersuchung des Weines. 7th Ed. Stuttgart. 1898. 6*s*.
III. Die Ursachen des Krankwerdens der Weine.—Bericht ü. d. Verh. d. XIV. D. Weinbau-Kongresses in Neustadt a. d. Hardt. 1895. 57.
NESTLER, Anton,
I. Über einen in der Frueht von Lolium temulentum L. entdeckten Pilz.—Ber. d. D. Bot. Ges. 1898. XVI. 207.—C. f. B. 2. Abt. 1899. V. 365.
NEUBAUER, C.,
I. Studien über den Rotwein.—Annalen der Önologie. 1872. II. 1.—Ch. C. 1872. 778.
NEUMANN, Albert,
See KOSSEL und NEUMANN.
NEUMANN, J.,
I. Über die Konservierung der Milch dureh Kaliumbichromat, Ammoniak und Ammoniakverbindungen.—Milehzeitung. 1893. XXII. 453.—Ch. C. 1893. II. 1109.—K. J. IV. 202.
NEUMANN, R.,
See LEHMANN und NEUMANN.
NEUMANN-WENDER,
I. Die Presshefe des Handels.—Zeitschrift f. Nahrungsmittel-Untersuehung, Hygiene u. Warenkunde. 1896. X. 153.—K. J. VII. 149.

NEUMAYER, Johann,
I. Untersuchungen über die Wirkungen der verschiedenen Hefearten, welche bei der Bereitung weingeistiger Getränke vorkommen, auf den tierischen und menschlichen Organismus.—Dissert. München. 1890.—Z. g. Br. 1890. XIII. 297.—C. f. B. 1. Abt. 1893. XIII. 611.—K. J. I. 34.

NEUMEISTER, Richard,
I. Bemerkungen zu Eduard Buchner's Mitteilungen über "Zymase."—Ber. d. D. Chem. Ges. 1897. XXX. 2963. — Ch. C. 1898. I. 394.—K. J. VIII. 284.
II. Über das Vorkommen und die Bedeutung eines eiweisslösenden Enzymes in jugendlichen Pflanzen. — Zeitschrift f. Biologie. 1893. XII. 447.—Ch. C. 1894. I. 828.

NEUVILLE,
I. Les ferments industriels de l'Extrême-Orient. Paris. 1902. 175.

NEWCOMBE,
I. Ann. of Botany. 1899. XIII. 49. — Bot. Centralbl. 1898. LXXIII. 105.

NEWLANDS, R., and LING, A. R.,
I. J. Fed. Inst. Brewing. 1901. VII. 181.

NIEDERHÄUSER, Emil,
I. Über italienische Weine.—Pharmaceutische Centralhalle. 1891. XXXII. 15.—Ch. C. 1891. I. 383.

NIEDERSTADT,
I. Über die Wirkung des Zentrifugierens auf die Verteilung der Bakterien in der Milch.—Zeitschrift f. Nahrungsmittel-Untersuchung u. Hygiene. 1893. VII. 3.—K. J. IV. 205.

NIELSEN, J. Chr.,
I. Sur le développement des spores du Sacch. membranæfaciens, du Sacch. Ludwigii et du Sacch. anomalus.—Compte rendu, etc., de Carlsberg. 1894. III. 176.—C. f. B. 2. Abt. 1895. I. 187.—K. J. V. 36.

NIEMANN, F.,
See JESERICH und NIEMANN.

NIEMILOWICZ,
See KRATSCHMER u. NIEMILOWICZ.

NIGGL, Max,
I. Das Indol ein Reagens auf verholzte Membranen. — Flora. 1881. XLIV. 545.

NISHIMURA, T.,
I. Bull. Coll. Agricult. Tokyo. III. 191.

NISHIMURA, Tojosaku,
I. Untersuchungen über die chemische Zusammensetzung eines Wasserbacillus.—A. f. Hygiene. 1893. XVIII. 318. — Ch. C. 1893. II. 1007.—K. J. IV. 71.

NISSEN,
I. Über das Verhalten der Kerne in den Milchdrüsen-Zellen bei der Absonderung.—Archiv f. mikroskop. Anatomie. 1886. XXVI.

NIVIÈRE, G., et HUBERT, A.,
I. Sur la gomme des vins.—Revue intern. des falsific. 1896. IX. 48.—Ch. C. 1896. I. 898.

NOBBE, Friedrich,
I. Versuche über Leguminosen-Knöllchen.—Tageblatt d. Vers. D. Naturf. u. Ärzte zu Bremen. 1890.—K. J. I. 132.

NOBBE, Fr., und HILTNER, L.,
I. Wodurch werden die knöllchenbesitzenden Leguminosen befähigt, den freien atmosphärischen Stickstoff für sich zu verwerten ? — D. landw. Versuchs-Stationen. 1893. XXXXII. 459.—K. J. IV. 215.

NOBBE, F., HILTNER, L., und SCHMID, E.,
I. Versuche über die Biologie der Knöllchenbakterien der Leguminosen, insbesondere über die Frage der Artcinheit derselben.—D. landw. Versuchs-Stationen. 1894. XXXXV. 1.—C. f. B. 2. Abt. 1895. I. 199.—Ch. C. 1894. II. 705.—K. J. V. 251.

NOBBE, F., SCHMID, E., HILTNER, L., u. HOTTER, E.,
I. Die Stickstoffassimilation der Leguminosen.—D. Landw. Versuchs-Stationen. 1891. XXXIX. 327.—C. f. B. 1892. XII. 685.—K. J. II. 199.
II. Über die physiologische Bedeutung der Wurzelknöllchen von Elæagnus angustifolius. — D. Landw. Versuchs-Stationen. 1892. XXXXI. 138.—K. J. III. 207.

Nocard, E., et Mollereau,
I. Sur une mammite contagieuse des vaches laitières.—Ann. Inst. Past. 1887. I. No. 3.—C. f. B. 1. Abt. 1888. III. 15.

Noll, Alfred,
I. Über die Bildung von Lävulinsäure aus Nucleinsäuren.—Z. f. physiolog. Chemie. 1898. XXV. 430.—Ch. C. 1898. II. 723.

Nomura,
I. Bot. Magazine. Tokyo. 1897. XI. No. 123, p. 31.

Nordhausen, M.,
I. Beiträge zur Biologie parasitärer Pilze.—Pringsheim's Jahrbücher f. w. Bot. 1898. XXXIII. 1.—C. f. B. 2. Abt. 1899. V. 527.

Nordtmeyer, H.,
I. Über Wasserfiltration durch Filter aus gebrannter Infusorienerde.—Z. f. Hygiene. 1891. X. 145.—C. f. B. IX. 644.—K. J. II. 22.

Nuttal, George H. F., u. Thierfelder, H.,
I. Tierisches Leben ohne Bakterien im Verdauungskanal.—Z. f. physiolog. Chemie. 1895. XXI. 109. 1896. XXII. 62.—Ch. C. 1896. I. 118; II. 50.

Oberdörffer, H. J.,
I. Über die Einwirkung des Ozons auf Bakterien. Dissertation. Bonn. 1889.—C. f. B. 1890. VII. 350.

Obermayer, F., und Kerry, R.,
I. Studien zur Kenntnis der Eiweissfäulnis.—Centralblatt f. Physiologie. 1894. VII. 806.—Ch. C. 1894. I. 825.—K. J. V. 74.

Obermüller, Kuno,
I. Über Tuberkelbacillenfunde in der Marktmilch.—Hygien. Rundschau. 1895. V. 878.—Ch. C. 1896. I. 126.

Obrastzow, S.,
See Iwanowski u. Obrastzow.

Ogston,
I. Über Abscesse.—Arch. f. klin. Chirurgie. 1880. XXV.
II. Report on Micro-organisms in Surgical Diseases.—Brit. Med. Journ. 1881, p. 369.

Ohlmüller,
I. Über die Wirkung des Ozons auf Bakterien.—Arbeiten a. d. K. Gesundheits-Amte. 1892. VIII. 228.—C. f. B. 1892. XI. 773.—K. J. III. 72.

Okada, K.,
I. Über einen (roten Farbstoff erzeugenden) Bacillus.—C. f. B. 1892. XI. 1.—K. J. III. 43.

Okamura u. Takakusu,
I. Quoted by Kozai, II.

Olivier, L.,
See Etard et Olivier.

Oltmanns, Friedrich,
I. Über positiven und negativen Heliotropismus. — Flora. 1897. LXXXIII. Heft 1.—Bot. Ztg. 1897. LV. 23.

Omeis, Th.,
I. Über den konzentrierten Traubenmost. — Forschungsberichte über Lebensmittel, Warenkunde und Hygiene. 1894. I. 474.—Ch. C. 1895. I. 183.

Omeliansky, V.,
I. Sur la fermentation de la cellulose.—C. R. 1895. CXXI. 653.—Ch. C. 1895. II. 1166.—Z. g. Br. 1896. XIX. 137.

Ono, N.,
I. Über die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize.—Journal of the College of Science, Imp. University of Tokyo. 1900. XIII. 141.—C. f. B. 2. Abt. 1902. IX. 154.—Ch. C. 1902. II. 1068.

Oppenheimer, Carl,
I. Die Fermente und ihre Wirkungen. Leipzig. 1900. 10*s.*—C. f. B. 2. Abt. 1900. VI. 706.
II. Die Fermente. 2nd Ed. 1903. 105.
III. *Ibid.* 2nd Ed. 1903, p. 242.

Oppenheimer,
I. Biologie der Milchkotbakterien des Säuglings.—C. f. B. 1889. VI. 586.

Ordonneau, Ch.,
I. Etude sur les produits volatils des vins et sur l'équation de fermentation des sucres par les Mycoderma cerevisiæ et ellipsoideus.—Bulletin de la Soc. chimique. 1886. XXXXV. 332.—C. R. 1886. CII. 217.—Z. g. Br. 1886. IX. 122.

Orlowski.
I. Wirkung des Arsens auf Wachstum u. chemische Zusammensetzung von Asp. niger. Dissert. St. Petersburg. 1902. (Russian.)—Bot. Centralbl. 1904. XCV. 302.

Ortloff, H.,
I. C. f. B. 2. Abt. 1900. VI. 676.

Osborne, A.,
I. Die Sporenbildung des Milzbrandbacillus auf Nährböden von verschiedenem Gehalt an Nährstoffen.—A. f. Hygiene. 1890. XI. 51.—K. J. I. 27.

Osborne, Thomas B., und Campbell, G. F.,
I. Die Nucleinsäure aus dem Embryo des Weizens und ihre Proteinverbindungen. — Journal of the American Chemical Society. 1900. XXII. 379.—Ch. C. 1900. II. 538.

Osborne, W. A.,
I. Z. f. physiolog. Chemie. 1899. XXVIII. 399.

Oser, Johann,
I. Untersuchungen über die Alkoholgärung.—Sitzgsber. d. Kais. Akademie der Wissenschaften Wien. Mathem.-naturw. Klasse. 1867. LVI. 2. Abt. 489.—Ch. C. 1869. I. 141.

Ost,
I. Chem. Ztg. 1896. XX. 762.
II. Ber. d. D. Chem. Ges. 1890. XXIII. 3006.

Ostertag, Robert,
I. Handbuch der Fleischbeschau. 3rd Ed. Stuttgart. 1899. 20*s*.

Osterwalder, A.,
I. Landw. Jahr. d. Schweiz. 1902. XVI. 498.
II. Weinbau u. Weinhandel. 1903. V. 169.

Osthoff,
I. Anlagen für die Versorgung der Städte mit Lebensmitteln. Markthallen, Schlachthöfe und Viehmärkte.—Weyl, Handbuch der Hygiene. 5. Bd. Jena. Gustav Fischer. 1892.—C. f. B. 1894. XVI. 718.

O'Sullivan, Cornelius,
I. Engl. Patent No. 19161, 1897.
II. Trans. Fed. Inst. Brewing. 1893. VI. 67.

O'Sullivan and Tompson,
I. Journ. Chem. Soc. 1890. LVII. 834.

Otto, Richard,
I. Beobachtungen und Ergebnisse bei der Untersuchung und Vergärung von Heidelbeermosten. —Landw. Jahrbücher. 1898. XXVII. 261.—C. f. B. 2. Abt. 1897. III. 428.—Ch. C. 1898. II. 734.—K. J. VIII. 123.

Oudemans,
I. Nederl. Kruidk. Arch. 1886. 2nd Series. IV. 535.
II. Arch. Néerlandaises des Sciences exactes et Natur. 1902. 2nd Series. VII. 288.
III. *Ibid.* 283.
IV. Nederl. Kruidk. Arch. 1904. 2nd Series. II. Suppl. 4, p. 1123.

Overbeck, A.,
I. Zur Kenntnis der Fettfarbstoffproduktion bei Spaltpilzen.—Nova Acta Academiæ Cæsareæ Leopoldino-Carolinæ Germanicæ Naturæ Curiosorum. 1891. LV. 399.—K. J. II. 85.

Overbeck, O.,
I. Über eine neue Methode zur Entdeckung freien Schwefels im Hopfen.—The Brewer's Journal. 1891. No. 307.—K. J. II. 142.

Overbeck, Otto Gerhard Christoph,
I. German Patent No. 107737, Dec. 21, 1898.—Ch. C. 1900. I. 1008.

Overton, E.,
I. Über die osmotischen Eigenschaften der lebenden Pflanzen- und Tierzelle.—Vierteljahrschrift d. naturf. Ges. in Zürich. 1895.—Ch. C. 1895. II. 726.—Z. g. Br. 1896. XIX. 47.

Pabst,
I. Neues Ferment zur Bereitung billigen moussierenden Getränkes aus Zuckerlösung.—Soc. industr. de Mulhouse. 1890. K. J. I. 143.

Pässler, Johannes,
I. Gerbt Tannin die tierische Haut? —Chemiker - Zeitung. 1894. XVIII. 363.—Ch. C. 1894. I. 802.

Paetow, U.,
See Herzfeld und Paetow.

Pagès, C.,
See Arthus et Pagès.
Palladin, W. J.,
I. C. f. B. 2. Abt. 1904. XIII. 353.
Pallas,
I. Sammlung historischer Nachrichten über die mongolischen Völkerschaften. St. Petersburg. 1776. I. 133.
Palm, R.,
I. Über den chemischen Charakter des violetten Farbstoffes im Mutterkorn, sowie dessen Nachweis im Mehle.—Zeitschr. f. analyt. Chemie. 1883. XXII. 319.
Pammel, L. H.,
I. Some Bacteriological Work in the Dairy. Extracts from the Iowa Agricult. Experim. Stat. Bull. No. 21, p. 6.—C. f. B. 1894. XVI. 128.
II. An Aromatic Bacillus of Cheese (Bacillus aromaticus). Extracts, etc., p. 1.—C. f. B. 1894. XVI. 128.
Pammel, Weems, and Lawson-Scribner,
I. Iowa Geological Survey. 1901.
Pantanelli,
I. Jahrb. wiss. Bot. 1904. XL. 303.
Panum, P. L.,
I. Das putride Gift, die Bakterien, die putride Infektion oder Intoxikation und die Septikämie.—Virchow's Archiv. 1874. LX. 328.—Bibliothek for Läger. 1856. 253.
Pasteur, Louis,
I. Mémoire sur les corpuscules organisés qui existent dans l'atmosphère.—Annales de Chim. et de Phys. 1862. LXIV. 6.—German transl. by von A. Wieler. Leipzig. Engelmann. 1892. 1*s.* 10*d.*
II. Influence de l'oxygène sur le développement de la levure et sur la fermentation alcoolique.—C. R. 1861. LII. 1260. 1863. LVII. 936.
III. Etudes sur la bière, ses maladies, causes qui les provoquent, procédé pour la rendre inaltérable, avec une théorie nouvelle de la fermentation. Paris. 1876. Gauthier-Villars.
IV. Faits nouveaux pour servir à la connaissance de la théorie des fermentations proprements dites. — C. R. 1872. LXXV. 784.
V. Note sur la production de l'alcool par les fruits.—C. R. 1872. LXXV. 1054.
VI. Etudes sur la maladie des vers à soie. 1870. I. 228.
VII. Mémoire sur la fermentation alcoolique.—Annales de Chimie et de Physique. 1860. 3ème série. LVIII. 323. — German transl. by Victor Griessmayer: Die Alkoholgärung. Augsburg. 1871.
VIII. Animalcules infusoires vivant sans gaz oxygène libre et déterminant des fermentations.—C. R. 1861. LII. 344 et 1260.—Journal f. prakt. Chemie. 1861. LXXXIII. 374.—Ch. C. 1864. 459.
IX. Nouvel exemple de fermentation déterminée par des animalcules infusoires pouvant vivre sans oxygène libre et en dehors de tout contact avec l'air de l'atmosphère. Fermentation du tartrate de chaux.—C. R. 1863. LVI. 416.—Journal f. prakt. Chemie. 1863. LXXXIX. 351. —Ch. C. 1864. 460.
X. Mémoire sur la fermentation lactique.—C. R. 1857. XXXXV. 913. 1858. XXXXVII. 224. 1859. XXXXVIII. 337 et 1149.
XI. Über die Asymmetrie bei natürlich vorkommenden organischen Verbindungen. 1860.—German transl. by. M. u. A. Ladenburg. Leipzig. Engelmann. 7*d.*—C. R. 1860. LI. 298.
XII. Etudes sur le vin, ses maladies, causes qui les provoquent, procédés nouveaux pour le conserver et pour le vieillir. Paris. 1866.
XIII. Mémoire sur la fermentation acétique.—Annales scientifiques de l'Ecole normale supérieure.—Paris. 1864.
XIV. Etudes sur le vinaigre. Paris. 1868. — German transl. by E. Borgmann ("Der Essig"). Braunschweig. 1878.

XV. Nouveau procédé pour la fabrication du vinaigre.—C. R. 1862. LV. 28.—Dingler's Journal. 1862. CLXV. 303. 1871. CCI. 67.
XVI. Des altérations spontanées ou maladies des vins, particulièrement dans le Jura.—Etudes sur les vins; deuxième partie.—C. R. 1864. LVIII. 142.
XVII. Observations relatives à une note de M. Duclaux sur la germination dans un sol riche en matières organiques, mais exempt de microbes.—C. R. 1885. C. 66.—Ch. C. 1885. 142.
XVIII. Examen critique d'un écrit posthume de Claude Bernard sur la fermentation alcoolique. Paris. 1879.
XIX. Nouveaux faits concernant la fermentation alcoolique.—C. R. 1859. XXXXVIII. 640.
XX. Procédé pratique de conservation et d'amélioration des vins.—C. R. 1865. LX. 899; LXI. 274, 865, 979. 1869. LXIX. 577, 645, 905, 973.
XXI. Mémoire sur la fermentation de l'acide tartrique.—C. R. 1858. XXXXVI. 615.
XXII. Note relative au Penicillium glaucum et à la dissymétrie moléculaire des produits organiques naturels.—C. R. 1860. LI. 298.
XXIII. C. R. 1858. XLVII. 1011.
XXIV. Ann. de Chimie et de Phys. 1872. 4e Sèrie. XXV. 145.
XXV. Etudes sur la bière. 1876. 100.
XXVI. *Ibid.*, p. 73.
XXVII. *Ibid.*, p. 76.
XXVIII. *Ibid.*, p. 148.
XXIX. Cited by Roux, III.
XXX. Etudes sur la bière. 1876. 150, 155, 176-178.
XXXI. Ann. de Chimie et de Phys. 1860. (3.) LVIII. 324.
XXXII. Die Alkoholgärung. — German transl. by Griessmayer. 1887. 27.
XXXIII. Etudes sur les vins. 636.
XXXIV. Ann. de Chimie et de Phys. 1860. (3.) LVIII. 323, 401.

Pasteur, L., et Joubert,
I. Sur les germes des bactéries en suspension dans l'atmosphère et dans les eaux.—C. R. 1877. LXXXIV. 206.—Ch. C. 1877. 214.
II. Sur la fermentation de l'urée.—C. R. 1876. LXXXIII. 1.

Pasteur, Joubert, et Chamberland,
I. La théorie des germes et ses applications à la Médecine et à la chirurgie. — C. R. 1878. LXXXVI. 1037.

Patouillard et Delacroix,
I. Bull. Soc. Mycol. de France. 1891. VII. 118.

Paul, Theodor, und Krönig, B.,
I. Über das Verhalten der Bakterien zu chemischen Reagenzien. —Zeitschrift f. physikal. Chemie. 1897. XXI. 414.—Bot. Ztg. 2. Abt. 1897. LV. 145.—Ch. C. 1897. I. 327.
II. Die gesetzmässigen Beziehungen zwischen Lösungszustand und Wirkungswert der Desinfektionsmittel.—Müneh. medic. Wochenschrift. 1897. XXXXIV. 304.—C. f. B. 1. Abt. 1897. XXI. 710.
See also Krönig und Paul.

Pautz und Vogel,
I. Z. f. Biol. 1895. XXXII. 304.

Payen, Anselme,
I. Extrait d'un Rapport adressé à M. le Ministre de la Guerre sur une altération extraordinaire du pain de munition.—Annales de Chimie et de Physique. 1843. 3e sér. IX. 5.
II. Mémoire sur le développement des végétaux.—Mémoires présentés à l'Académie des sciences de France. 1846. IX. 21.
III. C. R. 1859. XLIX. 521.

Payen, Anselme, et Persoz, J. F.,
I. Mémoire sur la diastase, les principaux produits de ses réactions, et leurs applications aux arts industriels.—Ann. de Chimie et de Physique. 1833. LIII. 73.

Pedersen, Rasmus,
I. Sur l'influence que l'introduction de l'air atmosphérique dans le moût qui fermente exerce sur la fermentation.—Compte rendu, etc., de Carlsberg. 1878. I. 38.

II. Recherches sur quelques facteurs qui ont de l'influence sur la propagation de la levure basse du Saccharomyces cerevisiæ.—Compte rendu, etc., de Carlsberg. 1878. I. 22.

Pedler,
I. Liebig's Ann. 1868. CXLVII. 243.

Peeters,
I. Belg. Patent No. 131555 of 1897.—Engl. Patents Nos. 26985 of 1897 and 17278 of 1898.—Ch. C. 1898. I. 964.—K. J. IX. 103.

Peglion, Victor,
I. Etudes sur la pourriture des raisins causée par le Botrytis cinerea.—Revue intern. de viticulture et d'œnologie. 1895, p. 414.—Zeitschr. f. Pflanzenkrankh. 1896. VI. 102.
II. Staz. sperim. agrarie ital. 1895. XXVIII. 369.
III. Rendic. della R. Accad. dei Lincei. 1897.—C. f. B. 2. Abt. 1901. VII. 754.

Pellet,
I. Chem. Zeit. 1900. XXIV. 356 (notice).

Pelouze u. Liebig,
I. Liebig's Ann. 1836. XIX. 241.

Pelouze, Théophile Jules, et Gay-Lussac, L. J.,
I. Sur l'acide lactique.—Annales de Chimie et de Physique. 1833. LII. 410.

Pelouze, Théophile Jules, et Gélis, A.,
I. Sur l'acide butyrique.—C. R. 1843. XVI. 1262.—Journal f. prakt. Chemie. 1843. XXIX. 453.

Penzo, Rudolf,
I. Beitrag zum Studium der biologischen Verhältnisse des Bacillus des malignen Ödems.—C. f. B. 1891. X. 822.

Perdrix, L.,
I. Sur les fermentations produites par un microbe anaérobie de l'eau.—Ann. Inst. Past. 1891. V. 286.—Ch. C. 1891. II. 252.—K. J. II. 240.

Péré, A.,
I. Sur la formation des acides lactiques isomériques par l'action des microbes sur les substances hydrocarbonées. — Ann. Inst. Past. 1893. VII. 737.—C. f. B. 1894. XVI. 121.—Ch. C. 1894. I. 411.—K. J. IV. 187.

Pereire u. Guignard,
I. German Patent No. 139,387.—Z. f. Spiritusind. 1903. XXVI. 131.

Pernossi, Leone,
See Fermi und Pernossi.

Perrey, A.,
See Hautefeuille et Perrey.

Perroncito, C., e Maggiora, A.,
I. Studien über bitteren Rotwein.—Weinlaube. 1884. 459.

Persoon, Christian Hendrik,
I. Mycologia europæa. — Erlangen. 1822. Sectio prima, p. 96.

Persoz,
I. Ann. de Chimie et de Phys. 1834. LVI. 361.

Persoz, Jean François,
See Payen et Persoz.

Perty, Maximilian,
I. Zur Kenntnis kleinster Lebensformen. Bern. 1852.

Peters, W. L.,
I. Die Organismen des Sauerteiges und ihre Bedeutung für die Brotgärung.—Bot. Ztg. 1889. XXXXVII. 405. Gives antecedent bibliography.—C. f. B. 1889. VI. 228.—Ch. C. 1889. II. 847.

Petersen, Anton,
I. Sarcina im Biere ohne irgend eine Krankheitserscheinung.—Z. g. Br. 1890. XIII. 1.—C. f. B. 1890. VII. 606.—K. J. I. 71.
II. Einige Bemerkungen über das Lüften der Würze.—Z. g. Br. 1888. XI. 53.

Petit, P.,
I. Sur les hydrates de carbone restant dans la bière.—C. R. 1897. CXXIV. 510.—Ch. C 1897. I. 828.—K. J. VIII. 129.
II. Sur une différence entre les levures hautes et basses.—C. R. 1897. CXXIV. 93.—Ch. C. 1897. I. 423.—K. J. VIII. 97.

III. Sur l'oxydation des moûts de bière.—C. R. 1894. CXVIII. 1055.
IV. C. R. 1899. CXXVIII. 1176.

Petit, P., et Labourasse, G.,
I. Sur la solubilisation des matières azotées du malt.—C. R. 1900. CXXXI. 349, 394. — Ch. C. 1900. II. 586, 680.

Petkow,
I. Notice in Chem. Ztg. 1902. XXVI. 41.

Petri, R. J.,
I. Zusammenfassender Bericht über Nachweis und Bestimmung der pflanzlichen Mikroorganismen der Luft.—C. f. B. 1887. II. 113.
II. Über die Widerstandsfähigkeit der Bakterien des Schweinerotlaufes in Reinkulturen und im Fleisch rotlaufkranker Schweine gegen Kochen, Schmoren, Braten, Salzen, Einpökeln und Räuchern. —Arbeiten a. d. Kaiserl. Gesundheitsamte. 1890. VI. 292.—C. f. B. 1890. VIII. 596.
III. Das Mikroskop. Von seinen Anfängen bis zur jetzigen Vervollkommnung für alle Freunde dieses Instruments. Berlin. 1896. R. Schoetz. 8*s*.

Petri, R. J., und Maassen, A.,
I. Über die Bereitung der Nährbouillon für bakteriologische Zwecke.—Arbeiten a. d. Kais. Gesundheitsamte. 1892. VIII. 30.—C. f. B. 1892. XII. 484. —K. J. III. 15.
II. Über die Herstellung von Dauermilch, unter Anlehnung an Versuche mit einem bestimmten neueren Verfahren.—Arbeiten a. d. K. Gesundheitsamte. 1891. VII. Heft 1.—K. J. II. 193.
III. Weitere Beiträge zur Schwefelwasserstoffbildung aerober Bakterien und kurze Angaben über Merkaptanbildung derselben. —Arb. a. d. Kais. Gesundheitsamte. 1893. VIII. 490.—C. f. B. 1894. XV. 908.—K. J. IV. 88.
IV. Beiträge zur Biologie der krankheitserregenden Bakterien insbesondere über die Bildung von Schwefelwasserstoff durch dieselben unter vornehmlicher Berücksichtigung des Schweinerotlaufes.—Arbeiten a. d. Kais. Gesundheitsamte. 1892. VIII. 318.

Peuch, F.,
I. Des effets de la salaison sur la virulence de la viande de pore charbonneux.—C. R. 1887. CV. 285.

Pfeffer, Wilhelm,
I. Über chemotaktische Bewegungen von Bakterien, Flagellaten und Volvocineen. — Untersuchungen a. d. Bot. Institut zu Tübingen. 1887. II. 582.—C. f. B. I. Abt. 1888. III. 684.
II. Über Elektion organischer Nährstoffe.—Pringsheim's Jahrb. f. w. Bot. 1895. XXVIII. 205.—K. J. VI. 65.
III. Pflanzenphysiologie. 2nd Ed. 2 vols. Leipzig. 1897.
IV. Über lokomotorische Richtungsbewegungen durch chemische Reize.—Untersuchungen a. d. Bot. Institut in Tübingen. 1884. I. 363.—Ber. d. D. Bot. Ges. 1883. I. 532.—Bot. Ztg. 1884. XLII. 133.
V. Über die regulatorische Bildung von Diastase.—Sitzgsber. d. kgl. sächs. Ges. d. Wiss. 1896. Mathem.-physik. Klasse. 513.—C. f. B. 2. Abt. 1897. III. 425. —K. J. VII. 235.
VI. Jahrb. wiss. Bot. 1895. XXVIII. 206.
VII. Verhandl. d. Ges. d. Naturf. u. Aerzte. 1899. II. 210.

Pfeifer, Fr.,
I. Über das Vorkommen von schwefliger Säure im Biere.—Z. g. Br. 1889. XII. 345.—Ch. C. 1889. II. 954.

Pfeiffer, Richard,
See Fraenkel und Pfeiffer.

Pflüger, E.,
I. Die Bestimmung des Glycogens nach Brücke und Külz. — Pflüger's Archiv f. d. gesamte Physiologie. 1899. LXXV. 120. —Ch. C. 1899. I. 1167.
II. Eine neue Methode zur Bestimmung des Glycogens.—Pflüger's Archiv, etc. 1899. LXXVI. 531, 543.—Ch. C. 1899. II. 572, 573.

Pfuhl, E.,
I. Über die Desinfektion der Typhus- und Cholera-Ausleerungen mit Kalk.—Z. f. Hygiene. 1889. VI. 97; VII. 363.—C. f. B. 1889. VI. 340. 1890. VII. 260.—Ch. C. 1889. I. 814. 1890. I. 411.

II. Weitere Fortschritte in der Flachsgewinnung. — A reprint from the Rigaer Industrie-Zeitung. 1895. Riga. N. Kymmel. —C. f. B. 1896. II. 275.

Phisalix, C.,
See Charrin et Phisalix.

Pichi, P.,
I. Sopra l'azione dei sali di rame nel mosto di uva sul Saccharomyces ellipsoideus. — Nuova Rass. di vitic. ed enol. Conegliano. 1891. Fasc. V.—C. f. B. 1892. XII. 662.—Ch. C. 1893. I. 184.
II. Annali della R. Scuola di Vitic. e di Enol. in Conegliano. 1892. I.

Pictet, Raoult, und Weyl, Th.,
I. Über die Herstellung von Dauermilch mit dem Apparate der Herren Neuhauss, Gronwald und Oehlmann. — Berliner klinische Wochenschrift. 1891. No. 41. —C. f. B. 1892. XII. 491.

Pictet, R., et Yung, E.,
I. De l'action du froid sur les microbes.—C. R. 1884. XCVIII. 747.—Z. g. Br. 1884. VII. 193.

Pierre u. Puchot,
I. Liebig's Ann. 1869. CLI. 299.

Pinner, A.,
See Krämer und Pinner.

Piorkowski,
I. Über die Einwanderung des Typhus-bacillus in das Hühnerei. —A. f. Hygiene. 1895. XXV. 145.—Ch. C. 1896. I. 266.

Pirotta e Riboni,
I. Studii sul latte. 1879. Pavia.
II. Rendiconti d. R. Istituto Lombardo. 1879.

Plagge,
I. Untersuchungen über Wasserfilter.—Veröffentlichungen a. d. Gebiete d. Militär-Sanitätswesens. Part 9. 1895.—Ch. C. 1896. I. 604.

Plagge und Trapp,
I. Die Methoden der Fleischkonservierung. — Veröffentlichungen aus dem Gebiete des Militär-Sanitätswesens.—Part 5. Berlin. 1893. — C. f. B. 1893. XIII. 768.—K. J. IV. 105.—*See* Trapp, A., I.

Planitz, Hans von der,
I. Das Bier und seine Bereitung einst und jetzt.—Z. g. Br. 1879. II. 13.

Planta, A. v., und Schulze, E.,
I. Ber. d. D. Chem. Ges. 1890. XXIII. 1692. 1891. XXIV. 2705.

Plath, H.,
I. Über die Nitrifikation des Ammoniaks und seiner Salze.— Landw. Jahrbücher. 1887. XVI. 891.—Ch. C. 1888. 344.

Plauchu, E.,
I. Recherches sur la formation des eaux sulfureuses naturelles.—C. R. 1877. LXXXIV. 235.—Ch. C. 1877. 137.

Plaut, Hugo,
I. Zur Konservierungstechnik.—C. f. B. 1889. V. 324.

Podwyssozki, W.,
I. Kefyr, kaukasisches Gärungsferment und Getränk aus Kuhmilch. St. Petersburg. 1884.

Poehl, Alexander,
I. Zur Lehre von den Fäulnis-Alkaloiden.—Ber. d. D. Chem. Ges. 1883. XVI. 1975.

Poggiale,
I. Sur une altération spéciale et extraordinaire du pain de munition.—Bulletin de l'Académie de médecine. 1871. XXXVI. 657.

Pohl, C., und Bauer, H.,
I. Apparate für kontinuierliche Fortpflanzung reingezüchteter ober- und untergäriger Hefe-rassen für Brauereien und Brennereien. German Patent No. 64372, March 17, 1892.—Z. g. Br. 1893. XVI. 3.

Pohl, Josef,
I. Über Fischgifte.—Ch. C. 1894. I. 1061.

Pollacci, Gino,
I. Sulla distribuzione del Fosforo nei tessuti vegetali.—Malpighia. 1894. VIII. 94.—Zeitschrift f. Pflanzenkrankheiten. 1895. V. 299.—Zeitschrift f. wissenschaftl. Mikroskopie. 1894. XI. 539.

Polzeniusz, F.,
See Godlewski u. Polzeniusz.

Popoff, D.,
I. Die Zeit der Erscheinung und die allmähliche Verbreitung der Mikroorganismen im Verdauungstraktus der Tiere. — Wratsch. 1891. No. 39.—C. f. B. 1. Abt. 1892. XI. 214.—K. J. II. 63.

Popoff, P. M.,
I. Über die Einwirkung von eiweissverdauenden Fermenten auf die Nucleinstoffe.—Z. f. physiolog. Chemie. 1894. XVIII. 533.—Ch. C. 1894. I. 289.
Popoff,
I. Über Sumpfgasfäulnis.—Pflüger's Archiv f. d. ges. Physiologie. 1875. X. 113.—Dingler's Journal. CCXVI. 191.—Ch. C. 1875. 470.
II. Sur un bacille anaérobie de la fermentation panaire. — Ann. Inst. Past. 1890. IV. 674.—C. f. B. 1891. IX. 104.—Ch. C. 1891. I. 548.—K. J. I. 143.
Popp und Becker,
I. Über die Verarbeitung erhitzter Milch in den Molkereien. — Hygien. Rundschau. 1893. III. 530.—K. J. IV. 201.
Porodko,
I. Jahrb. wiss. Bot. 1904. XLI. 1.
Potievin, H.,
I. Recherches sur le pouvoir antiseptique de l'aldéhyde formique. —Ann. Inst. Past. 1894. VIII. 796.—K. J. V. 93.
II. C. R. 1900. CXXXI. 1215.
Pottevin and Napias,
I. C. R. Soc. Biol. 1898. (10.) V. 237.
Pouchet, A.,
I. Sur la germination des levures, des fermentations, et sur les végétaux qu'elles produisent.—C. R. 1868. LXVII. 376, 549.
Poulsen, S. V.,
See Holm und Poulsen.
Power, W. H.,
I. Milk-Scarlatina in London.—Report of the Medical Officer of the Local Government Board for 1885–1886. No. 8*a*, p. 73.—C. f. B. 1887. II. 217.
Pozzi-Escot, Emm.,
I. Bull. Soc. Chimique. 1901. XXVII. 557.
II. *Ibid.* 1902. (3.) XXVII. 346.
III. *Ibid.* XXVII. 460.
IV. *Ibid.* XXVII. 459.
V. *Ibid.* XXVII. 692.
VI. Bull. de l'Assoc. chim. de sucr. et distill. 1906. XXIII. 1021.
VII. C. R. 1903. CXXXVII. 495.
VIII. Bull. Soc. Chimique. 1902. (3.) XXVIII. 280.
Prandtl, C.,
I. Dingler's Journ. 1865. CLXXVIII. 149.
Prazmowski, Adam,
I. Untersuchungen über die Entwicklungsgeschichte und Fermentwirkung einiger Bakterien-Arten. — Dissert. Leipzig. 1880.
II. Über Sporenbildung bei den Bakterien.—Biolog. Centralblatt. 1888. VIII. 301.—C. f. B. 1888. IV. 325.
III. Über den genetischen Zusammenhang der Milzbrand- und Heubakterien.—Biolog. Centralblatt. 1884. IV. 393.
IV. Die Wurzelknöllchen der Erbse. —Vers.-Stat. 1890. XXXVII. 161; XXXVIII. 1.—C. f. B. 1889. V. 805. 1890. VIII. 379. —K. J. I. 112.
Prechtl, J. B.,
I. Zur Geschichte des bayrischen Bieres.—Z. g. Br. 1879. II. 469.
Preyer, A.,
I. D. Tropenpflanzer. 1901. V.—Abst. in C. f. B. 2. Abt. 1902. VIII. 715.
Preyss, M.,
I. Über den auffallenden Eiweissgehalt der ungarischen Weine als Ursache ihrer geringen Haltbarkeit und dessen Gegenmittel. —Weinbau. 1875. I. 189.
Pringsheim, H. H.,
I. Ber. d. D. Chem. Ges. 1905. XXXVIII. 486.
Prinsen-Geerligs, H. C.,
I. Einige chinesische Sojabohnenpräparate. — Chemiker - Zeitg. 1896. XX. 67.—Ch. C. 1896. I. 607.—K. J. VII. 250.
II. Eine technisch angewandte Zuckerbildung aus Reis.—Chemiker-Zeitg. 1895. XIX. 1681, 1805.—C. f. B. 2. Abt. 1896. II. 122.—Ch. C. 1895. II. 872, 1019.—K. J. VI. 315.
III. Ist Methylalkohol ein normaler Bestandteil in Rum und Arac ?—Chemiker-Zeitg. 1898. XXII. 70.—Ch. C. 1898. I. 642.
IV. Ang-Khak, ein chinesischer Pilzfarbstoff zum Färben von Esswaren. — Chemiker - Zeitg. 1895. XIX. 1311.—C. f. B. 2. Abt. 1896. II. 234.—Ch. C. 1895. II. 469.—K. J. VI. 74.
V. Notice in Chem. Ztg. XXI. 150.
See also Went und Prinsen-Geerligs.

Prior, Eugen,
I. Über die Menge und Natur der beim Mälzen der Gerste, Darren und Maischen des Malzes und Kochen der Würze gebildeten Säuren.—Bayer. Brauerjournal. 1895. V. 181.—Z. g. Br. 1895. XVIII. 395.
II. Chemie und Physiologie des Malzes und Bieres. Leipzig. 1896. J. A. Barth.
III. Über die Erfahrungen beim Arbeiten mit Nürnberger Reinhefen. — Bayer. Brauerjournal. Nürnberg. 1891. I.
IV. Bayer. Braujournal. 1893. III. 2.
V. Chemie u. Physiologie d. Malzes u. d. Bieres. 1896. 397.
VI. Bayer. Braujournal. 1895. V. 49.
VII. *Ibid.* 1892. II. 1. 1894. IV. 469. 1895. V. 97.

Prior, E., und Schulze, H.,
I. Z. f. angew. Chemie. 1901. 208.

Prochownik und Spaeth,
I. Über die keimtötende Wirkung des galvanischen Stromes. — Deutsche medic. Wochenschrift. 1890. No. 26.—C. f. B. 1891. IX. 324.—K. J. I. 45.

Proskauer, Bernhard, u. Beck, M.,
I. Beiträge zur Ernährungsphysiologie des Tuberkelbacillus.—Z. f. Hygiene. 1894. XVIII. 128.—Ch. C. 1894. II. 995.—K. J. V. 74.
See also Fischer und Proskauer.

Proust, J. L.,
I. Recherches sur le principe qui assaisonne les fromages.—Journal de Physique. 1819. LXXXIX. 233.

Prove, Oskar,
I. Micrococcus ochroleucus, eine neue chromogene Spaltpilzform. —Cohn's Beiträge zur Biologie der Pflanzen. 1887. IV. 409.—C. f. B. 1887. II. 498.

Prudden, T. Mitchell,
I. On Bacteria in Ice and their Relations to Disease, with Special Reference to the Ice Supply of New York City.—New York Medical Record. 1887.—C. f. B. 1887. I. 650.

Purdie, T., and Walker, J. W.,
I. Resolution of Lactic Acid into its Optically Active Compounds. —Journal of the Chem. Soc. Transactions. 1892. LXI. 754. —Chem. News. 1892. LXVI. 352.—Ch. C. 1892. II. 352.—K. J. III. 171.
II. Darstellung der aktiven Milchsäuren und die Drehung von deren gelösten Metallsalzen.—Chem. News. 1895. LXXI. 278. —Ch. C. 1895. II. 157.

Puriewitsch, K.,
I. Über die Stickstoffassimilation bei den Schimmelpilzen.—Ber. d. D. Bot. Ges. 1895. XIII. 339. —Ch. C. 1896. I. 125.
II. Über die Wirkung des Lichtes auf den Atmungsprozess bei den Pflanzen.—Schriften d. Ges. d. Naturforscher in Kiew. 1890. XI. 211. (Russian.)
III. Über die Atmung der Schimmelpilze auf verschiedenen Nährlösungen.—Ber. d. D. Bot. Ges. 1898. XVI. 290.—C. f. B. 2. Abt. 1899. V. 223.—Ch. C 1899. I. 1251.
IV. Schriften d. Ges. d. Naturforscher in Kiew. 1899. XVI.
V. Comptes rendus Soc. de Biologie. 1897. 686.
VI. Ber. d. D. Bot. Ges. 1898. XVI. 368.
VII. *Ibid.* XIII. 339.
VIII. *Ibid.* 1896. XIV. 210.

Quevenne, T. A.,
I. Sur la levure et la fermentation vineuse.—Journal de Pharmacie. 1838. XXIV. 265, 329.—Journal f. prakt. Chemie. 1838. XIV. 328, 458.

Quinquaud, E.,
See Gréhant et Quinquaud, Schutzenberger et Quinquaud.

Rabinowitsch, Lydia,
I. Über die thermophilen Bakterien.—Z. f. Hygiene. 1895. XX. 154.—C. f. B. 2. Abt. 1895. I. 585.—Ch. C. 1895. II. 165.

Rabuteau,
I. Sur les effets des alcools de la série $C_nH_{2n} + 2O$.—C. R. 1875. LXXXI. 631.
II. C. R. 1878. LXXXVII. 500.

Raciborski, M.,
I. Mykologische Studien. — Anzeiger der Akademie d. Wissenschaften in Krakau. 1896. 377.
II. Cryptogamæ parasiticæ in insula Java lectæ exsiccatæ Buitenzorg. 1899. Fasc. II.
III. Bull. Acad. Sciences de Cracovie. Cl. des Sci. Math. et Nat. 1905. 693.

Radais, M.,
See Sauvageau et Radais.

Radenhausen, P.,
See Danilewsky und Radenhausen.

Radziszewski, Bronislaus,
I. Über die Phosphorescenz der organischen und organisierten Körper.—Annalen d. Chemie. 1880. CCIII. 305.

Rapp, Rudolf,
I. Einfluss des Sauerstoffes auf gärende Hefe.—Ber. d. D. Chem. Ges. 1896. XXIX. 1983.—C. f. B. 2. Abt. 1896. II. 680.—Ch. C. 1896. II. 899.—K. J. VII. 86.
II. Münch. med. Wochenschrift. 1902. XLIX. 1494.
See also Buchner und Rapp.

Rathgen, F.,
I. Über Konservierung antiker Bronzen. — Dingler's polyt. Journal. 1896. CCCI. 44.—Ch. C. 1896. II. 415.
II. Japans Volkwirthschaft u. Staatshaushalt. 1891.

Rátz, Stefan von,
I. Über die schleimige Milch.—Archiv f. Tierheilkunde. 1890. XVI. 100.—K. J. I. 87.

Rau, A.,
I. A. f. Hygiene. 1892. XIV. 225.

Raulin, Jules,
I. Sur les conditions chimiques de la vie des organismes inférieurs.—C. R. 1870. LXX. 634.
II. Etudes chimiques sur la végétation des Mucédinées particulièrement de l'Ascophora nigrans.—C. R. 1863. LVII. 228.
III. Etudes chimiques sur la végétation.—Annales des sciences naturelles. Botanique. 5e série. 1869. XI. 93.

Raum, Johannes,
I. Der gegenwärtige Stand unserer Kenntnisse über den Einfluss des Lichtes auf Bakterien und auf den tierischen Organismus.—Z. f. Hygiene. 1889. VI. 312.—C. f. B. 1889. VI. 261.
II. Zur Morphologie und Biologie der Sprosspilze.—Z. f. Hygiene 1891. X. 1.—Z. g. Br. 1891. XIV. 273.—C. f. B. 1891. X. 79.—Ch. C. 1891. I. 882.—K. J. II. 38.
III. Z. f. Hygiene. 1891. X. 17.

Raumer, E. v.,
I. Z. f. angew. Chemie. 1890. 421.

Raux, G.,
I. Quoted by Nomura, I.

Ravà, Jacopo,
I. L'acidità dell latte in rapporto alla Fabricazione dell Formaggio. Lodi. 1887. Constantino dell' Ava.—Milchzeitung. 1887. XVI. 422.

Ravaz, L.,
I. Sur une maladie de la Vigne causée par le Botrytis cinerea.—C. R. 1894. CXVIII. 1289.—C. f. B. 2. Abt. 1895. I. 311.
II. La pourriture des raisins.—Revue de viticulture. 1895. IV. 183.
See also Viala et Ravaz.

Ravaz, L., et Gouirand, G.,
I. Recherche sur le traitement de quelques maladies de la vigne. I. Pourriture grise (Botrytis cinerea).—Revue de viticulture. 1896. VI. 101.

Ray, Julien,
I. Sur le développement d'un champignon dans un liquide en mouvement. — C. R. 1896. CXXIII. 907.—Bot. Ztg. 2. Abt. 1897. LV. 68.

Raýman, Bohuslav, und Kruis, K.,
I. Chemisch-biologische Studien. —Mitteilungen der Versuchs-Station für Spiritusindustrie zu Prag. 1891. I.—C. f. B. 1. Abt. 1892. XII. 150. — Z. g. Br. 1892. XV. 94.—Ch. C. 1892. I. 211.—K. J. II. 125.
See also Kruis und Raýman.

Rechter, de, und Legros,
I. Notiz über die Desinfektion durch Schwefeldioxyd und das Gasgemisch Pictet.—Ch. C. 1894. I. 827.—Reprinted from Presse méd. belge, 1893, No. 42, and Journal de Pharmacie et de Chimie, 1894, XXIX. 236.

Reess, Max.
I. Botanische Untersuchungen über die Alkoholgärungspilze. 1870. Leipzig. A. Felix.
II. Über die systematische Stellung der Hefepilze.—Bot. Ztg. 1884. XXXXII. 651.—Z. g. Br. 1884. VII. 442.
III. Zur Naturgeschichte der Bierhefe.—Bot. Ztg. 1869. XXVII. 105.
IV. Botanische Untersuchungen ü. d. Alkoholgärungspilze. Leipzig 1870. 71.

Regel, E.,
I. Wirkung des Lichtes auf Pilze. —Sitzgsber. d. botan. Sect. d. St. Petersburger Naturforscher-Gesellschaft. 1881.—Bot. Ztg. 1882. XXXX. 29.

Regnard, P.,
I. Über Aufhaltung der Hefegärung durch Alkohole.—Comptes rendus de la Société de Biologie. 9e série. 1889. X. 124.—Centralblatt f. Physiologie. 1889. VI. 121.

Rehm, H.,
I. Die Pilze Deutschlands, Österreichs und der Schweiz. III. Abt.: Hysteriaceen und Discomyceten. Leipzig. 1896.

Reich,
I. Eine Unterleibstyphusepidemie infolge des Genusses ungekochter Molkereimilch. — Berliner klinische Wochenschrift. 1894. 702. —C. f. B. 1894. XVI. 704.

Reichard, Albert,
I. Studien über einen Sarcinaorganismus des Bieres.—Z. g. Br. 1894. XVII. 257.—K. J. IV. 197.
II. Über Blasengärung.—Z. g. Br. 1892. XV. 215.
III. Über Schaumbildung.—Z. g. Br. 1897. XX. 411.—K. J. VIII. 127.

Reichard, A., und Riehl, A.,
I. Zur Kenntnis und zur Bekämpfung der Sarcina-Krankheit. — Z. g. Br. 1895. XVIII. 59.—C. f. B. 2. Abt. 1895. I. 641.—K. J. VI. 201.
II. Versuche über Einwirkung der Hefegabe auf das Bier.—Z. g. Br. 1897. XX. 8. — K. J. I. 125.

Reichardt,
I. Verhandl. d. Zool. bot. Ges. in Wien. 1867. XVII. 335.

Reichert, Edward,
See Mitchell und Reichert.

Reimers, J.,
I. Über den Gehalt des Bodens an Bakterien. Dissert. Jena. 1889. Gives the antecedent bibliography.—C. f. B. 1. Abt. 1891. X. 489.

Rein,
I. Japan. 1886.

Reinhardt, M. O.,
I. Das Wachstum der Pilzhyphen. —Pringsheim's Jahrbücher f. wissenschaftl. Botanik. 1892. XXIII. 479.

Reinke, J.,
I. Über den Einfluss mechanischer Erschütterung auf die Entwicklung der Spaltpilze.—Pflüger's Archiv. 1880. XXIII. 434.

Reinke, Otto,
I. Die Klärung der Biere durch Licht.—W. f. Br. 1896. XIII. 400.—K. J. VII. 113.
II. Über den Vergärungsgrad. Vortrag.—W. f. Br. 1889. VI. 569.
III. Die Konservierung von Hefen. —W. f. Br. 1888. V. 745. 1891. VIII. 703. 1892. IX. 1009.—K. J. III. 162.
IV. W. f. Br. 1891. VIII. 809.

Reinmann, R.,
I. C. f. B. 2. Abt. 1900. VI. 131.

Reinsch, A.,
I. Die Bakteriologie im Dienste der Sandfiltrationstechnik.—C. f. B. 1894. XVI. 881.—K. J. V. 24.
II. Zur bakteriologischen Untersuchung des Trinkwassers.—C. f. B. 1891. X. 415.

Reiset, Jules,
I. Expériences sur la putréfaction et sur la formation des fumiers.—C. R. 1856. XXXXII. 53. 1889. CVIII. 708, 779.—Ch. C. 1889. I. 846.
II. Note sur la production du gaz nitreux pendant la marche des fermentations dans les distilleries. —C. R. 1868. LXVI. 177.—Ch. C. 1868. 939.

Reiss, Rudolf,
I. Über die Natur der Reservecellulose und über ihre Auf-

lösungsweise bei der Keimung der Samen. — Landwirtschaftl. Jahrbücher. 1889. XVIII. 711. —Ch. C. 1890. I. 165.

Renk,

I. Über Marktmilch in Halle.— Münchner medicinische Wochenschrift. 1891. No. 6 u. 7.— C. f. B. 1891. X. 193.

Renon,

I. Etude sur l'aspergillose chez les animaux et chez l'homme. Paris. 1867.

Rey-Pailhade, J. de,

I. Sur un corps d'origine organique hydrogénant le soufre à froid.— C. R. 1888. CVI. 1683.—Ch. C. 1888. 1085.

II. Nouvelles recherches physiologiques sur la substance organique hydrogénant le soufre à froid.—C. R. 1888. CVII. 43. —Ch. C. 1889. I. 439.

III. Sur de nouvelles propriétés chimiques de l'extrait alcoolique de levure de bière.—Bulletin de la Société chimique de Paris. Troisième série. 1890. III. 171. —C. f. B. 1. Abt. 1890. VIII. 106.—Ch. C. 1890. I. 682.— K. J. I. 32.

IV. Action de l'alcool et du soufre sur la levure de bière.—Comptes rendus de la Soc. de Biologie. 1893. (10.) II. 46.—K. J. IV. 149.

V. Etudes sur les propriétés chimiques de l'extrait alcoolique de levure de bière: formation d'acide carbonique et absorption d'oxygène.—C. R. 1894. CXVIII. 201.—Ch. C. 1894. I. 472.— K. J. V. 114.

VI. Rôles respectifs du philothion et de la laccase dans les graines en germination.—C. R. 1895. CXXI. 1162.—Ch. C. 1896. I. 562.—K. J. VI. 331.

VII. Existence du corps protéique prévu par M. G. Bertrand dans la constitution des oxydases.— Bulletin de la Société chimique. 1897. (3.) XVII. 756.—Ch. C. 1897. II. 595.—K. J. VIII. 274.

VIII. Fermentation chimique par la levure en milieu antiseptique. —Bulletin de la Soc. chim. 1900. (3.) XXIII. 666.—Ch. C. 1900. II. 729.

IX. C. R. 1902. (3.) XXVII. 6.

X. *Ibid.* 1904. (3.) XXXI. 987.

XI. Comptes Rendus Soc. Biol. 1898. (10.) V. 372.

XII. Bull. Soc. chimique. 1906. (3.) XXXV. 1030.

XIII. *Ibid.* XXXV. 1030.

Riboni,

See Pirotta e Riboni.

Richards, Herbert Maule,

I. Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. — Jahrbücher f. wissenschaftl. Botanik. 1897. XXX. 665.—Bot. Ztg. 2. Abt. 1897. LV. 377.—Z. g. Br. 1897. XX. 585.

Richardson,

I. The Action of Light in preventing Putrefactive Decomposition and inducing the Formation of Hydrogen Peroxide in Organic Liquids.—Transactions of the Chemical Society. 1893. XXXXIII. 1109.—C. f. B. 1894. XVI. 42.—K. J. IV. 120.

II. The Action of Light on Oxalic Acid. — Chem. News. 1894. LXIX. 226.—Ch. C. 1894. I. 1144.

Richardson, F. W.,

I. Journ. Federated Inst. Brewing. 1898. IV. 128.

Richet, Ch.,

I. De l'action de quelques sels métalliques sur la fermentation lactique.—C. R. 1892. CXIV. 1494.—C. f. B. 1892. XII. 866. —K. J. III. 170.

Richter, A.,

I. Abst. in Botan. Centralbl. 1902. LXXXIX. 621.

II. C. f. B. 2. Abt. 1902. VIII. 787.

Richter, Andreas,

I. Zur Frage der chemischen Reizmittel.—C. f. B. 2. Abt. 1901 VII. 417.

Richter, Karl,

I. Beiträge zur genaueren Kenntnis der chemischen Beschaffenheit der Zellmembran bei den Pilzen. —Sitzungsber. d. K. Akademie d. W. zu Wien. 1881. LXXXIII. I. 494.—Ch. C. 1881. 483.

Riehl, Albert,

See Reichard und Riehl.

Rietsch, M.,

See Martinand et Rietsch.

Rietsch, M., et Herselin, M.,
I. Sur la fermentation apiculée et sur l'influence de l'aération dans les fermentations à température élevée.—Progrès agricole et viticole. 1895.—C. R. 1895. CXXI 378.—Z. g. Br. 1896. XIX. 47.—Ch. C. 1895. II. 871.

Rijn, J. J. L. van,
I. Die Glykoside. Berlin. 1900. 10*s*.—C. f. B. 2. Abt. 1900. VI. 743.

Rindfleisch,
I. Untersuchungen über niedere Organismen.—Virchow's Archiv. 1873. LIV.

Ringel, T.,
I. Über den Keimgehalt der Frauenmilch.—Münchner medic. Wochenschrift. 1893. XXXX. 513.—C. f. B. 1893. XIV. 429.—Ch. C. 1893, II. 695.

Ringer, Sydney,
I. Einwirkung von Kalksalzen auf Caseïn und Milch.—Journal of Physiology. 1891. XI. 464; XII. 164.—Ch. C. 1891. I. 702. 1892. I. 172.—K. J. II. 259; III. 261.

Ripper, M.,
I. Weinbau u. Weinhandel. 1890. VIII. 168.
II. J. f. prakt. Chemie. 1892. XLVI. 428.
III. Forschungsberichte u. Lebensmittel. 1895. II. 12, 35.

Ripper, Maximilian,
I. Die schweflige Säure im Weine und deren Bestimmung.—Ch. C. 1891. I. 46. 1893. I. 280. 1895. I. 566, 815.

Rischlet und Tollens,
I. Ber. d. D. Chem. Ges. 1885. XVIII. 2611.

Riss,
I. Zeits. d. Landw. Vereins im Bayern. 1891. — Noticed in Koch's Jahresb. 1891. II. 170.

Rist, E., et Khoury, J.,
I. Ann. Inst. Past. 1902. XVI. 65.

Ritsert, E.,
I. Untersuchungen über das Ranzigwerden der Fette. Bern. 1890.—Ch. C. 1890. II. 507.
II. Bakteriologische Untersuchungen über das Schleimigwerden der Infusa.—Pharm. Zeitung. 1891. XXXVI. 715, 774.—C. f. B. 1892. XI. 730.—Ch. C. 1892. I. 236.—K. J. II. 223; III. 59.

Ritthausen, H.,
I. Chem. Ztg. 1897. XXI. 717.

Ritthausen, H., und Baumann,
I. Über Zerstörung von Fett durch Schimmelpilze. — D. landw. Versuchs - Stationen. 1896. XXXXVII. 389.—C. f. B. 2. Abt. 1896. II. 711.—Ch. C. 1896. II. 680.—K. J. VII. 46.

Roberts, W.,
I. Studies on Biogenesis.—Philosophical Transactions of the Royal Socicty of London. 1874. CLXIV. II. 474.

Robertson, J. Chalmers,
I. Symptoms of Irritant Poisoning in a Family, due to Diseased Bread.—The Lancet. 1887. II. 518.

Robin, Ch.,
I. Sur la nature des fermentations en tant que phénomènes nutritifs des assimilateurs des plantes.—Journal de l'anatomie et de physiologie. 1875, p. 379.
II. Des végét. qui croissent s. les anim. viv. 1847.

Robiquet,
I. Journ. de Pharm. et de Chim. 1852. XXII. 121.

Rochard, Felix,
I. Du parasitisme végétal dans les altérations du pain.—Annales d'Hygiène publique et de médecine legale. 1873. 2e série. XXXX. 83.

Roeder, Georg,
See E. Fischer u. G. Roeder.

Roeser, P.,
I. Contribution à l'étude de l'influence de la température sur les variations morphologiques et évolutives des micro-organismes.—Archives de médecine expérimentale et d'anatomie pathologique. 1890, p. 139.—C. f. B. VII. 476.—K. J. I. 39.
II. De la formation d'aldéhyde dans la fermentation alcoolique.—Ann. Inst. Past. 1893. VII. 41.—Ch. C. 1893. I. 837.—K. J. IV. 150.

Roesler, L.,
I. Die Anwendung der schwefligen Säure in der Kellerwirtschaft und der Schwefelsäure-Gehalt des Weines.—Mitteilungen der chem.-phys. Vers.-Stat. zu Klosterneuburg bei Wien. 1885. IV. 9.

Roger,
I. Fabrication des fromages de Brie, etc. Communication to the Société de l'Agriculture de Meaux. 1898.

Rogers, L. A.,
I. C. f. B. 2. Abt. 1903. X. 381.
II. *Ibid.* 1904. XII. 338.

Röhling, A.,
I. Morphol. u. physiol. Untersuchungen ü. einige Rassen d. Saech. apic. Dissert. Erlangen. 1905.

Röhmann, F.,
I. Nachtrag zur Arbeit von B. Bienstock: Über die Bakterien der Fäces.—Zeitschrift f. klin. Medicin. 1884. VIII. 43.—Ch. C. 1884. 646.

Rohn, Severin, u. Wichmann, H.,
I. Notiz über einen bemerkenswerten Fall von unreinem Tiefbrunnenwasser. — Mitteilungen d. Österr. Versuchs-Station f. Brauerei u. Mälzerei in Wien. Heft II.—C. f. B. 1889. V. 642.—Ch. C. 1889. II. 93.

Rohrer,
I. Über die Pigmentbildung des Bacillus pyocyaneus.—C. f. B. 1892. XI. 327.—K. J. III. 86.

Rolants, E.,
See Boidin et Rolants.

Roloff,
I. Über die Milzbrand-Impfung und die Entwicklung der Milzbrand-Bakterien.—Archiv f. wissenschaftl. u. prakt. Tierheilkunde. 1883. IX. 459.

Rommel, W.,
See Sitnikoff und Rommel.

Rommel, W., u. Sitnikoff, A.,
I. Bull. de l'Ass. belge des Chimistes. 1903. XVIII. 1049.

Rommier, A.,
I. Sur la diminution de la puissance fermentescible de la levure ellipsoidale de vin, en présence des sels de cuivre.—C. R. 1890. CX. 536.—C. f. B. 1892. XII. 662.—Ch. C. 1890. I. 803.—K. J. I. 78.
II. C. R. 1890. CX. 1341.

Roos, L.,
I. Die Mannitgärung der Weine.—Journal de pharmacie et de chimie. 1893. XXVII. 405.—Ch. C. 1893. I. 1098.—K. J. IV. 152.

Röse, B., und Schulze, E.,
I. Über einige Bestandteile des Emmenthaler Käses.—D. Landw. Versuchs - Stationen. 1885. XXXI. 115.

Rosenbach,
I. Gibt es Spaltpilze oder deren Keime in den Geweben, im Blute, Lymphe gesunder Menschen und Tiere? — Deutsche Zeitschrift für Chirurgie. 1880. XIII. 344.
II. Mikroorganismen bei den Wundinfektionskrankheiten des Menschen. Wiesbaden. 1884.

Rosenstiehl, A.,
I. C. R. 1900. CXXX. 195. 1902. CXXXIV. 119.

Rössler, Oskar,
I. Über die Kultivierung von Crenothrix polyspora auf festem Nährboden.—Archiv der Pharmacie. 1895. CCXXXIII. 189.—Ch. C. 1895. II. 173.

Roth, O.,
I. Über das Vorkommen von Tuberkelbacillen in der Butter.—Korrespondenzblatt f. schweizer. Ärzte. 1894. No. 17.—Hygien. Rundschau. 1894. IV. 1132.—Ch. C. 1895. I. 279.

Rothenbach, F.,
I. Z. f. Spiritusind. 1896. XIX. 8.

Rothenbach, F., u. Eberlein, L.,
I. Die deutsche Essigindustrie. 1905. IX. 233.

Rothert, W.,
I. Über Sclerotium hydrophilum Sacc., einen sporenlosen Pilz.—Bot. Ztg. 1892. L. 321.

Roux, E.,
I. De l'action de la lumière et de l'air sur les spores de la bactéridie du charbon.—Ann. Inst. Pas. 1887. I. 445.—Ch. C. 1888. 554.
II. Bull. Soc. Chim. XXXV. 37.
III. Ann. de la Brasserie et de la Distillerie. Paris. 1898. I. 512.

ROWLAND, Sydney,
I. Cheese and Butter as Possible Carriers of Typhoid and Cholera Infection.—Brit. Medic. Journal. 1895. I. 1392.—C. f. B. 1. Abt. 1895. XVIII. 204. — Ch. C. 1895. II. 875.
See also HARDEN and ROWLAND; MACFADYEN, MORRIS and ROWLAND.

ROWLANDSON,
I. On the Production of Butter.—Journal of the Royal Agricult. Society of England. 1852. XIII. 35.

ROZE,
I. C. R. 1897. CXXV. 982.

ROZIER, François,
I. Cours complet d'agriculture, etc., ou Dictionnaire universel d'agriculture. 1786. IV. 525.

RÜBNER, M.,
I. Die Wanderungen des Schwefels im Stoffwechsel der Bakterien.—A. f. Hygiene. 1893. XVI. 78. —C. f. B. 1893. XIV. 64, 66. —K. J. IV. 95.
II. Über das Vorkommen von Merkaptan.—A. f. Hygiene. 1893. XIX. 136.—Ch. C. 1894. I. 90.—K. J. IV. 97.
III. A. f. Hygiene. 1903. XLVIII. 260.
IV. *Ibid.* 1904. XLIX. 355.

RULLMANN, W.,
I. Chemisch-bakteriologische Untersuchungen von Zwischendeckenfüllungen mit besonderer Berücksichtigung von Cladothrix odorifera.—Forschungsber. über Lebensmittel, etc. 1895. II. 177. —C. f. B. 1. Abt. 1895. XVII. 884.—Ch. C. 1895. II. 372.
II. Weitere Mitteilungen über Cladothrix odorifera.—C. f. B. 2. Abt. 1896. II. 116. — K. J. VII. 58.

RUSSELL, H. L.,
I. The Effect of Mechanical Movement upon the Growth of Certain Lower Organisms.—The Botanical Gazette. 1892. No. 1. Bloomington. Indiana.
II. Untersuchungen über im Golf von Neapel lebende Bakterien.—Z. f. Hygiene. 1891. XI. With two plates.—K. J. II. 58.

RUSSELL, N. L.,
I. A Biological Study of Pasteurised Milk and Cream under Commercial Conditions.—C. f. B. 2. Abt. 1895. I. 741.

SAARE, Oskar,
I. Über Brennereibetrieb in Belgien und das Amyloverfahren.—Z. f. Spiritusind. 1900. XXIII. 419.

SACC,
I. De la panification aux Etats-Unis, et des propriétés du houblon comme ferment.—C. R. 1875. LXXXI. 1130. 1876. LXXXIII. 361.

SACCARDO, P.,
I. Sylloge fungorum omnium hucusque cognitorum. 16 vols. Patavii. 1882–1902.
II. *Ibid.* 1895. XI. 457.
III. Michelia. II. 547. — Fungi italici. Pl. 893. — Sylloge fungorum. 1886. IV. 85.
IV. Zusammenstellung der Penicillium spezies in Sylloge fungorum. IV. 78; X. 527; XI. 513; u. d. Aspergilleen in I. 25 (Eurotium); IV. 64; X. 524; XI. 591.

SACCARDO und SYDOW,
I. Sylloge fungorum. 1902. XVI. 818.

SADEBECK, R.,
I. Über Pythium Anguillulæ aceti. —Sitzungsbericht d. Ges. f. Botan. z. Hamburg. 1886. II. 39.—C. f. B. 1887. I. 50.
II. Untersuchungen über die Pilzgattung Exoascus und die durch dieselbe um Hamburg hervorgerufenen Baumkrankheiten.—Jahresbuch der wissenschaftl. Anstalten zu Hamburg für 1883. —Bot. Ztg. 1884. XXXXII. 655.
III. Kritische Untersuchungen über die durch Taphrina-Arten hervorgebrachten Baumkrankheiten. — *Ibid.* 1890. VIII.— C. f. B. 1. Abt. 1891. IX. 576.
IV. Die parasitischen Exoasceen.—Bot. Ztg. 1893. X.—C. f. B. 1. Abt. 1894. XV. 503.

SAIDA,
I. Ber. d. D. Bot. Ges. 1901. XIX. 107.

Saito, K.,
I. Journ. of the College of Science, Imp. Univ. Tokyo. 1904. XIX. Art. 18, p. 1.
II. Botan. Mag. Tokyo. 1905. XIX. 75.
III. Journ. of the College of Science, Imp. Univ. Tokyo. 1904. XVIII. Art. 5.
IV. Bot. Mag. Tokyo. 1903. XVIII. 75.

Salfeld,
I. Ein Versuch mit Impferden verschiedener Herkunft auf Naturboden bei Pferdebohnen und Erbsen. — Deutsche landw. Presse. 1892. 648.

Salkowski, E.,
I. Über die antiseptische Wirkung des Chloroformwassers. — Deutsche medicin. Wochenschrift. 1888. No. 16.—C. f. B. 1. Abt. 1888. IV. 188. 1889. V. 428.—Ch. C. 1888. 718. 1889. I. 613.
II. Über Zuckerbildung und andere Fermentationen in der Hefe.—Z. f. physiolog. Chemie. 1889. XIII. 506.—Ch. C. 1889. I. 591.
III. Über Autodigestion der Organe.—Z. f. klin. Medicin. 1890. —Ch. C. 1890. II. 524.
IV. Fermentative Prozesse in den Geweben.—Archiv d. Anat. u. Physiol., Physiol. Abt. 1890. 554.—Ch. C. 1891. I. 223.—K. J. II. 90.
V. Über die Kohlehydrate der Hefe.—Ber. d. D. Chem. Ges. 1894. XXVII. 497, 925.—Ch. C. 1894. I. 624, 864.—K. J. V. 114.
VI. *Ibid.* II.—*Ibid.* 3325.—Ch. C. 1895. I. 328.—K. J. VI. 50.
VII. Bemerkungen über den bei der Autodigestion der Hefe entstehenden Zucker.—Zeitschrift f. Biologie. 1895. XXXII. 468.—Ch. C. 1895. II. 870.—K. J. VI. 56.
VIII. Über das "Invertin" der Hefe.—Z. f. physiolog. Chemie. 1900. XXXI. 305. — Ch. C. 1901. I. 263.
IX. Über die Gärung der Pentosen. —Z. f. physiolog. Chemie. 1900. XXX. 478.—Ch. C. 1900. II. 920.
X. Maly's Jahresberichte. 1877. VII. 286.

Salomon, Alfred Gordon, und Mathew, W. de Vere,
I. Einfluss gewisser phosphorsaurer Salze auf die Alkoholgärung.—The Brewer's Journal. 1884. 149.—Z. g. Br. 1884. VII. 231.

Sanguineti, J.,
I. Contribution à l'étude de l'Amylomyces Rouxii de la levure chinoise et des moisissures ferments de l'amidon.—Ann. Inst. Past. 1897. XI. 264.—C. f. B. 2. Abt. 1897. III. 430.—Ch. C. 1897. I. 998.—K. J. VIII. 285.

Sanna-Salaris, G.,
See Malerba e Sanna-Salaris.

Sannino, Antonio,
See Sostegni e Sannino.

Sanson,
I. C. R. 1888. CVI. 208.

Sassen, A.,
See Woltering und Sassen.

Saussure, Théodore de,
I. Sur la fermentation vineuse.—Bibliothèque univ. de Genève. 1841. No. 63, p. 180.—Journal f. prakt. Chemie. 1841. XXIV. 47.

Sauvageau, C., et Radais, M.,
I. Sur les genres Cladothrix, Streptothrix, Actinomyces et description de deux streptothrix nouveaux.—Ann. Inst. Past. 1892. VI. 242.—C. R. 1892. CXIV. 559.—K. J. III. 45.

Savoff,
I. Recherches sur l'aspergillose pulmonaire. Nancy. 1905.

Savoure,
I. Arch. de Parasitologie. 1905.—Abst. in Bot. Centralbl. 1906. CI. 11.

Schacht, Hermann,
I. Die Kartoffelpflanze und deren Krankheiten. Berlin. C. Wigand.
II. Über die Veränderungen durch Pilze in abgestorbenen Pflanzenzellen.—Jahrbücher f. wissenschaftl. Botanik. 1863. III. 442.

Schaffer, F.,
I. Die Anwendung der eudiometrischen Methode zur Untersuchung von Milch, Lab und Wasser zu Käsereizwecken.—Schweizer. Wochenschrift f. Pharmacie. 1894. XXXII. 17.—Landw. Jahrbuch der Schweiz. 1893.—Ch. C. 1894. I. 607; II. 223.—Milchzeitung. 1894. XXIII. 349.

II. Über das Casein und die Wirkung des Labfermentes in der Kuhmilch.—Landw. Jahrb. d. Schweiz. 1887. I. 43.—Ch. C. 1887. 1359.
III. Über des Einfluss des sogen. Nachwärmens bei der Käsefabrikation auf die Reifungsprodukte der Käse. — Landw. Jahrbuch d. Schweiz. 1895. IX. 93.—C. f. B. 2. Abt. 1895. I. 760.
IV. Dissert. Erlangen. 1901, p. 15.
See also FREUDENREICH u. SCHAFFER; HESS, SCHAFFER u. LANG; NENCKI und SCHAFFER.

SCHAFFER, F., und BONDZYNSKI, St.,
I. Über das Casein und die Wirkung des Labfermentes in der Kuhmilch.—Landw. Jahrbuch d. Schweiz. 1887. I. 47. 1888. II. 32.—Ch. C. 1887. 1359.

SCHAFFER, F., und FREUDENREICH, Ed. von,
I. De la résistance des bactéries aux hautes pressions combinées avec une élévation de la température.—Annales de Micrographie. 1891. IV. 105.—K. J. II. 99.
II. Quantitative Untersuchungen über die in Naturweinen und Kunstweinen enthaltenen Hefen und Bakterien.—Landw. Jahrb. d. Schweiz. 1891. V. 79.—Ann. de Micrographie. 1892. V. 239.—C. f. B. 1892. XI. 467.—K. J. II. 62.

SCHANDER, R.,
I. Jahresb. d. Vereinigung d. Vertreter d. angew. Botanik. 1903–1904. 104.
II. Bericht d. Kgl. Lehranstalt zu Geisenheim f. d. Jahr. 1903–4, p. 123.
III. Jahresb. d. Vereinigung, etc., for 1903–4, 1905. 92.
IV. *Ibid.* for 1903–4, 1905. 85.

SCHARDINGER, F.,
I. C. f. B. 2. Abt. 1890. VIII. 144.

SCHARDINGER, Franz,
I. Reinkulturen von Protozoen auf festen Nährböden.—C. f. B. 2. Abt. 1896. XIX. 538.
II. Über eine neue, optisch aktive Modifikation der Milchsäure, durch bakterielle Spaltung des Rohrzuckers erhalten.—Monatshefte f. Chemie. 1890. XI 545.—Ch. C. 1891. I. 441.—K. J. I. 85.

SCHEDTLER, H.,
I. Beitrag zur Morphologie der Bakterien. Bacterium Zopfii Kurth.—Virchow's Archiv f. patholog. Anatomie. 1887. CVIII. 30.—C. f. B. 1887. II. 437.

SCHEELE, Karl Wilhelm,
I. Anmärkningar om sättet att conservera Attika.—Kongl. Vetenskaps Academiens nya Handlingar. 1782. III. 120.
II. Crell's Annalen. 1785. I. 61.

SCHEIBLER, C.,
I. Untersuchungen über die Natur der gallertartigen Ausscheidung (sog. "Froschlaich"), welche bei der Saftgewinnung aus Rüben beobachtet wird.—Zeitschrift des Vereins f. d. Rübenzucker-Industrie des Deutschen Reiches. 1869. 472. 1874. 309. 1875. 112. — Biedermann's Centralblatt. 1875. VIII. 131.
II. Zur Geschichte der Melitriose.—Neue Zeitschrift für Rübenzucker-Industrie. 1889. XXIII. 234.—Ch. C. 1890. I. 21.
III. Über das Vorkommen der Arabinsäure (Gummi) in den Zuckerrüben und über den Arabinzucker (Gummizucker).—Ber. d. D. Chem. Ges. 1873. VI. 612.

SCHEIBLER, C., und MITTELMEIER, H.,
I. Über die Inversionsprodukte der Melitriose.—Ber. d. D. Chem. Ges. 1889. XXII. 1678, 3118.—Ch. C. 1889. II. 557. 1890. I. 110.
II. Ber. d. D. Chem. Ges. 1885. XVIII. 1779.
III. *Ibid.* 1889. XXII. 3120.
IV. Z. f. angew. Chemie. 1891. 407.

SCHELLHORN, B.,
See WINDISCH und SCHELLHORN.

SCHENCK,
I. W. f. Br. 1905. XXII. 221.

Schenk, Samuel,
I. Über einen Micrococcus tetragenus concentricus in den Fäces. —Allgem. Wiener medic. Zeitschrift. 1892.

Scherpe, R.,
I. Die chemischen Veränderungen des Roggens und Weizens beim Schimmeln und Auswachsen.—Zeitschrift f. Untersuchung d. Nahrungs- und Genussmittel. 1899. II. 550.—C. f. B. 2. Abt. 1900. VI. 747.—Ch. C. 1899. II. 398.

Scheurer-Kestner, A.,
I. Sur un ferment digestif qui se produit pendant la panification. —C. R. 1880. XC. 369.—Ch. C. 1880. 214.

Scheurlen,
I. Über Saprol und die Saprolierung der Desinfektionsmittel.—A. f. Hygiene. 1893. XVIII. 35.
II. Weitere Untersuchungen über Saprol.—A. f. Hygiene. 1893. XIX. 347.—Gives a copious bibliography for saprol.—Ch. C. 1892. II. 118, 580, 585, 727. 1893. I. 890 II. 869. 1894. I. 522.
III. Geschichtliche und experimentelle Studien über den Prodigiosus.—A. f. Hygiene. 1896. XXVI. 1. — Ch. C. 1896. I. 1013.—K. J. VII. 54.

Scheurlen und Spiro,
I. Die gesetzmässigen Beziehungen zwischen Lösungszustand und Wirkungswert der Desinfektionsmittel.—Münchener medic. Wochenschrift. 1897. XXXXIV. 81. —Ch. C. 1897. I. 505.—K. J. VIII. 63.

Schewiakoff, W.,
I. Über einen neuen bakterienähnlichen Organismus des Süsswassers.—Verhandl. d. Naturhist.-med. Vereins Heidelberg. Bd. V.; auch Habilitationsschrift. Heidelberg. 1893. Winter.—C. f. B. 1893. XIV. 151.—K. J. IV. 57.

Schiel,
I. Elektrotherapeutische Studien. —Deutsches Archiv für klinische Medizin. 1875. XV. 190.

Schifferer, Anton,
I. Über die nicht-kristallisierbaren Produkte der Einwirkung der Diastase auf Stärke. Dissert. Basel. 1892. — Ch. C. 1892. II. 1011.—K. J. III. 250.

Schild, E.,
I. Formalin zur Diagnose des Typhusbacillus.—C. f. B. 1893. XIV. 717.—Ch. C. 1894. I. 162.
II. Eine Typhus-Epidemie mit nachweisbarer Entstehungsursache und die Diagnose des Typhusbacillus mittelst Formalin.—Z. f. Hygiene. 1894. XVI. 368.—C. f. B. 1894. XV. 692. —Ch. C. 1894. I. 872.

Schilow, P.,
I. Einfluss des Wasserstoffsuperoxyds auf einige pathogene Mikroorganismen. — Pharmac. Zeitschrift f. Russland. 1894. XXXIII. 99.—Ch. C. 1894. I. 689.

Schindler, S.,
I. Beiträge zur Kenntnis des Adenins, Guanins und ihrer Derivate. — Z. f. physiolog. Chemie. 1889. XIII. 432. — Z. g. Br. 1889. XII. 203. — Ch. C. 1889. I. 719.

Schiönning, H.,
I. Nouvelle et singulière formation d'ascus dans une levure.—Comptes rendus, etc., de Carlsberg. 1895. IV. 30.—C. f. B. 2. Abt. 1895. I. 441.—K. J. VI. 34.
II. Comptes rendus, etc., de Carlsberg. 1903. VI. 103.
III. Notice in Chem. Ztg. 1895. IX. 225.
See also Klöcker und Schiönning.

Schipin, D.,
I. Zur Bakteriologie des Kumys. Dissert. St. Petersburg. 1899. —C. f. B. 2. Abt. 1900. VI. 775.

Schirokikh, J.,
I. Über einen neuen Salpeter zerstörenden Bacillus.—C. f. B. 2. Abt. 1896. II. 204.—Ch. C. 1896. II. 111.—K. J. VII. 216.

Schlegel,
I. Kupferkalkmischung und Traubenfäule.—Weinbau und Weinhandel. 1894. XII. 539.

Schleiden, Matthias Jakob,
I. Grundzüge der wissenschaftlichen Botanik. 3rd Ed. 1849. I. 207.
II. Beiträge zur Phytogenesis.—Müller's Archiv f. Anatomie, Physiologie u. wissenschaftl. Medicin. 1838. 137.

Schlösing, Th.,
I. Sur la fermentation en masses du tabac pour poudre.—Mémorial des Manufactures de l'état. 1888. I. 514. 1889. II. 119.
II. Contribution à l'étude de la fermentation du râpé. Du rôle des transvasements.—*Ibid.* II. 192.
III. Sur la décomposition des nitrates pendant les fermentations.—C. R. 1868. LXVI. 237. —Ch. C. 1868. 940.

Schlösing, Th. fils, et Laurent, Emile,
I. Sur la fixation de l'azote libre par les plantes.—C. R. 1891. CXIII. 776, 1059. 1892. CXV. 659, 732. — Ann. Inst. Past. 1892. VI. 65, 824. — Ch. C. 1892. I. 170. 1893. I. 52.—K. J. II. 204, 206; III. 208.

Schlösing, Th., et Müntz, A.,
I. Sur la nitrification par les ferments organisés.—C. R. 1877. LXXXIV. 301; LXXXV. 1018.
II. Recherches sur la nitrification. —C. R. 1879. LXXXIX. 891, 1074.

Schlossberger, Julius Eugen,
I. Über die Natur der Hefe, mit Rücksicht auf die Gärungserscheinungen.—Annalen d. Chem. u. Pharm. 1844. LI. 193.

Schlossberger, J., und Doepping, O.,
I. Chemische Beiträge zur Kenntnis der Schwämme.—Annalen d. Chemie und Pharmacie. 1844. LII. 106.

Schmelck, L.,
I. Eine Gletscherbakterie.—C. f. B. 1888. IV. 545.—Ch. C. 1889. I. 48.

Schmid, E.,
See Nobbe, Hiltner und Schmid; Nobbe, Schmid, Hiltner u. Hotter.

Schmidt, B.,
I. Über den Einfluss der Bewegung auf das Wachstum und die Virulenz der Mikroben.—A. f. Hygiene. 1891. XIII. 247.—K. J. II. 73.

Schmidt, C.,
I. Liebig's Ann. 1847. LXI. 171.
II. Handwörterbuch der Chemie. 1848. III. 224.

Schmidt, Paul,
I. Milch, die Quelle einer Typhusepidemie. Dissert. Halle. 1893. —C. f. B. 1894. XV. 63.

Schmidt, R.,
I. Über verschimmelte Tapeten. Dissert. Erlangen. 1899.—C. f. B. 1. Abt. 1900. XXVII. 752.

Schmidt, R. H.,
I. Über Aufnahme und Verarbeitung von fetten Ölen durch Pflanzen.—Flora. 1891. LXXIV. 300.—Ch. C. 1892. I. 320.
II. Sitzgsber. d. physik. med. Societät Erlangen. 1898. Heft 30.

Schmidt-Muhlheim,
I. Untersuchungen über fadenziehende Milch.—Pflüger's Archiv. 1882. XXVII. 490.—Die landw. Versuchs - Stationen. 1892. XXVIII. 91. — Ch. C. 1882. 566.—Z. f. Spiritusind. 1882. V. 312.

Schmidt-Nielsen,
I. C. f. B. 2. Abt. 1902. IX. 145.

Schmiedeberg, O., und Harnack, E.,
I. Synthesen des Muscarins und über muscarinartig wirkende Ammoniumbasen. — Ch. C. 1876. 554.
See also Bergmann und Schmiedeberg.

Schmitz, Friedrich,
I. Über die Zellkerne der Thallophyten.—Verhandl. u. Sitzungsber. d. natur-hist. Vereins d. preuss. Rheinlande und Westphalens. 1879. XXXVI. 345.
II. Untersuchungen über die Struktur des Protoplasmas und der Zellkerne der Pflanzenzellen.—*Ibid.* 1880. XXXVII. 159.

Schmitz, J.,
I. Beiträge zur Anatomie und Physiologie der Schwämme.—Linnæa. 1843. XVII. 417.

Schmitz-Dumont, W.,
See Schroeder u. Schmitz-Dumont.

Schmoeger, M.,
I. Über Centrifugen-Käse.—Milchzeitung. 1883. XII. 483.

SCHNEIDER, A.,
I. Observations on some American Rhizobia.—Bull. of the Torrey Botan. Club. 1892. July.—K. J. III. 207.—Compare Ber. d. D. Bot. Ges. 1894. XII. 11.—Zeitschrift f. Pflanzenkrankheiten. 1895. V. 232.

SCHNEIDER, Paul,
I. Die Bedeutung der Bakterienfarbstoffe für die Unterscheidung der Arten. Inaugural-Dissertation. Basel. 1894.—C. f. B. 2. Abt. 1895. I. 633.—K. J. V. 62.

SCHNELL,
I. Erfahrungen bei der Hefereinzucht unter Verwendung rein gezüchteter Hefen zur Weinvergärung.—Zeitschrift f. angewandte Chemie. 1894. 417.—Ch. C. 1894. II. 498.—K. J. V. 173.

SCHNIRER, M. T.,
I. Zur Frage nach der Verbreitung der Tuberkelbacillen ausserhalb des Körpers. — Wien. medic. Presse. 1891. 3.—C. f. B. 1891. IX. 544.

SCHNURMANS STEKHOVEN, J. H.,
I. Saccharomyces Kefyr. Proefschrift. Utrecht. 1891.—K. J. II. 136.

SCHÖNBEIN, Christian Friedrich,
I. Über Ozon und Ozonwirkungen in Pilzen.—Phil. Magazin. XI. No. 70. 137.—Journal f. prakt. Chemie. 1856. LXVII. 496.

SCHÖNFELD, Franz,
I. Das Hefenwachstum in der Hauptgärung bei untergärigem Bier.—W. f. Br. 1896. XIII. 421.—C. f. B. 2. Abt. 1896. II. 462.—Ch. C. 1896. I. 1181.—K. J. VII. 89.
II. Über die Gärungs- und Nachgärungs-Verhältnisse mit besonderer Berücksichtigung des tatsächlichen Auftretens von Infektion in den obergärigen Brauereien Nord- und Mitteldeutschlands.—W. f. Br. 1895. XII. 546.—C. f. B. 2. Abt. 1895. I. 639.—K. J. VI. 208.
III. Das Infizieren von Flaschenbier durch Sarcina.—W. f. Br. 1897. XIV. 177.—C. f. B. 2. Abt. 1899. V. 162.—K. J. VIII. 142.
IV Studien über eine Bier-Sarcina.—W. f. Br. 1898. XV. 285.—K. J. IX. 136.
V. Erforschung der Quellen der Sarcina-Infektion im Brauereibetrieb.—W. f. Br. 1898. XV. 321.—C. f. B. 2. Abt. 1898. IV. 865.—K. J. IX. 138.
VI. Welche praktischen Massnahmen sind zu ergreifen zur Bekämpfung der Sarcina-Infektion.—W. f. Br. 1898. XV. 694.—K. J. IX. 140.
VII. Studien über eine Biersarcina.—W. f. Br. 1899. XVI. 665.—C. f. B. 2. Abt. 1900. VI. 262.—K. J. X. 154.
VIII. Einige Versuche zur Fortzüchtung verschiedener Sarcinen-Rassen.—W. f. Br. 1899. XVI. 681.—C. f. B. 2. Abt. 1900. VI. 376.—K. J. X. 157.
IX. Die Bakterieninfektionen bei den obergärigen Bieren.—W. f. Br. 1901. XVIII. 274.—C. f. B. 2. Abt. 1902. VIII. 282.
X. Die Stellhefe des Berliner Weissbieres.—W. f. Br. 1902. XIX. 146.—C. f. B. 2. Abt. 1902. IX. 168.
XI. W. f. Br. 1898. XV. 288.

SCHOLL, Hermann,
I. Untersuchungen über giftige Eiweisskörper bei Cholera asiatica und einigen Fäulnisprozessen.—A. f. Hygiene. 1892. XV. 172.—C. f. B. 1892. XII. 727.—Ch. C. 1892. II. 795.
II. Die Milch. Wiesbaden. 1891.

SCHOLTZ, W.,
I. Über den Nachweis von Arsen auf biologischen Wege in den Hautschuppen, Haaren, Schweiss und Urin.—Berliner klin. Wochenschrift. 1899. XXXVI. 913.—Ch. C. 1899. II. 1033.

SCHOSTAKOWITSCH, W.,
I. Über die Bedingungen der Konidien-Bildung bei Russthau-Pilzen.—Flora. 1895. LXXXI. 362.—C. f. B. 2. Abt. 1896. II. 235.

SCHOTTELIUS, M.,
I. Biologische Untersuchungen über den Micrococcus prodigiosus. Leipzig. 1887. Engelmann.—C. f. B. 1887. II. 439.

Schrank, J.,
I. Untersuchungen über den im Hühnerei die stinkende Fäulnis hervorrufenden Bacillus.—Wiener medic. Jahrbücher. 1888. 303.—C. f. B. 1889. V. 770. —Ch. C. 1889. II. 463.

Schreiner,
I. Über Kuhmilch, etc.—Tageblatt d. 50 Vers. D. Naturforscher u. Ärzte. 1877. — Ch. C. 1878. 588.

Schrohe, A.,
I. Behandlung von Wasser und alkoholischen Getränken mit Elektrizität, Ozon und Wasserstoffsuperoxyd zur Reinigung, Konservierung und Geschmacksverbesserung.—W. f. Br. 1891. VIII. 706.
II. Über einen 18 Proz. Alkohol ergebenden Gärungserreger.—Z. f. Spiritusind. 1891. XIV. 96. —C. f. B. 1. Abt. 1892. XII. 467.

Schröder, H.,
I. Über Filtration der Luft in Beziehung auf Fäulnis, Gärung und Krystallisation. — Annalen der Chemie u. Pharmacie. 1859. CIX. 35. 1861. CXVII. 273.—Ch. C. 1859. 410. 1861. 542.

Schröder, H., und Dusch, Th. von,
I. Über Filtration der Luft in Bezug auf Fäulnis und Gärung.—Annalen der Chemie u. Pharmacie. 1854. LXXXIX. 232.—Ch. C. 1854. 303.

Schroeder, J. von,
I. Über Gerbung mit Fichtenextrakt und Quebracho-Extrakt. —Deutsche Gerberzeitung. 1889. No. 38.

Schroeder, J. von, und Bartel, A.,
I. Über Gerbstoffverluste beim Gären der Gerbebrühen. — Deutsche Gerberzeitung. 1890. No. 67.

Schroeder, J. von, und Schmitz-Dumont, W.,
I. Beiträge zur Kenntnis der chemischen Natur der "Ascher."—Dingler's Journal. 1896. CCC. 161.—Ch. C. 1896. II. 278.

Schroeter, J.,
I. Über einige durch Bakterien gebildete Pigmente. — Cohn's Beiträge z. Biologie d. Pflanzen. 1870. Bd. I. Heft 2. 109.

Schröter,
I. In Rabenhorst's Kryptogamenflora Schlesiens. III. 220.
II. *Ibid.* III. 218.

Schröter u. Cohn,
I. Beiträge z. Biol. d. Pflanz. 1872. I. 110, 187. Pl. III. fig. 1.

Schrötter, Hermann von,
I. Vorläufige Mitteilungen über das Pigment von Sarcina aurantiaca und Staphylococcus pyogenes aureus.—C. f. B. 1. Abt. 1895. XVIII. 781.—Ch. C. 1896. I. 317.

Schrötter, Hugo,
I. Über eine im Fuselöl enthaltene Base.—Ber. d. D. Chem. Ges. 1879. XII. 1431.

Schüpphaus,
I. Journ. Am. Chem. Soc. 1892. XIV. 45.

Schütte,
See Tacke, Immendorf, Hessenland, Schütte und Minssen.

Schütz, J.,
I. Hofmeister's Beiträge z. chem. Physiologie u. Pathologie. 1903. III. 433.

Schützenberger, Paul,
I. Recherches sur la levure de bière.—Bulletin de la Société chimique de Paris. 1874. XXI. 204.—C. R. 1874. LXXVIII. 493, 698.
II. Die Gärungserscheinungen. 1876.
III. Ber. d. D. Chem. Ges. 1874. VII. 192.

Schützenberger, P., et Destrem, A.,
I. Recherches sur la levure de bière.—C. R. 1879. LXXXVIII. 287.—Z. g. Br. 1879. II. 271.

Schützenberger, P., et Quinquaud, E.,
I. Sur la respiration des végétaux aquatiques immergés. — C. R. 1873. LXXVII. 272.

Schukow, J.,
I. Über reine Weinhefen.—W. f. Br. 1899. XVI. 195.—C. f. B. 2. Abt. 1899. V. 411.
II. C. f. B. 2. Abt. 1896. II. 611.

Schulte im Hofe,
I. Über den Einfluss der Milchsäure bezw. Schwefelsäure auf den Stickstoffgehalt der Maische. —Z. g. Br. 1889. XII. 325.—Ch. C. 1889. II. 955.

Schultz,
I. Über das Umschlagen der Rotweine.—Weinlaube. 1877. IX. 303.
Schultz, N. K.,
I. Zur Frage von der Bereitung einiger Nährsubstrate.—C. f. B. 1891. X. 52.—K. J. II. 26.
Schultze, W.,
I. Über die Zerstörung des Biergeschmackes und -Geruches durch das Sonnen- oder Tageslicht im Kleinverkehr mit Bier.—Mitteilungen d. österr. Versuchs-Station f. Brauerei und Mälzerei in Wien. 1888. I. 10.—Z. g. Br. 1890. XIII. 162.
II. Über den Vergärungsgrad der Hauptgärung.—Der. bayr. Bierbrauer. 1876. 22.
Schulz, A.,
I. Über den Stoffbedarf und den Stoffumsatz des Kahmpilzes (Saccharomyces Mycoderma). — Annalen der Önologie. 1878. VII. 115. — Just's Botan. Jahresbericht. 1879. 84.
Schulz, Hugo,
I. Über Hefegifte.—Pflüger's Archiv. 1888. XXXXII. 517.—Ch. C. 1888. 583.—Z. g. Br. 1888. XI. 183.
II. Zur Kenntnis der Oxydation der Fette.—Pflüger's Archiv. 1877. XXXX. 398.—Ch. C. 1878. 47.
Schulze, Carl,
I. Die Anwendung des Pasteurisierens gegen Nachgärungen der Weine auf den Flaschen.—Landw. Jahrbücher. 1895. XXIV. 403.—C. f. B. 2. Abt. 1895. I. 833.—K. J. V. 202.
Schulze, Ernst,
I. Über die Cellulose.—Chemiker-Zeitung. 1895. XIX. 1465.—Ch. C. 1895. II. 595.
II. Die chemische Zusammensetzung der pflanzlichen Zellmembranen.—Ber. d. D. Chem. Ges. 1891. XXIV. 2277. — Ch. C. 1891. II. 472.
III. Ber. d. D. Chem. Ges. 1890. XXIII. 2579.
IV. Chem. Ztg. 1893. XVII. 1263.
V. Ber. d. D. Chem. Ges. 1889. XXII. 1191. 1891. XXIV. 2277.
See also Benecke und Schulze, Röse und Schulze.
Schulze, Franz,
I. Vorläufige Mitteilung der Resultate einer experimentellen Beobachtung über generatio æquivoca.—Poggendorff's Annalen d. Physik u. Chemie. 1836. XXXIX. 487.
Schulze, Franz Ferdinand,
I. Beiträge zur Kenntnis des Lignins. Rostock. 1856.
Schumacher, Emil,
I. Beiträge zur Morphologie und Biologie der Hefe.—Sitzungsberichte d. K. Akademie der Wiss. in Wien. Mathem.-naturw. Klasse. 1874. LXX. I. 157.
Schunck, Edward,
I. Über die Einwirkung des Krapp-Ferments auf Zucker.—Journal f. prakt. Chemie. 1854. LXIII. 222.—Ber. d. D. Chem. Ges. 1898. XXXI. 309. — Ch. C. 1898. I. 899.—K. J. IX. 319.
II. Mem. Manchester Lit. and Phil. Soc. 1853-54.—Ber. d. D. Chem. Ges. 1898. XXXI. 309.
Schuppan, P.,
Die Bakteriologie in ihrer Beziehung zur Milchwirtschaft.—C. f. B. 1893. XIII. 527.—Ch. C. 1893. I. 1033.—K. J. IV. 179.
Schuurmans-Stekhoven, J. H.,
I. Dissert. Utrecht. 1891.—Onderzoekingen gedonn in het Physiolog. Laborat. des Utrechtschen Hoogschol. Edited by T. W. Engelmann and C. A. Pekelharing. 4th Series. I. 2, p. 119.
Schwanhaeuser, Rudolf,
I. Beitrag zur experimentellen Untersuchung der Ursache der Gesundheitsschädlichkeit hefetrüber Biere.—Dissert. Greifswald. 1890.—C. f. B. 1. Abt. 1891. IX. 100.
Schwann, Theodor,
I. Vorläufige Mitteilung betreffend Versuche über Weingärung und Fäulnis.—Poggendorff's Annalen d. Physik u. Chemie. 1837. XXXXI. 184.
II. Mikroskopische Untersuchungen über die Übereinstimmung in der Textur und dem Wachstum der Tiere und Pflanzen. Berlin. 1839.

III. Über das Wesen des Verdauungsprozesses. — Joh. Müller's Archiv für Anatomie, Physiologie, etc. 1836. 90. — Poggendorff's Annalen. 1836. XXXVIII. 358.

Schwarz,
I. Liebig's Ann. 1852. LXXXIV. 82.

Schweinitz, E. A. von,
I. Kulturmedien für biochemische Untersuchungen. — The New York Medical Journal. 1893.— C. f. B. 1893. XIV. 330.

Schweitzer, R.,
See Bleisch und Schweitzer.

Schweizer, Eduard,
I. Das Kupferoxyd-Ammoniak, ein Auflösungsmittel für die Pflanzenfaser.—Vierteljahrsschrift d. Züricher Naturf. Ges. 1857. II. 395. — Journal f. prakt. Chemie. 1857. LXXII. 109.

Schweninger, F.,
I. Über die Wirkung faulender org. Substanzen auf den lebenden tierischen Organismus. — Gekrönte Preisschrift. München. 1866.

Schwickerath, Karl,
I. German Patent No. 113164, April 8, 1899.—Ch. C. 1900. II. 653.

Sclavo, Achille, und Gosio, B.,
I. Über eine neue Gärung des Stärke.—Staz. sperim. agrar. ital. 1890. XIX. 540.—Ch. C. 1891. II. 253.—K. J. II. 242.

Sédillot, Charles Emmanuel,
I. De l'influence des découvertes de M. Pasteur sur les progrès de la chirurgie. — C. R. 1878. LXXXVI. 634.

Seekamp, W.,
I. Über die Zersetzung der Weinsäure und Citronensäure durch das Sonnenlicht.—Annalen der Chemie und Pharmacie. 1894. CCXXLVIII. 373.—Ch. C. 1894. I. 854.

Segall,
See Buchner und Segall.

Seifert, Wenzel,
I. Die Organismen der alkoholischen Gärung in der Weinbereitung. — Weinlaube. 1901. XXXIII. 2.
II. Ber. d. chem. phys. Versuchsst. Klosterneuberg. 1899-1900.— Zeitschr. f. d. Landw. Versuchsw. in Österreich. 1900. Heft 3.
IV. Z. f. Nährungsmittel-Unters., etc. VII. 148.
V. Zeitschr. f. d. landw. Versuchswesen in Österreich. 1901. IV 221.
VI. *Ibid.* 1906. IX. 1019.

Seiter, O.,
I. Studien über die Abstammung der Saccharomyceten und Untersuchungen über Schizosaccharomyces octosporus. Dissert. Erlangen. 1896.—C. f. B. 2. Abt. 1896. II. 301.—Ch. C. 1896. II. 250.—K. J. VII. 32.

Sell, Eugen,
I. Über Cognac, Rum und Arak. I. Mitteilung (Cognac).—Arbeiten a. d. Kaiserl. Gesundheitsamte zu Berlin. 1890. VI. 335.—Ch. C. 1890. II. 637.
II. Desgl. II. Mitteilg. (Rum u. Arak).—*Ibid.* 1891. VII. 210. —Ch. C. 1891. I. 1097.

Selmi, Francesco,
I. Preparazione nuova dell' acido lactico, etc. Milano. 1844.— Journal de Pharmacie et de Chimie. 1846. X. 458.
II. Sulle ptomaïne ed alkaloidi cadaveri. Bologna. 1878.—Ber. d. D. Chem. Ges. 1878. XI. 808 u. 1691.
III. Nuovo processo generale per la ricerca delle sostanze venefiche. Bologna. 1875.—Ber. d. D. Chem. Ges. 1874. VII. 1642.

Senus, A. H. C. van,
I. Bijdrage tot de kennis der cellulosegisting. — Proefschrift. Leiden. 1890.—K. J. I. 136.

Serafini, A.,
I. Chemisch - bakteriologische Analysen einiger Wurstwaren. Ein Beitrag zum Studium der Nahrungsmittel-Konservierung. — A. f. Hygiene. 1891. XIII. 173.— K. J. II. 106.

Serafini, A., und Ungaro, G.,
I. Der Einfluss des Räucherns auf die Lebensfähigkeit der Bakterien.—Ann. dell' Ist. d'Igiene di Roma. 1891. II.—K. J. II. 106.

SESTINI, Fausto e Leone,
I. Über die ammoniakalische Gärung der Harnsäure.—D. Landw. Versuchs - Stationen. 1890. XXXVIII. 157.—Ch. C. 1890. I. 1064.—K. J. I. 100.

SETTE,
I. Memoria storico-naturale sull' arrossimento straordinario di alcune sostanze alimentose, osservato nella provincia di Padova l'anno 1819 letta all' Ateneo di Treviso, nella sera 28 Aprile 1820. Venezia. 1824. — Schweigger's Journal f. Chemie u. Physik. 1827. L. 396.

SEVERIN, S. A.,
I. Die im Miste vorkommenden Bakterien und deren physiologische Rolle bei der Zersetzung desselben.—C. f. B. 2. Abt. 1895. I. 97, 799.—Ch. C. 1896. I. 378.

SEYFFERT, H.,
I. Einiges über Reinzucht-Hefen und ihre Ernährung.—Z. g. Br. 1896. XIX. 318.—C. f. B. 2. Abt. 1896. II. 465.—K. J. VII. 123.
II. W. f. Br. 1904. XXI. 519.

SEYNES, J. de,
I. Sur le Mycoderma vini.—C. R. 1868. LXVII. 105.—Bot. Ztg. 1869. XXVII. 521.

SHIBATA,
I. Beiträge z. Chem. Physiologie u. Pathologie. 1904. V. 384.

SHIGA, K.,
I. Z. f. physiolog. Chemie. 1904. XLII. 502.

SIEBEL,
I. Nutzbarmachung der Hefe.—Ch. C. 1898. I. 1049.—K. J. IX. 104.

SIEBENMANN,
I. Die Fadenpilze Aspergillus u. Eurotium. 1882. 2nd Ed. as Die Schimmelmykosen des menschlichen Ohres. Wiesbaden. 1889.

SIEBER, Nadina,
I. Über die antiseptische Wirkung der Säuren.—Journal f. prakt Chemie. 1879. XIX. 433.—Ch. C. 1879. 525.
II. Die angebliche Umwandlung von Eiweiss in Fett beim Reifen des Käses.—Journal f. prakt. Chemie. (2.) 1880. XXI. 203.
See also MACFADYEN, NENCKI u. SIEBER; NENCKI und SIEBER.

SIGMUND, Wilhelm,
I. Über fettspaltende Fermente im Pflanzenreiche.—Monatshefte f. Chemie. 1890. XI. 272.—Ch. C. 1890. II. 628.
II. Beziehungen zwischen fettspaltenden und glykosidspaltenden Fermenten.—*Ibid.* 1892. XIII. 567.—Ch. C. 1892. II. 579.

SIMÁČEK, E.,
I. Centralbl. f. Physiologie. 1903. XVII. 477.
II. *Ibid.* XVII. 209.

SIMANOWSKY, N. P.,
I. Über die Gesundheitsschädlichkeit hefetrüber Biere und über den Ablauf der künstlichen Verdauung bei Bierzusatz.—A. f. Hygiene. 1886. IV. 1.—Reproduced in Z. g. Br. 1886. IX. 115.

SIROTININ,
See FLÜGGE und SIROTININ.

SITNIKOFF, A., und ROMMEL, W.,
I. Vergleichende Untersuchungen über einige sogen. Amylomyces-Arten.—Z. f. Spiritusind. 1900. XXIII. 391.—W. f. Br. 1900. XVII. 621.—C. f. B. 2. Abt. 1901. VII. 245.

SJÖBRING, Niels,
I. Über Kerne und Teilungen bei den Bakterien.—C. f. B. 1892. XI. 65.—K. J. III. 35.

SKALWEIT,
I. Bier-Untersuchungen.—Z. g. Br. 1878. I. 476. 1879. II. 331. 1880. III. 68.

SKERST, Oskar von,
I. Beiträge zur Kenntnis des Dematium pullulans.—W. f. Br. 1898. XV. 354.—C. f. B. 2. Abt. 1898. IV. 864.

SLATER, C.,
I. On a Red - Pigment - forming Organism, Bacillus corallinus.—The Quarterly Journal of Microscopical Science. 1891. Vol. 32. Part III.—K. J. II. 43.

SMITH, Allen J.,
I. A New Chromogenic Bacillus, Bacillus cœruleus. — Medical News. 1887. II. 758.—C. f. B. 1888. III. 401.

SMITH, Claud,
See CROSS, BEVAN u. SMITH.

SMITH, Theobald,
I. Das Gärungskölbchen in der Bakteriologie.—C. f. B. 1890. VII. 502.—K. J. I. 14.

II. The Fermentation Tube, with Special Reference to Anaërobiosis and Gas Production among Bacteria. — Reprint from the Wilder Quarter-Century Book. Ithaca. 1893, p. 187.

Soldan, Carl,
I. Die Gesamtarbeitsleistung der Hefen Saaz, Frohberg und Logos in Saccharose-, Dextrose- und Maltose-Lösung unter verschiedenen Versuchs- und Ernährungsbedingungen. Dissert. Erlangen. 1897.—K. J. IX. 82.

Söldner, Friedrich,
I. Die Salze der Milch und ihre Beziehungen zu dem Verhalten des Caseins.—D. Landw. Versuchs-Stationen. 1888. XXXV. 351.

Sommaruga, G. von,
I. Über Stoffwechselprodukte von Mikroorganismen. III.—Deutsche med. Wochenschrift. 1894. No. 48.—Ch. C. 1895. I. 96.—K. J. V. 74.

Sonntag, Hermann,
I. Über die Bedeutung des Ozons als Desinficienz.—Z. f. Hygiene. 1890. VIII. 95.—C. f. B. 1890. VIII. 778.

Sorauer, Paul,
I. Handbuch der Pflanzenkrankheiten. 2nd Ed. Berlin. 1886.—I. Bd.: Die nichtparasitären Krankheiten. 20*s.*, bound.—II. Bd.: Die parasitären Krankheiten. 14*s.*, bound.
II. Die bakteriose Gummosis der Zuckerrüben. — Zeitschrift f. Pflanzenkrankheiten. 1892. 280. —Blätter für Zuckerrübenbau. 1894. S. 9.—C. f. B. 2. Abt. 1895. I. 295.
III. Handbuch der Pflanzenkrankheiten. 3rd Ed. 1906. Bd. II.

Sorel, E.,
I. Etude sur l'Aspergillus oryzæ. —C. R. 1895. CXXI. 948.—C. f. B. 2. Abt. 1896. II. 120.—K. J. VI. 41.

Sorokin, N.,
I. Eine neue Spirillum-Art.—C. f. B. 1887. I. 466.
II. Noch einmal über Spirillum endoparagogicum.—C. f. B. 1890. VII. 123.—K. J. I. 19.
III. Zur Frage über das Ferment von Kumys.—Tagebuch d. Gesellschaft d. Ärzte der Universität Kasan. 1883. — Just's Botan. Jahresbericht pro 1885. I. 193.

Soskin, Selik,
I. Kritische Geschichte der Lehre von der Fettbildung.—Journal f. Landwirtschaft. 1894. XLII. 157.

Sostegni, Livio, e Sannino, Antonio,
I. Über die Entstehung von Schwefelwasserstoff bei der Alkohol-Gärung. — Staz. sper. agr. ital. 1890. XVIII. 434.—Ch. C. 1890. II. 112.—K. J. I. 66.
See also Berlese e Sostegni.

Soxhlet, Franz,
I. Ein verbessertes Verfahren der Milchsterilisierung. — Münchner medic. Wochenschrift. 1891. No. 19 u 20.—C. f. B. 1891. X. 203.—K. J. II. 187.
II. Chem. Ztg. 1899. XXIII. 851.

Soyka, J.,
I. Über ein Verfahren, Dauerpräparate von Reinkulturen auf festem Nährboden herzustellen. —C f. B. 1887. I. 542.

Soyka, J., und Král, F.,
I. Vorschläge und Anleitungen zur Anlegung von bakteriologischen Museen.—Z. f. Hygiene. 1888. IV. 143.—C. f. B. 1888. IV. 188.

Spaeth, Eduard,
I. Nachweis des Mutterkorns im Mehl.—Pharmac. Central-Halle. 1896. XVII. 542. — Chem.-Zeitg. Repertorium. 1896. XX. 244.
II. Die chemische und mikroskopische Untersuchung des Harnes. Leipzig. 1897.

Spaeth,
See Prochownik u. Spaeth.

Spallanzani, Lazaro,
I. Dissertazione di fisica animale e vegetabile. Modena. 1765.
II. Opuscoli di fisica animale e vegetabile. Modena. 1776. German translation by Donndorf. Leipzig. 1779.

Spieckermann u. Bremer,
I. Landw. Jahrbücher. 1901. XXXI. 81.

Spilker, W., und Gottstein, A.,
I. Über die Vernichtung von Mikroorganismen durch die Induktionselektrizität. — C. f. B. 1891. IX. 77.—Ch. C. 1891. I. 549. —K. J. II. 96.

Spina, A.,
I. Bakteriologische Versuche mit gefärbten Nährsubstanzen.—C. f. B. 1887. II. 71.

Spiro,
See Scheurlen und Spiro.

Spitta, Albert,
See Buchner und Spitta.

Sprengel, Karl,
I. Die Lehre vom Dünger. Leipzig. 1839.

Städeler, G.,
I. Untersuchungen über das Fibroïn, Spongin und Chitin, nebst Bemerkungen über den tierischen Schleim.—Annalen der Chemie und Pharmacie. 1859. CXI. 12.

Stagnitta-Balistreri,
I. Die Verbreitung der Schwefelwasserstoffbildung unter den Bakterien.—A. f. Hygiene. 1893. XVI. 10.—C. f. B. 1893. XIII. 755.—K. J. IV. 99.

Stahl, Georg Ernst,
I. Zymnotechnia fundamentalis sive fermentationis theoria generalis. Halle. 1697. — German translation: Frankfurt. 1734.

Stahl, J.,
I. Formalin. — Pharmaceutische Zeitg. 1893. XXXVIII. 173.— Ch. C. 1893. I. 750.

Stanley, Arthur,
See Frankland, Stanley and Frew.

Stavenhagen, A.,
I. Zur Kenntnis der Gärungserscheinungen.—Ber. d. D. Chem. Ges. 1897. XXX. 2422, 2963. —Ch. C. 1897. II. 1188. 1898. I. 395.—K. J. VIII. 281; IX. 314.

Steenbuch, Chr.,
I. Zur mikroskopischen Untersuchung des Mehles. Eine Methode, wodurch die Gewebselemente leicht isoliert werden können.—Ber. d. D. Chem. Ges. 1881. XIV. 2449.—Ch. C. 1882. 60.

Steickel, C.,
I. Engl. Patent No. 5124 of 1895.

Steinhaus, J. Th.,
I. Beitrag zur Lehre von den sogenannten sporogenen Körnern. —Sitzungsprotokolle der biolog. Sektion der Warschauer Naturforscher - Gesellsch. 1889. — Biolog. Centralblatt. 1889. IX. 574.

Steinhof,
I. Über das Blauwerden der Milch. —Neue Annalen der Mecklenburg. Landwirtschaftsgesellschaft, Rostock. 1838. XXII. 512.

Steinmetz, C.,
I. Kurze Mitteilungen über einige Versuche zur Frage der fäulniswidrigen Eigenschaften der Kohlensäure. — C. f. B. 1894. XV. 677.—Ch. C. 1894. II. 252. —K. J. V. 97.

Sterling, S.,
I. Die peptonisierenden Bakterien der Kuhmilch.—C. f. B. 2. Abt. 1895. I. 473.—Ch. C. 1895. II. 608.

Stern, Arthur L.,
I. The Nutrition of Yeast.—Transactions of the Chemical Society. 1899, p. 201.—Ch. C. 1899. I. 132.—K. J. IX. 84.
II. The Nutrition of Yeast. Part II.: On the Odour of the Gases evolved during Fermentation. —Journal of the Federated Institutes of Brewing. 1899. V. 399.—K. J. X. 109.
III. The Nutrition of Yeast. Part III.—Transactions of the Chemical Society. 1901. LXXIX. 943. —Ch. C. 1901. II. 139.
IV. Some Final Considerations on the Nutrition of Yeast. Part IV. —Journal of the Federated Institutes of Brewing. 1902. VIII. 690.
V. J. Federated Institutes of Brewing. 1902. No. 16. 199.—W. f. Br. 1903. XX. 198.
VI. Proc. Chem. Soc. 1898. CXCVIII. 182.

Stettner, Th.,
I. Das Antinonnin. Ein neues Mittel gegen den Hausschwamm und andere Pilze.—Süddeutsche Bauzeitung. 1892. No. 60.— Z. g. Br. 1893. XVI. 6.—Ch. C. 1893. I. 395.—K. J. IV. 114.

Steuber, L.,
I. Über die desinfizierende Wirkung von gelöschtem Kalk auf Hefe.—Z. g. Br. 1896. XIX. 41.—C. f. B. 2. Abt. 1896. II. 163.—Ch. C. 1896. I. 582.—K. J. VII. 132.
II. Beiträge zur Kenntnis der Gruppe Saccharomyces anomalus.—Z. g. Br. 1900. XXIII. 3. —C. f. B. 2. Abt. 1900. VI. 217.

Steudel, H.,
I. Über die Konstitution des Thymins.—Z. f. physiolog. Chemie. 1900. XXX. 539.—Ch. C. 1900. II. 1152. 1901. I. 443.

Stevens, F. L.,
I. The Effect of Aqueous Solutions upon the Germination of Fungus Spores. — Botanical Gazette. 1898. XXVI. 377.—C. f. B. 2. Abt. 1899. V. 610.

Stift, A.,
I. Über die pflanzlichen Schädlinge der Zuckerrübe.—C. f. B. 2. Abt. 1895. I. 489.
II. Über tierische Schädlinge der Zuckerrübe.—C. f. B. 2. Abt. 1895. I. 398.

Stocky, Alb.,
See Hanuš und Stocký.

Stoecklin, Henri de,
I. Recherches cliniques et expérimentales sur le rôle des levures trouvées dans les angines suspectes de diphtérie.—Archives de médecine expérimentale et d'anatomie pathologique. 1898, p. 1. — Baumgarten's Jahresbericht. 1897. XIII. 748.

Stoehr, C.,
See Brandes und Stoehr.

Stöhr,
I. J. f. pr. Chem. 1893. XLVII. 439.

Stoklasa, Julius,
I. Chemische Untersuchungen auf dem Gebiete der Phytopathologie.—Z. f. physiolog. Chemie. 1895. XXI. 79.—C. f. B. 2. Abt. 1896. II. 126.
II. Studien über die Assimilation elementaren Stickstoffes durch die Pflanzen.—Landw. Jahrb. 1895. XXIV. 827.—Ch. C. 1896. I. 253.
III. Österr. Chem.-Ztg. 1903. VI. 289.
IV. Ber. d. D. Bot. Ges. 1904. XXII. 460.
V. *Ibid.* 1905. XXXVIII. 664.
See also Vanha und Stoklasa.

Stoklasa, J., u. Czerny,
I. Ber. d. D. Chem. Ges. 1903. XXXVI. 622.
II. *Ibid.* XXXVI. 4058.

Stoklasa, J., Cerny, F., Jelinek, J., u Vitek,
I. C. f. B. 2. Abt. 1904 XIII. 86.
II. Zeits. f. d. landw. Versuchswesen in Österr. 1904. VII. 755.

Stoklasa, J., Jelinek, und Vitek,
I. Beiträge z. chem. Physiologie u. Pathologie. 1903. III. 460.

Stoklasa, J., u. Simácek, E.,
I. Centralbl. f. Physiologie. 1903. XVII. 209.

Stoll, O.,
I. Beiträge z. morphol. u. biolog. Characteristik v. Penicillium arten. Dissert. Würzburg. 1904.

Stone, W. E.,
I. Zur Kenntnis der Pentaglucosen. —Ber. d. D. Chem. Ges. 1890. XXIII. 3791.—Ch. C. 1891. I. 313.

Stone, W. E., und Tollens, B.,
I. Gärungsversuche mit Galactose, Arabinose, Sorbose und anderen Zuckerarten. — Annalen der Chemie. 1888. CCXXXXIX. 257.—Ch. C. 1889. I. 316.

Storch, W.,
I. Untersuchungen über Butterfehler und Säuerung des Rahmes. —Milchzeitung. 1890. XIX. 304.—K. J. I. 85.
See also Friis, Lunde und Storch.

Straub,
I. Forschungsber. u. Lebensmittel. 1895. II. 382.

Strecker,
I. Liebig's Ann. 1854. XCII. 80.

Strohmer, F.,
I. Bakterienwirkungen in der Zuckerfabrikation. — Österr.-ungar. Zeitschr. f. Zuckerindustrie und Landwirtschaft. 1891. XX. 7.—Neue Zeitschr. f. Rübenzuckerindustrie. 1891. XXVI. 161.—Ch. C. 1891. I. 897.—K. J. II. 224.

Strub, Emma,
I. Über Milchsterilisation.—C. f. B. 1890. VII. 665.—K. J. I. 89.

Strubell, A.,
I. Untersuchungen über den Bau und die Entwicklung des Rübennematoden, Heterodera Schachtii Schmidt.—Bibliotheca zoologica. Heft 2. Cassel. 1888. Th. Fischer.—C. f. B. 1887. I. 603. 1889. VI. 423.

Struve, Heinrich,
I. Über Kephir.—Ber. d. D. Chem. Ges. 1884. XVII. 314, 1364.

Strzyzowski, Casimir,
See Galli-Valerio und Strzyzowski.

Sturgis, W. C.,
I. Stem-rot.—Annual Report of the Connecticut Agricultural Experiment Station for 1891. New Haven. 1892, p. 184.
II. Preliminary Report on the so-called "Pole-burn" of Tobacco. —*Ibid.*, p. 168.

Stutzer, Adolf,
I. Über das Vorkommen von Nuclein in den Schimmelpilzen und in der Hefe.—Z. f. physiolog. Chemie. 1882. VI. 572.—Ch. C. 1882. 597.—Z. g. Br. 1882. V. 324.
See also Burri, Herfeldt und Stutzer; Burri und Stutzer.

Suchsland, E.,
I. Über Tabaksfermentation.—Ber. d. D. Bot. Ges. 1891. IX. 79.—C. f. B. 1892. XII. 723.—K. J. II. 230.
II. Über das Wesen der Tabakfermentation und über die sich daraus ergebende Möglichkeit, den Fermentationsprozess behufs Veredelung der Tabake zu beeinflussen.—Periodische Mitteilungen des Tabak-Vereins zu Mannheim. 1892. No. 38. 211.

Sugg,
See Ermengem und Sugg.

Svoboda, A.,
See Klaudi und Svoboda.

Swan, A. P.,
I. C. f. B. 2. Abt. 1896. II. 1.

Swan, P.,
I. On the Resisting Vitality of the Spores of Bacillus megaterium to the Conditions of Dryness.—Annals of Botany. 1893. VII. 153.—K. J. IV 110.

Symmers, W. St. Clair,
I. Preliminary Note on a New Chromogenic Micro-organism found in the Vesicles of Herpes labialis: Bacillus viridans. —British Medical Journal. 1891. No. 1615, p. 1252.—C. f. B. 1892. XII. 165.—K. J. III. 43.
II. Note on a Peculiar Movement of Certain Intracellular Particles in Yeast Cells.—Transactions of the British Institute of Preventive Medicine. London. 1898. I.—C. f. B. 1. Abt. 1898. XXIII. 794.

Tacke, Br.,
I. Über die Entwicklung von Stickstoff bei der Fäulnis.—Landw. Jahrbücher. 1887. XVI. 917.—C. f. B. 1888. III. 588.

Tacke, Immendorf, Hessenland, Schütte und Minssen,
I. Über das Verhalten der Bakterien der Leguminosenknöllchen gegen Ätzkalk.—Mitteilungen d. Ver. z. Förderung der Moorkultur im deutschen Reiche. 1895. XIII. 389.—C. f. B. 2. Abt. 1896. II. 161.—Ch. C. 1896. II. 252.

Tahara u. Kitao,
I. Abst. in Chem. Centralbl. 1889. I. 732.

Takahashi, T.,
I. Bull. College Agric. Tokyo. 1902. IV. 395.

Tanret, C.,
I. Action du nitrate d'ammoniaque sur l'Aspergillus niger.—C. R. 1896. CXXIII. 948.—Ch. C. 1897. I. 116.—K. J. VII. 42.
II. Action des nitrate, sulfate, chlorhydrate et phosphate d'ammoniaque sur l'Aspergillus niger. —Bulletin de la Société chimique de Paris. 1897. IIIe série. XVII. 914.—Ch. C. 1898. I. 71.
III. Recherches sur les champignons.—Bulletin de la Société chimique de Paris. 1897. IIIe séric. XVII. 921.—Ch. C. 1898. I. 71.—K. J. VIII. 88.
IV. Sur un nouveau principe immédiat de l'ergot de seigle, l'ergostérine.—Journal de pharmacie et de chimie. 1889. (5.) XIX. 225.—Ch. C. 1889. I. 421.
V. C. R. 1888. CVI. 418.
VI. *Ibid.* 1902. CXXXIV. 1586.

Tappeiner, H.,
I. Untersuchungen über die Gärung der Cellulose, insbesondere über deren Lösung im Darmkanale.—Zeitschrift f. Biologie. 1884. XX. 52.—Ch. C. 1884. 180.

Teichert,
I. Milchztg. 1903. XXXII. 785.

Telesnin, L.,
I. C. f. B. 2. Abt. 1904. XII. 205.

Tensi, W.,
I. Der Johannis- und Stachelbeerwein und die Bereitung der übrigen Beerenweine. Stuttgart. Ulmer. 1*s*.

Tereschtschenko, N. A.,
See Kubarew u. Tereschtschenko.

Thausing, Julius,
I. Die Theorie und Praxis der Malzbereitung und Bierfabrikation. 5th Ed. 1898. Leipzig. Gebhardt. Price, with atlas, bound, 40*s*.

Thenard,
I. Ann. de Chimie. 1803. XLVI. 294.

Thierfelder, Hans,
See Fischer und Theirfelder; Günther und Thierfelder; Nuttal und Thierfelder.

Thom,
I. Journ. of Mycology. 1905. 117.
II. Bull. Bureau of Animal Industry, U.S. Dep. Agriculture. 1906.

Thomas, Pierre,
I. Sur la nutrition azotée de la levure.—C. R. 1901. CXXXIII. 312.—Ch. C. 1901. II. 649.
II. C. R. 1903. CXXXVI. 1015.
III. *Ibid.* 1902. CXXXIV. 610.

Thomson, Robert D.,
I. Über die Resultate der Brotgärung und über den nährenden Wert des Brotes und Mehles verschiedener Länder. — The London, Edinburgh and Dublin Philosophical Magazine. 1843, p. 321.—Journal f. prakt. Chemie. 1844. XXXI. 188.

Thomson, Robert T.,
I. Über die Natur und die chemischen Wirkungen der Essigmutter.—Annalen der Chemie und Pharmacie. 1852. LXXXIII. 89.

Thörner, W.,
I. Untersuchung der Milch auf Tuberkelbazillen. — Chemiker-Zeitg. 1892. XVI. 791.—Ch. C. 92. II. 78.—K. J. III. 177.

Thudichum,
I. J. f. prakt. Chem. 1882. XXV. 19. 1896. LIII. 49.

Thumm, K.,
I. Beiträge zur Kenntnis der fluoreszierenden Bakterien. — Arbeiten aus d. Bakteriolog. Institute d. Techn. Hochschule zu Karlsruhe. I. 291.—C. f. B. 2. Abt. 1895. I. 586.—Ch. C. 1895. II. 876.

Thylman u. Hilger,
I. A. f. Hygiene. 1888. VIII. 451.

Tiegel,
I. Über die fiebererregenden Eigenschaften des Microsporon septicum. Dissertation. Bern. 1871. — Correspondenzblatt f. schweizerische Ärzte. 1871. 275.

Tieghem, Philippe Edouard Léon van,
I. Sur les prétendus cils des Bactéries.—Bulletin de la Société Botanique de France. 1879. XXVI.
II. Traité de Botanique. 1883.
III. Observations sur les bactériacécs vertes.—Bull. de la Société Bot. de France. 1880. XXVII. 174.
IV. Sur le Bacillus Amylobacter et son rôle dans la putréfaction des tissus végétaux.—Bull. de la Société Bot. de France. 1877. XXIV.
V. Identité du Bacillus Amylobacter et du Vibrion butyrique de Pasteur. — C. R. 1879. LXXXIX. 5.—Z. g. Br. 1879. II. 415.
VI. Sur la fermentation de la cellulose.—Bull. de la Société Bot. de France. 1879. XXVI. 28.—C. R. 1879. LXXXVIII. 205.
VII. Sur la gomme de sucrerie.—Annales des Sciences naturelles. Botanique. 6e série. 1878. VII. 180.
VIII. Sur la fermentation ammoniacale.—C. R. 1864. LVIII. 210.

XI. Action de la lumière sur la végétation du Penicillium glaucum dans l'huile.—Bull. de la Société Bot. de France. 1881. XXVIII. 186.
XII. Ann. d. Sci. Nat. Bot. 1867. VIII. 240.
XIII. C. R. 1867. LXV. 1091.—Ann. de l'Ecole Normale Supér. 1869. VI. 27.

Tiemann, F., und Gärtner, A.,
I. Die chemische und mikroskopisch-bakteriologische Untersuchung des Wassers. 4th Ed. 1895. Braunschweig. Vieweg. 24*s.*, bound.

Timm, H.,
I. Die Obst- und Gemüseverwertung für Haushaltungs- und Handelszwecke. Stuttgart. Ulmer. Boards, 3*s.* 8*d.*
II. Der Johannisbeerwein und die übrigen Obst- und Beerenweine. 3rd Ed. Stuttgart. 1896. E. Ulmer. 3*s.*, bound.

Timpe, Hermann,
I. Über die Beziehungen der Phosphate und des Caseins zur Milchsäuregärung. — A. f. Hygiene. 1893. XVIII. 1.—C. f. B. 1894. XV. 425.—K. J. IV. 193.

Tiraboschi,
I. Annali di Botanica. 1905. II. 137.
II. *Ibid.* II. 150.

Tischutkin, N.,
I. Die Rolle der Bakterien bei der Veränderung der Eiweissstoffe auf den Blättern von Pinguicula. —Ber. d. D. Bot. Ges. 1889. VII. 346.—C. f. B. 1890. VII. 288.
II. Über die Rolle der Mikroorganismen bei der Ernährung insektenfressender Pflanzen. — Acta horti petropol. 1892. XII. 1.—C. f. B. 1893. XIII. 134.—K. J. III. 57.

Tizzoni, Guido, u. Cattani, G.,
I. Über die Widerstandsfähigkeit der Tetanusbacillen gegen physikalische und chemische Einwirkungen.—Archiv f. experimentelle Pathologie u. Pharmakologie. 1891. XXVIII. 41.—C. f. B. 1. Abt. 1891. IX. 487.

Todur,
I. Contribution à l'étude de l'action des sels inorganiques et organiques d'argent sur diverses espèces d'Aspergillus. Nancy. 1905.

Tollens, B.,
I. Über eine Lampe zur Herstellung von Formaldehyd.—Ber. d. D. Chem. Ges. 1895. XXVIII. 261.—Ch. C. 1895. I. 729.
II. Kurzes Handbuch der Kohlenhydrate. I. Bd. 2nd Ed. 1898. II. Bd. 1895.
III. Über den Nachweis der Pentosen mittelst der Phloroglucin-Salzsäure-Absatzmethode. — Ber. d. D. Chem. Ges. 1896. XXIX. 1202.—Ch. C. 1896. II. 65.
IV. Ber. d. D. Chem. Ges. 1898. XXXI. 2150.
V. J. Fed. Inst. Brewing. 1898. IV. 438.—J. f. Landwirtschaft. 1901. XLIX. 29.
VI. Ber. d. D. Chem. Ges. 1885. XVIII. 26, 2611.—Liebig's Ann. 1886. CCXXXII. 169.
VII. Handbuch d. Kohlenhydrate. 1896. II. 186.
See also Feilitzen und Tollens; Stone und Tollens.

Tollens, B., u. Glaubitz, H.,
I. Über den Pentosangehalt verschiedener Materialien, welche zur Ernährung dienen, und über den Verbleib des Pentosans bei den Operationen, welchen die obigen Materialien unterworfen werden.—Journal f. Landwirtschaft. 1897. XLV. 97.—Ch. C. 1897. I. 613.

Tolomei, Giulio,
I. Das Gerinnen der Milch in der Gewitterluft. — Milchzeitung. 1891. XX. 519.—K. J. II. 186.
II. Die Nitrifikation in Mauern.—Atti R. Accad. dei Lincei Roma. 1894. (5.) Vol. 3, p. 356.—Ch. C. 1894. I. 1115.—K. J. V. 265.
III. Einwirkung des Lichtes auf die Essiggärung.—Staz. sperim. agrar. ital. 1891. XX. 380.—Ch. C. 1891. II. 254.—K. J. II. 230.
IV. Einwirkung der Elektrizität auf die Essiggärung. — Staz. sperim. agrar. ital. 1891. XX. 380.—Ch. C. 1891. I. 458.—K. J. I. 139.
V. Über ein lösliches Ferment, welches sich im Weine findet.—Atti R. Accad. dei Lincei Roma. 1896. V. 52.—Ch. C. 1896. I. 777.—K. J. VII. 247.

VI. Über die Fermentation der Oliven und die Oxydation des Olivenöles.—Atti R. Accad. dei Lincei Roma. 1896. V. 122. —Ch. C. 1896. I. 879.—K. J. VII. 246.

VII. Einwirkung des Lichtes auf Saccharomyces ellipsoideus. — Accad. dei Lincei. Rend. 1892. (5.) I. 320.—K. J. III. 119.

Topf, G.,
I. Einige Beobachtungen über die Reinzucht und Beurteilung der Bierhefen.—Z. g. Br. 1888. XI. 285.

Trabut,
I. Bull. Soc. Bot. de France. 1895. XLII. 33.

Trapp, A.,
I. Die Methoden der Fleischkonservierung. Dissertation. Berlin. 1893.—K. J. IV. 105.
See also Plagge und Trapp.

Traube, Moritz,
I. Théorie der Fermentwirkungen. Berlin. 1858. F. Dümmler.—Poggendorff's Annalen. 1858. CLXXI. 331.
II. Über das Verhalten der Alkoholhefe in sauerstoffgasfreien Medien.—Ber. d. D. Chem. Ges. 1874. VII. 872, 1756.

Trécul, Auguste,
I. Sur le Bacillus Amylobacter.—C. R. 1865. LXI. 156, 436. 1867. LXV. 513.
II. Remarques sur des levures lactiques et alcooliques.—C. R. 1872. LXXV. 1160, 1169.

Treviranus, Ludolf Christian,
I. Über die Neigung der Hülsengewächse zu unterirdischer Knollenbildung. — Bot. Ztg. 1853. XI. 393.

Trevisan, Victor Graf,
I. Prospetto della Flora Euganea. Padova. 1842.

Tribondeau,
I. C. R. Soc. Biol. Bordeaux. 1901. LIII. 1905. LV. 104.

Trillat, A.,
I. Sur les propriétés antiseptiques de la formaldéhyde.—C. R. 1892. CXIV. 1278; CXV. 290.—Ch. C. 1892. I. 524; II. 224. 1893. I. 1047. 1894. II. 169, 857. 1896. I. 798.
II. Analyse qualitative et quantitative de la formaldéhyde.—C. R. 1893. CXVI. 891.—Ch. C. 1893. I. 1047.

Trombetta, Sergi,
I. Die Fäulnisbakterien und die Organe und das Blut ganz gesund getöteter Tiere.—C. f. B. 1891. X. 664.—Gives also the earlier bibliography.

Trommsdorf,
I. Tageblatt d. Frankfurter Naturforschersvers. 1867. 52.

Tscheppe, Ad.,
I. Gegorene Milch.—Pharmaceutical Journal. 1889, p. 66.—Ch. C 1889. II. 457.

Tschirch, Alexander,
I. Beiträge zur Kenntnis der Wurzelknöllchen der Leguminosen.—Ber. d. D. Bot. Ges. 1887. V. 58.—C. f. B. 1887. I. 634.
II. Das Kupfer vom Standpunkte der gerichtlichen Chemie, Toxikologie und Hygiene. Mit besonderer Berücksichtigung der Reverdissage der Konserven und der Kupferung des Weins und der Kartoffeln. Stuttgart. 1893. F. Enke.—Gives numerous bibliographical references.
III. Angewandte Pflanzen-Anatomie. 1889. Wien u. Leipzig. I. Bd. 191.
IV. Microchemische Reaktionsmethoden im Dienste der techn. Mikroskopie.—Archiv d. Pharmacie. 1882. CCXX. 801.—Compare Bot. Ztg. 1883. XLI. 598.

Tsukamoto, M.,
I. Bull. Coll. Agric. Tokyo. 1897. II. 406.

Tubeuf, K. Freiherr von,
I. Pflanzenkrankheiten durch kryptogame Parasiten verursacht. Berlin. 1895. J. Springer. 16*s.*

Tulasne, Louis-René et Charles,
I. Fungi hypogæi. Paris. 1851.
II. Selecta fungorum carpologia. Paris. 1861.

Tumas, L.,
I. Über die Bedeutung der Bewegung für das Leben niederer Organismen. — Petersburger Medicin. Wochenschrift. 1882. No. 18.

TURPIN,
I. Mémoire sur la cause et les effets de la fermentation alcoolique et acéteuse.—Mémoires de l'Académie d. sc. de l'Institut de France. 1840. XVII. 95.—C. R. 1838. VII. 369.

TURRÓ, R
I. Contribucion ad estudio de la esporulacion de Bacillus anthracis.—Gaceta medica catalana. 1891. No. 3-4.—C. f. B. 1891. X. 91.—K. J. II. 74.

UDRÁNSKY,
I. Z. f. physiolog. Chemie. 1889. XIII. 539.

UFFELMANN, J.,
I. Verdorbenes Brot.—C. f. B. 1890. VIII. 481.—K. J. I. 143.

UHL,
I. Untersuchungen der Marktmilch in Giessen.—Z. f. Hygiene. 1892. XII. 475.—C. f. B. 1893. XIV. 67.—Ch. C. 1893. I. 168.—K. J. III. 176.

ULPIANI e SARCOLI,
I. Atti R. Accad. dei Lincei. 1902. XI. 173.

UNGARO, G.,
See SERAFINI und UNGARO.

UNNA, P. G.,
I. Die Entwicklung der Bakterienfärbung.—A reprint from C. f. B. 1888. III. 24. 1*s.* 6*d.*

URECH,
I. Ber. d. D. Chem. Ges. 1885. XVIII. 3048.

URY, Jakob,
I. Über die Schwankungen des Bacterium coli commune in morphologischer und kultureller Beziehung. — Diss. Strassburg. 1894.—C. f. B. 1894. XVI. 579.

USCHINSKY,
I. Über eine eiweissfreie Nährlösung für pathogene Bakterien nebst einigen Bemerkungen über Tetanusgift. — C. f. B. 1893. XIV. 316.

USPENSKI, A. B.,
I. Zur Bakteriologie des Kwass. Dissert. St. Petersburg. 1891. Russian.

UYEDA,
I. The Bot. Mag. Tokyo. 1902. XV. 160.

VANDAM, Léon,
I. Etude sur un bacille visqueux des bières anglaises: Bacillus viscosus III.—Bull. de l'Association belge des Chimistes. 1895. IX. 245.—W. f. Br. 1896. XIII. 31.—Z. g. Br. 1896. XIX. 161.

VANDEVELDE, A. J. J.,
I. Untersuchungen über Plasmolyse; Bestimmung der Giftigkeit von Alkoholen. — Handelingen van III. Vlammsch Natuur-en Geneeskündig Congres. Antwerpen. 1899.—Ch. C. 1900. I. 481.
II. Bull. Assoc. belge des Chimistes. 1903. XVII. 398.

VANDEVELDE, G.,
I. Studien zur Chemie des Bacillus subtilis.—Z. f. physiolog. Chemie. 1884. VIII. 367.—Ch. C. 1884. 645

VANHA, Johann, und STOKLASA, Julius,
I. Die Rüben-Nematoden (Heterodera, Dorylaimus und Tylenchus). Mit Anhang über die Enchytraciden. Berlin. 1896. P. Parey.

VARRY, G.,
I. Ann. de chim. et de phys. 1835. LX. 69.

VASSILLIÈRE, E., CHARVET et GAYON, U.,
I. Appareils à pasteuriser les Vins. Bordeaux. 1897. 5*s.* 6*d.*

VAUDIN, L.,
I. Sur un élément d'erreur dans la recherche du riz ajouté à la farine de froment.—Journal de Pharm. et de Chimie. (6.) 1899. IX. 431.

VAUGHAN, Victor C.,
I. Preliminary Note on the Chemistry of Tyrotoxicon.—Medical News. 1887, p. 369.—C. f. B. 1887. II. 497.
II. Über die Anwesenheit von Tyrotoxicon in giftigem Eis und giftiger Milch und seine wahrscheinliche Beziehung zur Cholera infantum.—A. f. Hygiene. 1887. VII. 420.—C. f. B. 1888. III. 400.

VERBNO LASZCZYNSKI, Boleslaw de,
I. Über das Vorkommen eines peptonisierenden Enzyms (Peptase) im Malz und Versuche zur Trennung der stickstoffhaltigen Bestandteile in Malz, Würze und Bier.—Z. g. Br. 1899. XXII. 71.—Ch. C. 1899. I. 698.

Vereinbarungen zur einheitlichen Untersuchung Beurteilung von Nahrungs- und Genussmitteln,
I. 1897. Heft 1, 7. 1899. Heft 2, 118.
II. *Ibid.* 1899. Heft 2, 118.

Vergnette-Lamotte, de,
I. Des effets de la chaleur pour la conservation et l'amélioration des vins.—C. R. 1865. LX. 895. 1869. LXIX. 693, 801, et 1048.

Viala, Pierre,
I. Les maladies de la vigne. 3rd Ed. 1893. Coulet, Montpellier; G. Masson, Paris. 20*s*. — Zeitschrift f. Pflanzenkrankheiten. 1894. IV. 250.

Viala, P., et Ravaz, L.,
I. Sur les périthèces du Rot blanc de la vigne.—C. R. 1894. CXIX. 443. 1897. CXXIV. 105.—C. f. B. 2. Abt. 1895. I. 298. 1897. III. 601.

Vieth, P.,
I. Die Behandlung der aus Molkereien wegzugebenden Magermilch bei herrschender Maul- und Klauenseuche. — Milchzeitung. 1894. XXIII. 329.—C. f. B. 1894. XVI. 745.

Vignal, William,
I. Contribution à l'étude des Bactériacées. Le mesentericus vulgatus.—Paris. 1889.—C. f. B. 1890. VII. 61.

Villiers, A.,
I. Sur la fermentation de la fécule par l'action du ferment butyrique.—C. R. 1891. CXII. 435, 536; CXIII. 144.—C. f. B. 1891. X. 283.—K. J. II. 243.

Vincenzi, Livio,
I. Über die chemischen Bestandteile der Spaltpilze.—Z. f. physiolog. Chemie. 1887. XI. 181.—Ch. C. 1887. 243.

Vines, Sydney H.,
I. The Influence of Light upon the Growth of Unicellular Organisms. —Arb. d. Botan. Inst. in Würzburg. 1878. II. 133.

Virchow, Rudolf,
I. Die Sarcina.—Archiv f. patholog. Anatomie und Physiologie. 1847. I. 264.

Viron, L.,
I. Die Rolle der Schizophyten bei den Umsetzungen, welche sich in den "destillierten Wässern" abspielen.—Journal de Pharmacie et de Chimie. 1891. XXIII. 586.—Ch. C. 1891. II. 181.—K. J. II. 226.

Voelcker,
I. On the Composition of Cheese and on the Practical Mistakes in Cheese-making.—Journal of the Royal Agricultural Society of England. 1861. XXII. 61.

Voit, Fritz,
I. Das Verhalten der Galaktose beim Diabetiker.—Zeitschr. f. Biologie. 1893. XXIX. 147.—Ch. C. 1893. I. 364.

Vorderman, A. G.,
I. Analecta op Bromatologisch gebied. — Geneeskundig Tijdschrift voor Nederl.-Indie. 1893. XXXIII. No. 3, p. 369.
II. Geneesk. Tijdschrift voor Nederl.-Indïe. 1894. XXXIV. No. 5.

Vrancken, J.,
I. Les levures de fond; leurs causes, leurs effets, moyens de les éviter.—Bulletin de l'association des anciens élèves de l'école de brasserie de Louvain. 1897. No. 4.

Vries, Hugo de,
I. Over blauwe Kaas.—Maanblad der Hollandsche Maatschappy von Landbouw. 1887. No. 5.—Milchzeitung. 1888. XVII. 861.—C. f. B. 1889. V. 383.
II. Wachstumsgeschichte des roten Klees. — Landw. Jahrbücher. 1877. VI. 936.

Vuillemin, P.,
I. Arch. de Parasitologie. 1904. VIII. 540.
II. C. R. 1904. CXXXVIII. 1350.

Vuylsteke, Julius,
I. Ein Beitrag zur Entwicklungsgeschichte der Mischsaaten von Saccharomyceten. — Z. g. Br. 1888. XI. 537. 1889. XII. 1.—Annales de Micrographie. 1888. I. 193.—C. f. B. 1. Abt. 1889. V. 766.

Wagener,
I. Österr. Monatschr. f. d. Orient. 1881. No. 12.

WAGER, Harold,
I. The Nucleus of the Yeast Plant. —Annals of Botany. 1898. XII. 499. Gives the antecedent bibliography.—C. f. B. 2. Abt. 1899. V. 225.—K. J. IX. 31.
II. On the Presence of Centrospheres in Fungi.—Annals of Botany. 1894. VIII. 321.

WAGNER, Paul,
I. Die geringe Ausnützung des Stallmist-Stickstoffes und ihre Ursachen. — Deutsche landw. Presse. 1895. XXII. No. 11–14.—Chem.-Zeitg. Repert. 1895. XIX. 71.—Ch. C. 1895. I. 894.
II. Ber. d. D. Chem. Ges. 1888. XXI. 1238.

WAHL, G.,
I. Die Anzahl der Hefezellen im Bier.—Der Braumeister. 1889. 307.—Z. g. Br. 1889. XII. 297.

WAHL, R., und HANTKE, E.,
I. Über die Eiweisskörper in Bierwürze und Bier. — American Brewer's Review. 1894. VII. 492.—Ch. C. 1894. I. 788.—K. J. V. 135.

WAHL, Robert, und HENIUS, Max,
I. United States Patent (America) No. 540471 of 1895.

WAHRLICH, W.,
I. Bakteriologische Studien. I.: Zur Frage über den Bau der Bakterienzelle.—Scripta botanica horti bot. Petropolis. 1890–91. III. 30.—C. f. B. 1892. XI. 49. —K. J. II. 51.

WALKER, J. Wallace,
See PURDIE und WALKER.

WALLACE, Schippen,
I. Cases of Cheese-poisoning. — Medical News. 1887. II. 69.—C. f. B. 1887. II. 523.

WALTHER, P.,
I. Über Fick's Theorie der Labwirkung und Blutgerinnung. — Pflüger's Archiv. 1891. XXXXVIII. 529.—K. J. II. 257.

WARBURG,
I. Untersuch. a. d. Bot. Inst. z. Tübingen. 1886. II. 54.

WARD, H. Marshall,
I. Experiments on the Action of Light on Bacillus anthracis.—Royal Society of London. 1892. December 15.—C. f. B. 1. Abt. 1893. XIII. 568.—K. J. III. 77; IV. 122.
II. The Ginger-beer Plant, and the Organisms composing it.—Philosophical Transactions. 1892. CLXXXIII. B. 125. With 6 plates.—Proceedings of the R. Society. 1892. L. No. 304–305.—C. f. B. 1892. XI. 689.—K. J. II. 133; III. 138.
III. On the Characters, or Marks, employed for classifying the Schizomycetes. — Annals of Botany. 1892, April.—C. f. B. 1. Abt. 1892. XII. 789.
IV. On the Biology of Bacillus ramosus (Fraenkel), a Schizomycete of the River Thames.—Fourth Report to the Royal Society Water Research Committee.—Proceedings of the R. Society. 1895. LVIII. 1.—Bot. Ztg. 2. Abt. 1896. LIV. 119.
V. On the Tubercular Swellings on the Roots of Vicia faba.—Philosophical Transactions. 1887. CLXXVIII. 539.—Biedermann's Centralblatt. 1887. XVI. 787. —Ch. C. 1888. 113.
VI. A Lily Disease.—Annals of Botany. 1888. II. 319.—C. f. B. 1889. V. 842.—Bot. Ztg. 1889. XXXXVII. 306.
VII. Further Experiments on the Action of Light on Bacillus anthracis.—Communic. made to the Royal Society. 1893.—C. f. B. 1. Abt. 1894. XV. 1019.—Ch. C. 1893. II. 61.—K. J. IV. 122; V. 100.
VIII. The Brewer's Guardian.—Abst. in W. f. Br. 1892. XIX. 75.
IX. Proc. Roy. Soc. 1892. L. Nos. 304 and 305.

WARINGTON, Robert,
I. The Chemical Actions of Some Micro-organisms. London. 1888. —Chem. News. 1888. LVII. 346.—C. f. B. 1889. VI. 498.—Ch. C. 1888. 1034.
II. Curdling of Milk by Micro-organisms.—The Lancet. 1888. I. No. 25.—Journal of the Chemical Society. 1888. LIII. 727.—C. f. B. 1888. IV. 394.
III. On Nitrification. — Chemical News. 1891. LXIII. 296.—Ch. C. 1890. I. 774. 1891. II. 202.—K. J. III. 215.

WARMING, Eugen,
I. Om nogle ved Danmarks Kyster levende Bacterier. — Videnskabelige Meddelelser fra den naturhistoriske Forening i Kjöbenhavn. 1875. Nos. 20 to 28.

WARSCHAWSKY, J.,
I. C. f. B. 2. Abt. 1904. XII. 400.

WASSERZUG, E.,
I. Variations de forme chez les baetérics. — Ann. Inst. Past. 1888. II. Nos. 2 and 3.—C. f. B. 1. Abt. 1888. III. 783.
II. Sur les spores ehez les levures.—Bulletin de la Société botanique de Franee. 1888. XXXV.—C. f. B. 1. Abt. 1888. IV. 232.

WATSON, D.,
I. Engl. Patent No. 22846 of 1897.

WEBSTER, W.,
I. Reinigung der Abwässer und Sehmutzwässer.—Ch. C. 1891. I. 336.

WEGENER,
I. Verfahren zur Nutzbarmachung von Hefe.—K. J. IX. 104.

WEGNER,
I. Über Dextran.—Zeitschrift f. Zuekerindustrie. 1890. XL. 789.—Ch. C. 1890. II. 1000.

WEHMER, C. (Karl),
I. Zur Zersetzung der Oxalsäure dureh Lieht- und Stoffweehselwirkung.—Ber. d. D. Bot. Ges. 1891. IX. 218.—Ch. C. 1891. II. 665.—K. J. II. 233.
II. Dureh Botrytis hervorgerufene Blattfäule von Zimmerpflanzen, nebst einigen kritisehen Bemerkungen zur Speciesfrage.—Zeitschrift f. Pflanzenkrankheiten. 1894. IV. 204.
III. Beiträge zur Kenntnis einheimischer Pilze. Erstes Heft. 1893. Hannover. Hahn.—Ch. C. 1893. II. 457.—K. J. IV. 241, 268.
IV. *Ibid.* Zweites Heft. 1895. Jena. G. Fiseher.—Bot. Ztg. 2. Abt. 1895. LIII. 377. 1896. LIV. 9, 44.—C. f. B. 2. Abt. 1897. III. 434.—K. J. VI. 72.
V. Entstehung und physiologische Bedeutung der Oxalsäure im Stoffweehsel einiger Pilze.—Bot. Ztg. 1891. XLIX. 233.—Z. g. Br. 1891. XIV. 283.—K. J. II. 110.
VI. Zur Oxalsäuregärung durch Aspergillus niger.—C. f. B. 2. Abt. 1897. III. 102.—Ch. C. 1897. I. 768. — K. J. VIII. 234.
VII. Über die Wirkung einiger Gifte auf Hefe u. Gärung.—Chemiker-Ztg. 1899. XXIII. 163.—C. f. B. 2. Abt. 1899. V. 236.—Ch. C. 1899. I. 795.—K. J. X. 160.
VIII. Aspergillus oryzæ, der Pilz der japanischen Sake-Brauerei.—C. f. B. 2. Abt. 1895. I. 150.—K. J. VI. 315.
IX. Einige Beobachtungen über den Einfluss des Alters und der Temperatur auf die Entwicklungsfähigkeit von Mycelpilzsporen.—C. f. B. 2. Abt. 1897. III. 104.—Ch. C. 1897. I. 996.
X. Uber zwei weitere, freie Citronensäure bildende Pilze.—Chemikerzeitung. 1897. XXI. 1022.—Ch. C. 1898. I. 269.—K. J. VIII. 235.
XI. Die ehinesische Hefe und der sogen. Amylomyces (= Mucor Rouxii).—C. f. B. 2. Abt. 1900. VI. 353. 1901. VII. 599.—Ch. C. 1900. II. 55.
XII. Über die Verflüssigung der Gelatine durch Pilze.—Chemiker-Ztg. 1895. XIX. 2038.—C. f. B. 2. Abt. 1896. II. 92.—Ch. C. 1896. I. 49.—K. J. VI. 73.
XIII. Der javanische Ragi und seine Pilze. I.—C. f. B. 2. Abt. 1900. VI. 610.
XIV. *Ibid.* II.—*Ibid.* 2. Abt. 1901. VII. 313.
XV. Chem. Ztg. 1897. XXI. 73.
XVI. *Ibid.* 1902. XXVI. No. 22.
XVII. Die Pilzgattung Aspergillus Genf. 1901.
XVIII. C. f. B. 1. Abt. 1903. XXXV. 140.
XIX. *Ibid.* 2. Abt. 1896. II. 140.
XX. Ber. d. D. Bot. Ges. 1893. XI. 499.
XXI. C. f. B. 2. Abt. 1897. III. 149.
XXII. Hedwigia. 1894. XXXIII. 211.

XXIII. Beiträge z. Kenntnis einheimischer Pilze. 1896. Heft 2. 73-77.
XXIV. *Ibid.* 68.
XXV. Ber. d. D. Bot. Ges. 1904. XXII. 476. *See also* XXI.
XXVI. *Ibid.* 1891. IX. 223.
XXVI*a*. *Ibid.*, p. 163.
XXVII. Liebig's Ann. 1892. CCLXIX. 383.
XXVIII. Beiträge z. Kenntnis einheimischer Pilze. Hannover u. Leipzig. 1892. Heft 1.—Sitzgsber. d. K. Akad. Wiss. Berlin. 1893. 519.
XXIX. Chem. Ztg. 1897. XXI. 1022.
XXX. Bull. Soc. Chimique. 1893. 728.—C. R. 1893. CXVII. 332.
XXXI. Abhandlungen d. D. Seefischereivereins. 1898. III. 1.
XXXII. C. f. B. 2. Abt. 1905. XIV. 682.
XXXIII. Bot. Ztg. 2. Abt. 1898. LVI. 53.
XXXIV. Beiträge z. Kenntnis einheimischer Pilze. 1896. Heft 2, p. 70.

Weibel, E.,
1. Untersuchungen über Vibrionen. —C. f. B. 1887. II. 465. 1888. IV. 225.

Weibel, L.,
See Bungener und Weibel.

Weidenbaum, A.,
I. Über die morphologischen und physiologischen Unterschiede zwischen Oidium albicans und Oidium lactis.—Arbeiten d. St. Petersburger Naturforscher-Gesellschaft. Abt. f. Botanik. 1891. 26.—C. f. B. 1. Abt. 1892. XI. 569.

Weidenbaum, Josef,
I. Zur quantitativen Bestimmung des Glycogens.—Pflüger's Archiv. 1899. LXXV. 113.—Ch. C. 1899. I. 1167.

Weidmann,
I. Untersuchungen über die Zusammensetzung und den Reifungsprozess des Emmenthaler Käses. — Landw. Jahrbücher. 1882. XI. 587.

Weigmann, Heinrich,
I. Über den jetzigen Stand der bakteriolog. Forschung auf dem Gebiete des Käsereifungsprozesses. —C. f. B. 2. Abt. 1896. II. 150.
II. Die Methoden der Milchkonservierung speziell das Pasteurisieren und Sterilisieren der Milch. With 22 Figs. Bremen. 1893. Heinsius.
III. Die Säuerung des Rahmes mittelst Bakterien-Reinkulturen. —Landw. Wochenbl. f. Schleswig-Holstein. 1890. No. 29.—C. f. B. 1892. XI. 762.
IV. Neue Mitteilungen über Rahmsäuerung mittelst Reinkulturen von Säurebakterien.—Milchzeitung. 1890. XIX. 945.—C. f. B. 1892. XI. 762.
V. Erfahrungen über die Rahmsäuerung mit Bakterien-Reinkulturen.—Landw. Wochenbl. f. Schleswig-Holstein. 1892. No. 16.—C. f. B. 1892. XI. 762.
VI. Die Bakteriologie im Dienste der Milchwirtschaft.—Milchzeitung. 1891. XX. 227.
VII. Der Organismus der sogenannten langen Wei.—Milchzeitung. 1889. XVIII. 982.—Ch. C. 1890. I. 431.
VIII. Über die Lochbildung und Blähung der Käse.—Milchzeitung. 1890. XIX. 741.—K. J. I. 92.
IX. Über bittere Milch.—Milchzeitung. 1890. XIX. 881.—K. J. I. 88.
X. Über den Anteil der Milchsäurebakterien an der Reifung der Käse.—C. f. B. 2. Abt. 1899. V. 630.—Ch. C. 1899. II. 724.
XI. Milchztg. 1890. XIX. 743.

Weigmann, H., und Zirn, G.,
I. Über das Verhalten der Cholerabakterien im Käse.—C. f. B. 1894. XV. 286.—Ch. C. 1894. I. 968.—K. J. V. 225.
II. Über seifige Milch.—C. f. B. 1894. XV. 463.—Ch. C. 1894. I. 166.—K. J. V. 215.

Weinland,
I. Z. f. Biologie. 1901. XXXVIII. 16, 606.

Weinland, E.,
I. Z. f. Biologie. 1901. XLII. 55. 1902. XLIII. 86.

Weis, Fr.,
I. Über das proteolytische und ein eiweisscoagulierendes Enzym in keimender Gerste (Malz).—Z. f. physiolog. Chemie. 1900. XXXI. 79.—Ch. C. 1901. I. 56.

Weleminsky, Friedrich,
I. Über Sporenbildung bei Dematium pullulans de Bary.—C. f. B. 2. Abt. 1899. V. 297.

Weller, H.,
I. Über das Vorkommen von Alkohol in der Milch.—Forsehungsberichte ü. Lebensmittel u. ihre Beziehungen z. Hygiene, etc. 1897. IV. 206.—Ch. C. 1897. II. 527.

Wells, J. G.,
See Morris und Wells.

Welte, Eugen,
I. Über das Verschimmeln des Brotes.—A. f. Hygiene. 1895. XXIV. 84; XXV. 104.—Ch. C. 1895. II. 543. 1896. I. 54.—K. J. VI. 301.

Went, C., und Prinsen Geerligs, C.,
I. Over suiker en alcoholvorming door organismen in verband met de verwerking der naproducten in de rietsuikerfabriken.—Mededeelingen van het proefstation voor suikerriet in West-Java te Kagok-Tegal. 1895. — Verhandeling. d. Koninkl. Akad. v. Wetenschappen te Amsterdam. 1895. Ser. II. Deel 4. No. 2.—C. f. B. 2. Abt. 1895. I. 501, 504.—Ch. C. 1894. II. 633.—K. J. V. 152.
II. Verh. d. K. Akad. Wetenschappen te Amsterdam. 1894. Ser. II. IV. No. 2.
III. Die deutsehe Zuckerindustrie. 1894. XIX. 1043.

Went, F. A. F. C.,
I. Ann. d. Sei. Nat. Bot. 7th Series. I.
II. Verhandl. d. Koninkl. Akad. Wetensch. Amsterdam. 1904. Aug. 26, p. 83.
III. Reeueil des travaux bot. Néerland. 1904. No. 1.—Abst. in W. f. Br. 1904. XXI. 698.
IV. C. f. B. 2. Abt. 1901. VII. 544.
V. Jahrb. wiss. Bot. 1901. XXXVI. 611.

Wermischeff,
I. Reeherehes sur les microbes acétifiants.—Ann. Inst. Past. 1893. VII. 213.—K. J. IV. 248.

Werner, C.,
I. Die Bedingungen der Konidienbildung bei einigen Pilzen. Dissert. 1898.—C. f. B. 2. Abt. 1899. V. 289.

Wernke,
I. Dissert. Dorpat. 1879. Quoted by Will, I.

Wesenburg, G.,
I. C. f. B. 2. Abt. 1902. VIII. 627.

Weyl, Theodor,
See Kitasato und Weyl; Pictet und Weyl.

Wichmann, Heinrich,
I. Über Wasserfiltration.—Mitteilungen d. Österr. Versuchs-Station f. Brauerei und Mälzerei in Wien. 1892. Heft V.—C. f. B. 1893. XIII. 22.—K. J. III. 73.
II. Biologische Untersuehung des Wassers für Brauereizwecke.—Mitteilungen d. Österr. Versuchs-Station für Brauerei und Mälzerei. 1892. V.—C. f. B. 1893. XIII. 207.—K. J. III. 134.
III. Über die Ascosporenzüchtung auf Thon.—C. f. B. 1. Abt. 1893. XIV. 62.—K. J. IV. 39.
IV. Neuere Hefereinzucht-Apparate.—Mitteilungen d. Österr. Versuehs-Station f. Brauerei, etc. 1894. VI. 39. 1902. X. 3.—K. J. V. 189.
See also Rohn und Wichmann.

Wiener, Emil,
See Gruber und Wiener.

Wierigo,
I. Sitzungsberichte des Odessaer naturw. Vereines. 1886 u. 1887.—Wratseh. 1886. 352. 1887. 576. Russian.

Wiesner, Julius,
I. Die heliotropischen Erscheinungen im Pflanzenreiche.—Denkschriften der Wiener Akademie. Mathem.-naturw. Klasse. 1879. XXXIX. Abth. 1. 143–209. 1882. XLIII. Abth. 1. 1–92.
II. Untersuchungen über den Einfluss, welchen Zufuhr und Entziehung von Wasser auf die Lebentätigkeit der Hefezellen äussern.—Sitzungsber. d. Akademie d. Wissenschaften in Wien. Mathem.-naturw. Klasse. 1869. LIX. 2. Abt. S. 495.

WIGAND, Alb.,
I. Entstehung und Fermentwirkung der Bakterien.—Marburg. 1884. Elwert.
II. Das Protoplasma als Fermentorganismus. Marburg. 1888.

WIGGERS, H. A. L.,
I. Untersuchung über das Mutterkorn. Annalen der Pharmacie. 1832. I. 129.

WIJSMANN, H. P.,
I. De diastase beschouwed als mengsel van Maltase en Dextrinase. Amsterdam. 1889. Spin & Co.—Recueil des travaux chimiques des Pays-bas. 1890. IX. 1.—Z. g. Br. 1890. XIII. 186.
II. Über den Stickstoffgehalt von Saccharomyces ellipsoides.—Z. g. Br. 1891. XIV. 381.—Ch. C. 1891. II. 759.—K. J. II. 120.

WILCKENS, Martin,
See ADAMETZ u. WILCKENS.

WILDIERS, E.,
I. Nouvelle substance indispensable au développement de la levure.—La Cellule. 1901. XVIII. 313.

WILEY,
I. Journ. Franklin Inst. 1897. CXLIII. 293.

WILFARTH, H.,
I. Über die Stickstoff-Aufnahme der Pflanzen.—Tageblatt der Naturforscher-Versammlung zu Bremen. 1890.—K. J. I. 131.

WILHELM, C.,
I. Beiträge z. Kenntnis d. Pilzgattung Aspergillus. Dissert. Berlin. 1877. 36.—Bot. Zeitung. 1881. XXXIX. 534

WILHELMI, Armand,
I. Beiträge zur Kenntnis des Saccharomyces guttulatus.—C. f. B. 2. Abt. 1898. IV. 305. Gives the antecedent bibliography.—K. J. IX. 34.

WILL, Heinrich,
I. Über die Wirkung einiger Desinfektionsmittel auf Hefe.—Z. g. Br. 1893. XVI. 151 *et seq.*, 411. 1894. XVII. 43. Has many references to the older literature.—Ch. C. 1893. II. 60. 1894. I. 159, 964.—K. J. IV. 171; V. 201.
II. Untersuchungen über die Verunreinigungen gebrauchter Trubsäcke.—Z. g. Br. 1892. XV. 77.—C. f. B. XII. 148.—K. J. III. 166.
III. Ein Beitrag zur Kenntnis der sogen. Glutinkörperchen in der Würze, im Bier und in der Hefe.—Z. g. Br. 1894. XVII. 187. 1897. XX. 77.—Ch. C. 1894. II. 392.
IV. Die braun gefärbten Ausscheidungen (Hopfenharz-Ausscheidungen), welche der Bierhefe beigemengt sind; deren Bau im normalen und abnormalen Zustand, sowie deren Beschaffenheit.—Z. g. Br. 1894. XVII. 315.
V. Bemerkung zu einer Arbeit von Paul Lindner (VII.).—Z. g. Br. 1896. XIX. 675.
VI. Notiz betreffend den Nachweis von wilden Hefenarten in Brauereihefen und Jungbieren sowie das Vorkommen von Saccharomyces apiculatus in denselben.—Z. g. Br. 1893. XVI. 29.—Ch. C. 1893. I. 547; II. 689.—K. J. IV. 170.
VII. Über einen ungeformten Eiweisskörper, welcher der untergärigen Bierhefe beigemengt ist, und dessen Beziehung zu dem sogen. gelatinösen Netzwerk, welches beim Eintrocknen der Bierhefe entsteht, nebst einigen Beobachtungen über Netzbildung in der Kahmhaut.—Z. g. Br. 1897. XX. 447.—C. f. B. 2. Abt. 1898. IV. 130.—Ch. C. 1897. II. 869.
VIII. Vergleichende Untersuchungen an vier untergärigen Arten von Bierhefe. Teil I. bis V.—Z. g. Br. 1895. XVIII. 1, 217.—C. f. B. 2. Abt. 1895. I. 449. 1896. II. 752. 1898. IV. 367.
IX. Zwei Hefearten, welche abnorme Veränderungen im Bier veranlassen.—Z. g. Br. 1891. XIV. 145.—C. f. B. 1891. X. 521.—Ch. C. 1891. II. 353. This gives a figure.—K. J. II. 150.
X. Die Hefenzelle, deren Aussehen und Beschaffenheit in den verschiedenen Stadien der Entwicklung und des Zerfalles unter

dem Mikroskop.—Allgem. Brauer- und Hopfen-Zeitung. 1892. XXXII. 1088.
XI. Schielige Biere. Bemerkungen zu einer Arbeit von C. J. Lintner (IV.).—Z. g. Br. 1891. XIV. 81. —K. J. II. 149.
XII. Studien über die Proteolyse durch Hefen. I. u. II.—Z. g. Br. 1898. XXI. 139. 1901. XXIV. 113.—C. f. B. 2. Abt. 1898. IV. 753. 1901. VII. 794.—Ch. C. 1898. I. 1141.
XIII. Eine Mycoderma-Art und deren Einfluss auf Bier.—Z. g. Br. 1899. XXII. 391. 1900. XXIII. 185.—C. f. B. 2. Abt. 1899. V. 842. 1900. VI. 561. —Ch. C. 1899. II. 449.—K. J. X. 153.
XIV. Gerbstoff-Reaktionen an Hefezellen und deren Beimengungen aus gehopfter Würze.—Z. g. Br. 1900. XXIII. 325.—C. f. B. 2. Abt. 1900. VI. 807.
XV. Vergleichende Untersuchungen an vier untergärigen Arten von Bierhefe. Teil VI.—Z. g. Br. 1898. XXI. 443. 1899. XXII. 151. 1902. XXV. 241. —C. f. B. 2. Abt. 1899. V. 726. 1902. IX. 135.—K. J. IX. 86; X. 93.
XVI. Untersuchungen über das Ausarten der Brauereihefe.—Z. g. Br. 1898. XXI. 243.—C. f. B. 2. Abt. 1898. IV. 808.—K. J. IX. 91.
XVII. Über Sporen- und Kahmhautbildung bei Unterhefe.—Z. g. Br. 1887. X. 357.—C. f. B. 1. Abt. 1887. II. 592.
XVIII. Über die mechanische Bierklärung durch Späne und Filtration und ihren Einfluss auf die Qualität und Haltbarkeit des Bieres. Vortrag.—Z. g. Br. 1896. XIX. 616.—K. J. VII. 113.
XIX. Welche Erfahrungen sind mit der Reinhefe im Brauereibetrieb gemacht worden? Vortrag.—Z. g. Br. 1897. XX. 591. K. J. VIII. 110.
XX. Zur Frage der alkoholischen Gärung ohne Hefezellen.—Z. g. Br. 1898. XXI. 291.—C. f. B. 2. Abt. 1899. V. 195.—Ch. C. 1898. II. 439.—K. J. IX. 318.
XXI. Einige Beobachtungen über die Lebensdauer getrockneter Hefe.—Z. g. Br. 1896. XIX. 453. 1897. XX. 91. 1898. XXI. 75. 1899. XXII. 43. 1900. XXIII. 11. 1901. XXIV. 3. 1902. XXV. 49. 1903. XXVI. 57.—C. f. B. 2. Abt. 1897. III. 17. 1898. IV. 485. 1899. V. 527. 1900. VI. 226. 1901. VII. 438. 1902. IX. 69. 1903. X. 251.—K. J. VII. 106; VIII. 90; IX. 85; X. 172; XI. 127.
XXII. Die Farbe des Bieres und die Hefe.—Z. g. Br. 1901. XXIV. 501.—Ch. C. 1901. II. 714.
XXIII. Hefewasser zur biologischen Analyse.—Z. g. Br. 1901. XXIV. 289.—C. f. B. 2. Abt. 1901. VII. 892.—Ch. C. 1901. II. 139.
XXIV. Die Methoden, welche bei der Reinzüchtung von Hefe und ähnlichen Organismen durch Einzellkultur auf festen Nährböden zur Feststellung der Lage der ausgewählten Zellen in den Kulturen zur Anwendung kommen. —C. f. B. 2. Abt. 1896. II. 483. —K. J. VII. 8.
XXV. Z. g. Br. 1900. XXIII. 748.
XXVI. *Ibid.* 1898. XXI. 307.
XXVII. *Ibid.* 1902. XXV. 33.
XXVIII. *Ibid.* 1895. XVIII. 249.
XXIX. *Ibid.* 1897. XX. 59.
XXX. *Ibid.* 1895. XVIII. 1. 1898. XXI. 1899. XXII. 1902. XXV. 1904. XXVII.
XXXI. *Ibid.* 1903. XXVI. 283.
XXXII. *Ibid.*, p. 284.
XXXIII. *Ibid.* 1905. XXVIII. 128.
XXXIV. *Ibid.* 1896. 453.
XXXV. *Ibid.* 1897. XX. 363.
XXXVI. *Ibid.* 1896. XIX. 20.
XXXVII. *Ibid.* 1898. XXI. 139. 1901. XXIV. 113.

WILL, H., u. WANDERSCHECK,
I. Z. g. Br. 1906. XXIX. 73.

WILLCOX, W. H.,
See WOOD and WILLCOX.

WILLIAMS,
I. Chinese Commercial Guide. 4th Ed. 1856.

Wilm,
I. Über die Einwanderung von Choleravibrionen ins Hühnerei.—A. f. Hygiene. 1895. XXIII. 145.—C. f. B. 2. Abt. XVII. 196, 892.—Ch. C. 1895. II. 171.

Wilson,
I. Über Atmung der Pflanzen.—Flora. 1882. LXXV. 93.

Windisch, K.,
I. Notice in Chem. Ztg. 1901. XXV. 240.
II. *Ibid.* 1902. XXVI. 865.
III. Arb. Kais. Ges. Amt. 1892. VIII. 175.
IV. *Ibid.* 1895. XI. 285.
V. Die Chem. Untersuchung u. Beurtheilung d. Weines. 1896. 42, 43.
VI. *Ibid.* 1896. 100.

Windisch, Wilhelm,
I. Sterilisierung von Kellern, Tennen, Fässern, etc., mittelst Dämpfen von Formaldehyd, sowie das Verhalten von Formaldehyd gegen Hefen und Bakterien.—W. f. Br. 1894. XI. 1531.—Ch. C. 1895. I. 276.—K. J. V. 95.
II. Über die Bildung und den Verbleib des Furfurols, sowie dessen Bedeutung im Brauereibetriebe.—W. f. Br. 1898. XV. 189.—Ch. C. 1898. I. 1213.—K. J. IX. 161.
III. Wie kommen die unedlen faden Geschmacksstoffe beim Maischprozess ins Bier.—W. f. Br. 1897. XIV. 393.
IV. W. f. Br. 1899. XVI. 653.
V. *Ibid.* XVI. 72.
VI. *Ibid.* 1895. XII. 655.
VII. *Ibid.* 1900. XVII. 91.

Windisch, W., und Schellhorn, B.,
I. Über das eiweissspaltende Enzym der gekeimten Gerste.—W. f. Br. 1900. XVII. 334.—Ch. C. 1900. II. 489.

Winkler, Willibald,
I. Zur Charakterisierung der Duclaux'schen Tyrothrix - Arten, sowie über die Variabilität derselben und den Zusammenhang der peptonisierenden und Milchsäure-Bakterien.—C. f. B. 2. Abt. 1895. I. 609.—Ch. C. 1895. II. 1050.—K. J. VI. 250.

Winkler, W.,
I. C. f. B. 2. Abt. 1902. VIII. 721.
II. Jahres. f. prak. Pharmazie. XXVI. 209.

Winogradsky, Sergius,
I. Sur le rouissage du lin et son agent microbien.—C. R. 1895. CXXI. 742.—C. f. B. 2. Abt. 1896. II. 273.—Ch. C. 1896. I. 50.
II. Sur l'assimilation de l'azote gazeux de l'atmosphère par les microbes.—C. R. 1893. CXVI. 1385. 1894. CXVIII. 353.—Ch. C. 1893. II. 283. 1894. I. 590.—C. f. B. 1894. XVI. 129.—K. J. IV. 231; V. 255.
III. Recherches sur l'assimilation du nitrogène libre de l'atmosphère par les microbes.—Archives des sciences biologiques de St. Pétersbourg. 1895. III. 295.—Ch. C. 1895. I. 1123.—Revue mycologique. 1897, p. 95.—K. J. VI. 273.
IV. Über Eisenbakterien. — Bot. Ztg. 1888. XXXXVI. 261.—C. f. B. 1888. IV. 65.—Ch. C. 1888. 1035.
V. Beiträge zur Morphologie und Physiologie der Bakterien. I. Zur Morphologie und Physiologie der Schwefelbakterien. 1888. Leipzig. A. Felix. — C. f. B. 1889. V. 57.
VI. Über Schwefelbakterien.—Bot. Ztg. 1887. XXXXV. 489.—C. f. B. 1887. II. 590.—Ch. C. 1888. S. 1034. 1890. I. 1062.
VII. Recherches sur les organismes de la nitrification. I.–IV.—Ann. Inst. Past. 1890. IV. 213, 257, 760. 1891. V. 92.—C. R. 1890. CX. 1013.—C. f. B. 1890. VIII. 175 u. 392. 1891. IX. 351 u. 603.—Ch. C. 1890. I. 1061; II. 110. 1891. I. 327; II. 491. 1892. I. 216.—K. J. I 101; II. 210.
VIII. Contributions à la morphologie des organismes de la nitrification.—Archives des seiences biologiques publ. par l'Inst. Imp. de médec. expérim. à St. Pétersbourg. 1892. I. 87.—C. f. B. 2. Abt. 1895. I. 243.—K. J. III. 221.

IX. Sur la formation et l'oxydation des nitrites pendant la nitrification.—C. R. 1891. CXIII. 89.—K. J. II. 212.
X. Zur Mikrobiologie des Nitrifikationsprozesses. — C. f. B. 2. Abt. 1896. II. 415.—Ch. C. 1896. II. 636.—K. J. VII. 209.
XI. Uber die Wirkung äusserer Einflüsse auf die Entwicklung von Mycoderma vini.—Arbeiten d. St. Petersburger naturforsch. Gesellschaft. 1884. XIV. 132. —Botan. Centralblatt. 1884. XX. 165.

Winter, Georg,
I. Heliotropismus bei Peziza Fuckeliana de By. — Bot. Ztg. 1874. XXXII. 1. It has some bibliographical references.
II. Die Pilze Deutschlands, Österreichs und der Schweiz. I. Abt.: Schizomyceten, Saccharomyceten und Basidiomyceten. Leipzig. 1884.
III. *Ibid.* II. Abt.: Gymnoasceen und Pyrenomyceten. Leipzig. 1887.
IV. In Rabenhorst's Kryptogamenflora Deutschlands. 2nd Ed. 1887. I. 2. Abt. 48, 61.

Winter, H.,
I. Untersuchungen über das Zuckerrohr. Dissert. Halle. 1891.—Mededeelingen van het Proefstation Midden Java. Semarang 1890.

Winterstein, E.,
I. Zur Kenntnis der Pilzcellulose.—Ber. d. D. Bot. Ges. 1893. XI. 441. 1895. XIII. 65.—Ch. C. 1893. II. 756. 1895. I. 962.
II. Die in den Membranen der Pilze enthaltenen Bestandteile.—Z. f. physiolog. Chemie. 1894. XIX. 521; XX. 342.—Ch. C. 1894. II. 524. 1895. I. 280.
III. Über ein stickstoffhaltiges Spaltungsprodukt der Pilzcellulose.—Ber. d. D. Chem. Ges. 1894. XXVII. 3113, 3508. 1895. XXVIII. 167.—Ch. C. 1895. I. 29, 392, 690.—C. f. B. 2. Abt. 1895. I. 500.
IV. Zur Kenntnis der in den Membranen der Pilze enthaltenen Bestandteile. II.—Z. f. physiolog. Chemie. 1896. XXI. 134.—Ch. C. 1896. I. 114.

Wirgin, G.,
I. Z. f. Hygiene. 1902. XL. 307.

Wisselingh, C. van,
I. Mikrochemische Untersuchungen über die Zellwände der Fungi.—Pringsheim's Jahrbücher. 1898. XXXI. 619.—C. f. B. 2. Abt. 1899. V. 193.

Wittlin, J.,
I. Über die angebliche Umänderung von Tyrothrix tenuis (Duclaux) in ein Milchsäure-Bacterium.—C. f. B. 2. Abt. 1896. II. 475.—K. J. VII. 193.

Wladika, Julius,
I. Zur Kenntnis der organischen Säuren in Fichtenbrühen.—Der. Gerber. 1890. No. 368.

Wladimiroff, Alexander,
I. Biologische Studien an Bakterien. I. Über das Verhalten beweglicher Bakterien in Lösungen von Neutralsalzen.—Z. f. Hygiene. 1891. X. 89.—K. J. II. 66.
II. *Ibid.* II. Osmotische Versuche an lebenden Bakterien.—Zeitschrift f. physikalische Chemie. 1891. VII. 524.—K. J. II. 66.

Wohl,
I. Ber. d. D. Chem. Ges. 1898. XXXI. 1796.

Wolff,
I. C. R. 1900. CXXXI. 1323.

Wolff, Emil,
I. Beiträge zur Theorie und Praxis der Düngung. A reprint from the 12th ed. of Praktischen Düngerlehre. Berlin. 1892. Parey.

Wolffhügel, G.,
I. Über den Wert der schwefligen Säure als Desinfektionsmittel.—Mitteil. a. d. K. Gesundheits-Amte. 1881. I. 1888.—Ch. C. 1882. 334.
See also Koch und Wolffhügel.

Wolffin, Alexander,
I. Hygienische Studien über Mehl und Brot.—A. f. Hygiene. 1894. XXI. 268.—Ch. C. 1894. II. 895.—K. J. VI. 300.

Wollny, R.,
I. Auf kaltem Wege sterilisierte eiweisshaltige Nährböden.—C. f. B. 1892. XI. 752.—K. J. III. 17.

Woltering, P., und Sassen, A.,
I. Über Reinigung von eisenhaltigem Grundwasser.—Nederl. Tijdschr. Pharm. 1895. VII. 304.—Ch. C. 1895. II. 1166.—Z. g. Br. 1896. XIX. 191.

Wood,
I. Enzyme Aetion in Lower Organisms.—Proeeedings of the Royal Soeiety of Edinburgh. 1889. XVII. 27.—C. f. B. 1890. VIII. 266.

Wood, Joseph T.,
I. Methods of Baeteriologieal Researeh, with some Aeeount of Bran Fermentation.—Journal of the Soeiety of Chemieal Industry. 1890. January 31 and Deeember 11.
II. Gärung in der Lederindustrie.—Chemieal News. 1893. LXVIII. 109.—Ch. C. 1893. II. 1013.—K. J. IV. 256.
III. Fermentation in the Leather Industry.—Journal of the Soeiety of Chemieal Industry. 1894. XIII. 218.—Ch. C. 1894. I. 941.—K. J. V. 271.

Wood, Joseph T., and Willcox, W. H.,
I. Further Contribution on the Nature of Bran Fermentation.—Journal of the Soeiety of Chemieal Industry. 1893. XII. 422.—Ch. C. 1893. II. 214.—K. J. IV. 256.

Wood-Smith, R. F.,
I. The Baeteriology of Yeast. Part I.—Journal of the Federated Institutes of Brewing. 1898. IV. 115.—W. f. Br. 1898. XV. 161.—Ch. C. 1898. I. 1141.—K. J. IX. 134.

Woronin, M.,
I. Über die bei der Sehwarzerle (Alnus glutinosa) und der gewöhnliehen Gartenlupine (Lupinus mutabilis) auftretenden Wurzelansehwellungen. — Mémoires de l'Aead. des seienees de St. Pétersbourg. 1866. 7ème série. T. 10. No. 6.—Bot. Zeitg. 1866. XXIV. 329.

Wortmann, Julius,
I. Untersuehungen über das diastatisehe Ferment der Bakterien.—Z. f. physiolog. Chemie. 1882. VI. 287.—Ch. C. 1882. 593.
II. Mitteilung über die Verwendung von konzentriertem Most für Pilzkulturen. Bot. Ztg. 2. Abt. 1893. LI. 177.—C. f. B. 1893. XIV. 816.
III. Über den sogen. Stopfengesehmaek der Weine und seine Bekämpfung. Vortrag.—Ber. ü. d. XV. D. Weinbau-Kongress in Heilbronn. 1896. 44.—Weinbau und Weinhandel. 1896. No. 45.—K. J. VII. 148.
IV. Untersuehungen ü. d. Auftreten u. Verhalten von Dematium pullulans im gärenden Most.—Jahresber. d. kgl. Lehranstalt zu Geisenheim a. Rh. für 1891–1892. 52.—K. J. III. 165.
V. Die seitherigen Erfahrungen der Praxis mit reinen Hefen und die Konsequenzen, welehe sieh hieraus für die Züehtung sowie die Anwendung der Reinhefen ergeben.—Ber. ü. d. Verh. d. XIII. D. Weinbau-Kongresses in Mainz. 1894. 57.—C. f. B. 2. Abt. 1895. I. 249.—Ch. C. 1895. I. 937.—K. J. V. 168.
VI. Einige Beobaehtungen über das Verhalten der Hefen im Weinberge.—Berieht der kgl. Lehranstalt, ete., zu Geisenheim a. Rh. pro 1897–98. 75.—Weinbau u. Weinhandel. 1898. 278.—Z. g. Br. 1898. XXI. 715.
VII. Vorkommen und Wirkung lebender Organismen in fertigen Weinen und ihre Bedeutung für die Praxis der Weinbereitung.—Landw. Jahrbüeher. 1898. XXVII. 631.—C. f. B. 2. Abt. 1899. V. 229.—Ch. C. 1899. I. 71.
VIII. Über die Herkunft der Weinhefen.—Berieht d. Kgl. Lehranstalt, ete., zu Geisenheim a. Rh. pro 1895–96. 82.—K. J. VII. 35.
IX. Über die vermeintliehe Hefebildung von Aspergillus oryzæ.—*Ibid.* 84.—K. J. VII. 36.
X. Über die Ursaehe des zögernden Eintrittes der Gärung der 1895 er Moste.—Weinbau und Weinhandel. 1895. No. 45.—K. J. VI. 148.

XI. Über künstlich hervorgerufene Nachgärungen von Weinen in der Flasche und im Fasse.—Landw. Jahrbücher. 1897. XXVI. 473.—C. f. B. 2. Abt. 1898. IV. 588.—Ch. C. 1897. II. 506.

XII. Untersuchungen über das Umschlagen der Weine.—Weinbau u. Weinhandel. 1899. S. 294.—C. f. B. 2. Abt. 1900. VI. 298.—Ch. C. 1900. I. 1311.—K. J. X. 145.

XIII. Zur Kenntnis der Reizbewegungen.—Bot. Ztg. 1887. XXXXV. 785.

XIV. Ein Beitrag zur Biologie der Mucorineen.—Bot. Ztg. 1881. XXXIX. 368.

XV. Anwendung und Wirkung reiner Hefen bei der Weinbereitung. Berlin. 1895. Parey. 2*s.* 6*d.*—K. J. VI. 172.

XVI. Landw. Jahrbücher. 1892. XXI. 901.

XVII. Vorkommen u. Wirkung lebender Organismen infestigen Weinen u. ihre Bedeutung f. d. Weinbereitung. Berlin. 1890.

XVIII. Die wissenschaftl. Grundlagen d. Weinbereitung u. Kellerwirtschaft. Berlin. 1905, p. 45.

XIX. Chem.-Ztg. 1898. XXII. 790.

XX. Landw. Jahrbücher. 1894. XXIII. 552.

XXI. Bericht d. Kgl. Lehranstalt, etc., zu Geisenheim pro 1900, 1901. 92.

Wossnesenski,

I. Influence de l'oxygène sous pression augmentée sur la culture du Bacillus anthracis.—C. R. 1884. LXXXXVIII. 314.

Wróblewski, A.,

I. Gärung ohne Hefezellen.—Centralblatt f. Physiologie. 1898. XII. 697.—Ber. d. D. Chem. Ges. 1898. XXXI. 3218.—Ch. C. 1899. I. 500.—K. J. IX. 321.

II. Zusammensetzung des Buchner'schen Hefepresssaftes.—Ber. d. D. Chem. Ges. 1898. XXXI. 3218.—C. f. B. 2. Abt. 1899. V. 161.—K. J. X. 320.

III. Über den Buchner'schen Hefepresssaft. — Journal f. prakt. Chemie. 1901. CLXXII. 1.—C. f. B. 2. Abt. 1900. VI. 59.—Ch. C. 1899. II. 672.—K. J. X. 324.

IV. Centralbl. f. Physiologie. 1899. XIII. 284.

V. J. f. pr. Chem. 1901. 2nd Series. LXIV. 1.

IV. [The reference thus made in Chap. 65 should be VI.] Ber. d. D. Chem. Ges. 1898. XXXI. 1134.

Wulff, Carl,

I. Beiträge zur Kenntnis der Nucleinbasen. — Z. f. physiolog. Chemie. 1893. XVII. 468.—Ch. C. 1893. I. 421.

Wurm, Emanuel,

I. Über Essigbildung mittelst Bakterien.—Dingler's Journal. 1880. CCXXXV. 225.

Wurtz,

I. Liebig's Ann. 1855. XCIII. 107.

Wurtz, R., et Foureur, A.,

I. Note sur un procédé facile de culture des microorganismes anaérobies.—Archives de médecine expérimentale et d'anatomie pathologique. 1889, p. 523.—C. f. B. 1889. VI. 710.

Wurtz, R., et Leudet, R.,

I. Note sur l'identité du bacille lactique de Pasteur avec le Bacillus lactis aërogenes. — Comptes rendus de la société de Biologie. 1893. 531.—K. J. IV. 196.

Wüthrich, E., und Freudenreich, E. von,

I. Über den Einfluss der Fütterung auf den Bakteriengehalt des Kuhkotes.—C. f. B. 2. Abt. 1895. I. 873.—Ch. C. 1896. I. 452.

Wyschnegradsky,

I. Liebig's Ann. 1878. CXC. 350.

Wysmann,

See Wijsmann.

Wyss, O.,

I. Über den Milchschlamm und darin sich findende Mikroorganismen.—Tageblatt der 62 Versammlung Deutscher Naturforscher u. Ärzte. 1889. 501.—C. f. B. 1889. VI. 587.

Wyssokowitsch (auch Wyssokowicz),
I. Einfluss des Ozons auf das Wachstum der Bakterien.—Mitteilungen aus Dr. Bremer's Heilanstalt für Lungenkranke in Görbersdorf. 1890. Wiesbaden. Bergmann.—C. f. B. 1890. VIII. 662.—K. J. I. 45.

Yabe, K.,
I. Über einen vegetabilischen Käse aus Sojabohnen.—Landw. Versuchs - Stationen. 1895. XXXXV. 438.—C. f. B. 2. Abt. 1895. I. 413.—Ch. C. 1895. I. 894.—K. J. VI. 293.
II. Preliminary Note on Saké Yeast.—Bulletin of the Imperial University of Tokyo, College of Agriculture. 1896. II. 219.—K. J. VII. 94.
III. On the Origin of Saké Yeast (Saccharomyces saké).—Bulletin, etc. 1897. III. 221.—C. f. B. 2. Abt. 1898. IV. 554.
IV. On Two New Kinds of Red Yeast. — Bulletin, etc. 1897. III. 233.—C. f. B. 2. Abt. 1898. IV. 555.
V. Quoted in Chem. Centralbl. 1894. II. 1048.
VI. Imp. University of Tokyo, College of Agricult. Bull. No. 3, p. 232.—Abst. in Chem. Centralbl. 1897. II. 818.
See also Kosai und Yabe.

Yoshii, T.,
See Kellner und Yoshii.

Yoshimura, K.,
I. Notiz über das Verhalten von Hippursäure im Boden.—College of Agriculture Bulletin. 1895. II. 221.—Ch. C. 1896. I. 56.

Yung, E.,
See Pictet et Yung.

Zacharias, E.,
I. Beiträge zur Kenntnis des Zellkerns und der Sexualzellen. Bot. Ztg. 1887. XXXXV. 281.
II. Über Chromatophilie.—Ber. d. D. Bot. Ges. 1893. XI. 188.—Gives the antecedent bibliography.

Zahn, F. W.,
I. Untersuchungen im Blute gesunder Tiere.—Virchow's Archiv. LXXXXV. 401.

Zaleski, St. Szcz. von,
I. Chemische Untersuchungen und Unternehmungen in Sibirien. Mitteilungen über Darstellung von Arakà oder Ojrán, berauschendes Getränk aus Milch.—Chem.-Ztg. 1895. XIX. 77.—K. J. VI. 225.

Zalewski, A.,
I. O tworzeniu się zarodnikow w komórkach drożdży.—Rozprawy, etc., Akademii Umiejętności w Krakowie. 1886. XIII. 124. — Botan. Centralblatt. 1886. XXV. 1.

Zander, Enoch,
I. Vergleichende und kritische Untersuchungen zum Verständnisse der Jodreaktion des Chitins. —Dissert. Erlangen.—Pflüger's Archiv. 1897. LXVI. 545.—Ch. C. 1897. I. 1237.

Zangemeister, Wilhelm,
I. Kurze Mitteilungen über Bakterien der blauen Milch.—C. f. B. 1. Abt. 1895. XVIII. 321.—Ch. C. 1896. I. 316.

Zega, A., und Majstorovic, R.,
I. Der Mais als Volksnahrung in Serbien.—Chemiker-Ztg. 1899. XXIII. 544.—Ch. C. 1899. II. 317.

Zeidler, A.,
I. Beiträge zur Kenntnis einiger in Würze und Bier vorkommender Bakterien.—W. f. Br. 1890. VII. 1213.—C. f. B. 1891. IX. 10.—K. J. I. 72.

Zenthöfer,
I. Über das Verhalten von Cholerakulturen in Hühnereiern.—Z. f. Hygiene. 1893. XVI. 362.—C. f. B. 1894. XV. 752.—Ch. C. 1894. I. 872.

Zimmermann,
I. C. f. B. 2. Abt. 1902. VIII. 218.
II. *Ibid.* 1901. VIII. 923.

Zimmermann, Albrecht,
I. Über die Fixierung der Plasmolyse.—Zeitschrift f. wissenschaftl. Mikroskopie. 1892. IX. 181.
II. Die Morphologie und Physiologie des pflanzlichen Zellkernes. Jena. 1896. Fischer. 5*s*.

III. Morphologie und Physiologie der Pflanzenzelle. Leipzig. 1887.

IV. Sammel-Referate aus dem Gesamtgebiete der Zellenlehre. — Botan. Centralblatt. 1893. Beihefte. 206, 420.

ZIMMERMANN, O. E. R.,

I. Über die Organismen, welche die Verderbnis der Eier veranlassen.—VI. Bericht d. naturwiss. Ges. zu Chemnitz. 1878.—Gives the antecedent bibliography.

II. Das Genus Mucor. Chemnitz. 1871.

ZIPPEL,

I. Vergiftungsversuche mit Penicillium glaucum.—Zeitschrift f. Veterinärkunde. 1894. VI. 57. — Baumgarten's Jahresbericht. 1894. X. 461.—Deutsche Zeitschrift f. Tiermedizin, etc. 1894. XX. 448.

ZIRN, Georg,

See WEIGMANN und ZIRN.

ZOEBL, A.,

I. Braunspitzige Gerste.—Allgem. Brauer- und Hopfenzeitung. 1892. No. 106.—Bot. Centralbl. 1892. LII. 344.

ZÖRKENDÖRFER, C.,

I. Über die im Hühnerei vorkommenden Bakterienarten nebst Vorschlägen zu rationellen Verfahren der Eikonservierung.—A. f. Hygiene. 1893. XVI. 369. —C. f. B. 1893. XIV. 141.—K. J. IV. 107.

ZOPF, Wilhelm,

I. Die Spaltpilze. 3rd Ed. Breslau. 1885.

II. Zur Kenntnis der Organismen des amerikanischen Baumwollsaatmehls. I. Bacterium vernicosum.—Beiträge zur Physiologie und Morphologie niederer Organismen. Leipzig. 1892. A. Felix.—C. f. B. 1893. XIII. 276.—K. J. III. 91.

III. Über Bacterium merismopedioides.—Ber. d. Botan. Vereins d. Provinz Brandenburg. 1882.

IV. Über Pilzfarbstoffe.—Bot. Ztg. 1889. XXXXVII. 53.—Ch. C. 1889. I. 291.

V. Über Ausscheidung von Fettfarbstoffen (Lipochromen) seitens gewisser Spaltpilze.—Ber. d. D. Bot. Ges. 1891. IX. 22.—Ch. C. 1891. I. 673.—K. J. II. 84.

VI. Entwicklungsgeschichtliche Untersuchungen über Crenothrix polyspora, die Ursache der Berliner Wasserkalamität. — Berlin. 1879.

VII. Zur Morphologie der Spaltpflanzen. Leipzig. 1882.

VIII. Über die Ursache der Rotfärbung eines neuen Wasserspaltpilzes aus der Familie der Cladotricheen, Sphærotilus roseus.—Beiträge z. Phys. u. Morph. nied. Org. Heft 2. Leipzig. 1892. A. Felix.—C. f. B. 1893. XIII. 234.—K. J. III. 84.

IX. Zur Kenntnis des regressiven Entwicklungsganges der Beggiatoen nebst einer Kritik der Winogradsky'schen Auffassung betreffs der Morphologie der roten Schwefelbakterien. — Beiträge z. Phys. u. Morph. nied. Org. Heft 5. 37. Leipzig. 1895.—K. J. VI. 29.

X. Die Pilze in morphologischer, biologischer und systematischer Beziehung. Breslau. 1890.—Z. g. Br. 1890. XIII. 399.

XI. Die Conidienfrüchte von Fumago.—Nova acta der Ksl. Leop.-Carol. Deutschen Akad. d. Naturf. 1878. XXXX. No. 7, p. 20.

XII. Kritische Bemerkungen zu Brefeld's Pilzsystem.—Beiträge z. Phys. u. Morph. nied. Org. Heft 3. Leipzig. 1893.—C. f. B. 1. Abt. 1893. XIV. 453.

XIII. Oxalsäuregärung (an Stelle von Alkoholgärung) bei einem typischen (endosporen) Saccharomyceten, S. Hansenii n. sp.—Ber. d. D. Bot. Ges. 1889. VII. 94.—C. f. B. 1. Abt. 1889. V. 796.

XIV. Ber. d. D. Chem. Ges. 1889. VII. 95.—Ber. d. D. Bot. Ges. 1900. XVIII. 32.

XV. Ber. d. D. Chem. Ges. 1889. VII. 94.

See also LIESENBERG und ZOPF.

Zschokke, A.,
I. Über den Bau der Haut und die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte.—Landw. Jahrbuch der Schweiz. 1897. XI. 153.—V. Jahresber. d. Versuchs-Station zu Wädensweil. 1894–95. S. 56.—C. f. B. 2. Abt. 1898. IV. 839.

Zukal, H.,
I. Öster. Botan. Zeitschr. 1893. No. 5.

II. Sitz. d. K. Akad. Wiss. Wien. Math.-naturw. Kl. 1. Abt. 1887. XCVI. Nov. Heft.

III. *Ibid.* 1889. 1. Abt. XCVIII. 561.

INDEX.

An asterisk affixed to a page number indicates that a figure occurs there.