

5
PRACTICAL MANAGEMENT
OF PURE YEAST

THE APPLICATION AND EXAMINATION
OF BREWERY, DISTILLERY, AND
WINE YEASTS

BY

ALFRED JÖRGENSEN

DIRECTOR OF THE LABORATORY OF PHYSIOLOGY AND TECHNOLOGY OF
FERMENTATIONS, COPENHAGEN

TRANSLATED BY
R. GREY

Second Edition

REVISED AND GREATLY EXTENDED BY THE AUTHOR

129432
3 | 10 | 13
Brewing Trade Review

13 LITTLE TRINITY LANE, LONDON, E.C.

1913

CONTENTS

	PAGE
Preface to Second Edition	II
Preface to First Edition	13-15
Introduction	17-18
 I.—THE ANALYSIS	 19-72
General remarks	21
Rapid analysis for purposes of control	21
Analysis required in particular cases	22
Proper method of taking samples	22-23
 I. ANALYSIS OF BREWERY YEAST	 23-39
(a) The ordinary microscopical examination of yeast, bacteria, moulds and the deposits in beer	23-25
(b) Rapid analysis for controlling the growth of wild yeasts—Bottom fermentation—How and when the samples should be taken—Cultures on moist gypsum blocks and their examination—Top fermentations—Taking samples—Plate cultures, to find acid-producing bacteria in top yeast— Measures to be taken when the yeast is found to be very much infected	25-32
(c) Analysis of the growth of bottom and top yeasts at rather advanced stages of the fermentation. Old growths—General growth of young yeast without separation of the cells—Partial separation of	

Analysis of Brewery Yeast—*continued*.

the cells, before the young growth has been
produced 32-37

(d) Detailed analysis required in particular cases, to
determine the character of the types of yeast
present in the mixture 37-39

II. ANALYSIS OF THE YEAST IN DISTILLERIES AND YEAST FACORIES 39-48

The microscopical examination 39-40

Examination of wild yeast 40-41

Examination of acetic acid bacteria, Mycoderma and
moulds 41-43

Lactic acid bacteria at the different stages of the
acidifying process and in the mash 43-45

How to take samples in the distillery 45-47

How to take samples in the air-yeast factory 47-48

III. ANALYSIS OF WINE YEAST 48-52

Examination for bacteria 49

Examination to ascertain if the yeast is growing or is in
a state of rest 49-50

Mycoderma 50

Analysis in reference to the predominating type 51-52

IV. WATER ANALYSIS 52-61

General remarks—Types that readily gain a footing,
live in water, and can thus be introduced into the
brewery or distillery, &c. 52-53

How to take samples from the tanks and wells 53-54

How to take samples from water-pipes 54-55

Water analysis, Principles and method of 55

CONTENTS

7

PAGE

Acetic acid bacteria	55-56
Mycoderma	56
Yeast (wild yeast and culture yeast), &c.	56-59
Observations in regard to the length of time taken by the different types to develop in the flasks	59
Putrefactive bacteria	59-60
The resistance that the selected race of yeast exercises towards organisms found in water	60-61

V. AIR ANALYSIS 61-66

Only a small number of the germs found in the air are able to develop during fermentation ; circumstances such as want of cleanliness and freedom from disturb- ance must be in their favour—An examination of the method of working will often disclose the real source of infection long before it can be found out by an analysis of the air	61-62
Examination of the air over the coolers	63
General method of air analysis	63-65
How to forward germs to the laboratory and the ex- amination of the contents of the flasks	65-66

VI. NUTRITIVE LIQUIDS AND GELATINE 66-72

Hopped brewery wort and its manipulation in the different flasks	67-69
Distillery wash and wort from yeast factories	69-70
Wine must and other liquids specially made up	70
Yeast water—Yeast water and dextrose—Cane-sugar solution—Nutritive gelatine—Agar-agar	71-72

II.—THE PURE CULTURE OF ALCOHOL- YEASTS

73-128

The pure cultures preferred to commercial yeast—By proper selection between the types or races it is always possible to find types that will alone carry through the primary and after fermentations 73-78

How to take the samples for the development of pure cultures 78-79

I. THE PREPARATION OF A PURE CULTURE 79-91

The employment of yeast as it is or after preparation 79-80

The moist chamber 80-81

The marking off of cells in the gelatine on the ruled cover slip 81-82

Inoculation of the flasks with colonies 82

Development of cultures in Pasteur flasks 82

Observation of fermentations in these flasks 82

Microscopical examination and spore cultures of the young growths 83

The growth of yeast in Pasteur flasks 83

Comparative fermentations in cylinder glasses 84-86

Development of the selected race in Pasteur flasks and Carlsberg flasks 86-90

The collection and transference of a sample to a travelling can 90-91

Small cultures that can be sent through the post on cotton-wool 91

II. EMPLOYMENT OF PURE CULTURES IN PRACTICE 91-118

(a) The development of the culture in a small tun 91-102

The so-called acclimatisation of pure cultures 91-92

CONTENTS

9

PAGE

Brewery bottom yeasts—The appearance of the fermentations in the first small tuns are always different from the normal. Care must be exercised if the difference is very marked 92-93

Points to which attention must be paid during the development of the culture in the small tuns 93-98

Brewery top-fermentation yeast 98

Distillery yeast 98-99

Yeast in the air-grown yeast factories 99-100

Wine yeast 100-101

Yeast for fermenting the juice of fruits and berries 101-102

(b) Development of a yeast culture in a propagating machine 102-118

Directions how to use the large model 103-110

Directions how to use the small model 110-111

General remarks about the fermentations in these cylinders 112-115

Employment of the cylinders in distilleries and yeast factories 116

Withdrawal of samples for analysis from the cylinders 116-117

Analysis of the samples withdrawn 117-118

II. DEGENERATION OF THE YEAST 118-124

A systematic selection between the yeasts is only possible when one single type is employed for the work 118-119

There are a large number of yeast types or races that are of practical use 119

There are definite limits to the possibility of a particular type bringing all its qualities to the front in practice 119-120

If work is carried on under ordinary conditions of daily work the selected race of pure yeast will be found to work uniformly as regards the most essential features 120-121

Degeneration of the Yeast—*continued*.

On the other hand, the continued influence of a particular kind may bring about a great change in the character of the race of yeast employed	121
Impure and degenerated yeast	122
If a type degenerates, all cells have not changed their character	122
The yeast may be regenerated by a selection between the cells	123
Improvement of a pure culture yeast by selection	123-124

IV. PRESERVATION OF YEAST

124-128

Many yeast types may be preserved for a considerable length of time without those particular properties that are valuable undergoing any change	124
The process of preservation	124-125
Raising a young growth from the preserved culture	125-127
Preservation of yeast in practice	127-128

PREFACE TO SECOND EDITION

THE present (second) edition is a re-modelling of the first edition, due regard being paid throughout to the advancement of the science since that time, especially in its *technical* aspect.

I venture to hope that the volume will be found able to serve as a guide for Brewers, Distillers, Yeast-makers, Manufacturers of Wine, Cider, &c., as also for analysts.

I need not add that I shall be pleased to give in a course of instruction, or by correspondence, all such particulars as could not be accommodated in this compendium, the object of which is to present an easy view of the subject-matter, which would be impossible if it were clogged with too many details.

ALFRED JÖRGENSEN.

COPENHAGEN, *June 1913.*



PREFACE TO FIRST EDITION

THE accompanying small book, making its appearance to supply a real want, is purely practical in its aims.

In regard to the constant control of the fermentations in the different branches of the industry, practical men have expressed a wish for a simple, comprehensive account of the way in which *samples*, sent to this Laboratory by men engaged in the fermentation industry, *are analysed*; also for a short, concise account of the leading features of *the treatment of pure culture yeast*; and for a short, complete review of the knowledge that has been acquired in this sphere of work by the constant co-operation of the Laboratory with the men who carry on the industry.

It is well known that it is easier *to put the problems before the analyst in proper form* when the limitations of analysis are known. Likewise it is easier for the practical man *to draw correct conclusions* from the information that is supplied to him, when he is acquainted with the principal features of the working methods of the Laboratory. A correct understanding of the principles on which analytical work is based is absolutely

essential for *taking samples in the proper manner* in the brewery or distillery, &c., as the case may be.

Analysts have also desired a short description, of a practical nature, of the outlines of *the elementary methods of analysis of alcohol-yeasts and of their pure culture*, and of the methods used here in the Analytical Laboratory and taught in the author's Laboratory for students.

It is evident that this short account cannot be regarded by analysts as a collection of receipts enabling them to carry through what work there may be to do. In order to employ these methods of analysis freely, and also to understand the principles on which they are based, it is necessary to have had practice in them under personal supervision, and to have studied a series of types of the Organisms of Fermentation.

This small hand-book, also, cannot do more than deal in a purely elementary manner with the most frequently occurring problems. The solution of the many special problems that arise demands at the outset more advanced study and greater experience.

The accompanying treatise will, then, be useful in making it easier for the practical man and the analyst to work together, as it reviews the most important methods of work, both practical and theoretical, employed in the control of yeast, each originating from a different basis.

This small book is, in a certain sense, a supplement

to my book "Micro-organisms and Fermentation" (C. Griffin and Co., London). The latter book aims at affording a theoretical, historical, and critical account of the subject, accompanied by a description of a series of types of Fermentation Organisms. The practical side of the problem, therefore, both as regards the methods of analysis and pure cultivation, could only be dealt with as regards its chief features.

Theoretical principles on which the methods are based, references to the literature of the subject, names of authors, &c., must be sought for in the above-named larger work.

(Signed) ALFRED JÖRGENSEN.

THE LABORATORY OF PHYSIOLOGY AND
TECHNOLOGY OF FERMENTATIONS,
COPENHAGEN, 1901.

INTRODUCTION

WHILST carrying on fermentations in practice, one tries to discover the particular types of fermentative organisms that answer the necessary requirements under given conditions; and one attempts to find out the conditions that are necessary for the free and unhindered production of those properties that are of value, and one also naturally endeavours to guard against the competition of wild yeasts and bacteria.

It is clearly evident that such proceedings *occupy some time before the results are clearly seen*. The micro-biological work that will engage our attention in the following pages concerns *the characteristic features* of the life-history of microscopical organisms. Life expresses itself in divers ways. The properties of organisms are manifold, and their condition of vigour changes unceasingly. The composition of the nutritive liquid (wort, mash, must, &c.) plays an important part. But in analytical work, as well as when making practical use of the results of such analysis, one must confine oneself to attending to *one* particular phase of this capability of exhibiting manifold properties; the particular piece of work must always exhibit one view only. Consequently, a definite series of such pieces of work must often

be carried out, each starting from a different standpoint, before one can possibly form any impartial opinion about the task before one. That one rapid experiment may be followed by a lucky result is the exception ; and it will not always reveal the real reason of the success met with in practice ; only when the real reason is discovered can one secure oneself from relapse. Only by *steady, systematic study of the conditions prevailing in practice* is it possible to understand the fluctuations, unavoidable in any Fermentation Industry, where living organisms have to be used.

I
THE ANALYSIS



I

THE ANALYSIS

THE examinations of the organisms of fermentation, undertaken in the present day in connexion with the different fermentation industries, are of two kinds—a distinction being made between, on the one hand, an analysis briefly carried out in order that information may be rapidly obtained as to the actual state of fermentations, and, on the other hand, a searching analysis carried out with the object of obtaining in detail definite information about the nature of the micro-organisms found to be present, and the special rôle which they play in practice.

When carrying out *rapid analysis* for purposes of general control, the question naturally enough suggests itself whether and to how large an extent a growth of foreign undesired organisms is present. An accurate analysis of this kind is usually sufficient for the purpose, as in the present day it is not at all a difficult matter to procure a reliable pure culture of a selected type of yeast. Such an analysis must be, then, carried out very quickly, and at short intervals, in order to be in a position to grapple with any irregularity as quickly as possible after its appearance. Experience has shown that a continued control of this nature is most essential, and it plays now a very

important part in fermentation industries properly carried on.

A *detailed analysis* would be carried out when certain definite problems require solution. For example, when more detailed information is required regarding the germs of different organisms found in the water or in the air ; again, to ascertain if at a particular stage of the fermentation a particular type of foreign organisms has obtained mastery, so that it is in practice important to obtain definite information about the character of the race of micro-organism concerned, and whence it came ; or when "sickness" appears, and it is desired to trace out the evil to one of several possible causes, amongst others that a micro-organism may have crept in about which the rapid general analysis gave no special information ; or again, perhaps it is desired to isolate *the different types of culture yeast* contained in the yeast mass in order to select a type of yeast from among them. Such investigations can be carried out with accuracy by experienced zymotechnologists, but require longer time than a general brief survey.

It is of the utmost importance to these different biological analyses that sufficiently large samples should be taken in the proper manner from fermenting vessels, casks, &c., to ensure an average sample of the organisms being obtained for examination. Later on, each single question that arises will be closely inquired into. It is now only necessary to note that for the biological analyses average samples of the liquid itself are not required. We must consider *in which portion of the liquid the living organisms are to be found* that

are the object of our research. The distribution is not always the same. To take as a simple example the bottom fermentation tun, where fermentation proceeds slowly and evenly. We must remember that during the whole course of the fermentation secretions are brought up to the surface of the liquid, and often, as an examination will show, peculiar growths are found amongst these secretions—for example, film-forming types are found. When one is not careful to first remove such film growths, and then afterwards immerse the sample glass sufficiently deep in the liquid, one obtains very incorrect information as to how the yeast *generally grows in the tun*.

The liquid itself may be subjected to *micro-chemical* analysis in order to obtain information about the secretions that are present, and often very valuable information can be obtained from such an examination under the microscope—for example, information whether a wort does or does not contain unconverted starch.

I. ANALYSIS OF BREWERY YEAST

(a) A SIMPLE MICROSCOPICAL EXAMINATION

The information obtained by a simple microscopical examination of the yeast, whether an examination is made of the yeast suspended in the fermenting liquid or of the thick mass of yeast thrown up to the surface or down to the bottom of the tun, is for the most part only about the foreign bodies mixed with the mass of the yeast, namely, the bacteria, mould spores, and secretions thrown out of suspension from the liquid.

As to the yeast itself, the following conclusions may be formed by this examination: namely, whether the majority of the cells *have the same appearance*, that is to say, if they show uniformly clear plasma, or whether vacuoles and small granulations have made their appearance, or whether the plasma is much granulated and the vacuoles have become irregular in size and shape; or, again, whether the preparation under the microscope shows *irregular growth*—in that the above-named different growths of yeast cells are detected mixed in the yeast mass. Further, the cells may show differences as regards size, shape, and refractive powers.

In by far the greater number of cases the cells of a normal growth of a yeast type will have the same appearance in regard to the above-mentioned characteristic features.

Inexperienced workers will often have a difficulty in distinguishing between bacteria and secretions (albuminoid matter, hop resin, &c.). Beer contains, for example, secretions that can be confusingly like cocci forms of bacteria. Solvents (*e.g.* dilute soda solution, or acetic acid, or a mixture made up of ether and alcohol) are generally employed for the preparation of the microscopic slides in order to distinguish between secretions and bacteria. Such an examination must be undertaken with great care (strong solutions must never be used), and it must be clearly understood that an absolutely certain test of this nature is impossible. Sometimes, under certain conditions, the secretions are able to resist the action of the mediums employed; at other times the bacteria

may be rendered so transparent that it is impossible to distinguish them. The best plan is to accustom the eye by examining and comparing several freshly prepared preparations, the contents of which are known, to work with accuracy in detecting the difference in the *refractive power and colour* of the matter in suspension, there being almost always a noticeable difference in these respects between secretions of albuminoid matter, &c., and bacteria.

The *very important question* whether the yeast mass contains "wild" yeasts or not cannot be decided by a *simple microscopical examination*, but recourse must be had to *spore cultures*.

(b) ANALYSES FOR THE RAPID DETECTION OF WILD YEASTS

Spore Cultures, using blocks of gypsum for growing them on, are of the greatest value, as it is thus possible to make an average estimate of the value of the sample by examining a large number of cells in a short time. This is accounted for by the fact that the difference between the spores of "culture" yeasts and wild yeasts is of so distinct a character that one quickly notices it on placing the slide under the microscope.

Such an analysis only has its real value when the sample for examination is taken in the right manner and at the right time. It is not advisable to take a sample from the thick liquid yeast at the bottom of the tun, because the wild yeast cells found there are often either not able to form spores, or only form them late and to a slight extent.

It is, therefore, necessary to examine *the yeast cells*

suspended in the beer during fermentation. In the *advanced stages of primary fermentation* the wild yeasts produce young cells, capable of rapidly forming spores.

A sample of the yeast suspended in the beer will, on analysis (made in the manner about to be described), give a clear indication as to the composition of the yeast used in the fermentation, and how the yeast which is settled out will develop, when it in its turn comes to be used for carrying on fermentation.

In the case of bottom fermentations the samples for analysis should be withdrawn *a short time before racking.* Small bottles fitted with corks are used for this purpose. The bottles are packed before use in white paper, and sterilised by being subjected to a heat of 150° C. (302° F.) for a couple of hours. The corks are sterilised by being exposed to the action of formalin vapour in a closed flask. The man taking the sample takes the bottle out of the paper (his hands should be quite clean) just before using it, blows the head a little off the beer, plunges the bottle below the surface, opens it, and lets it fill itself, and then puts the cork in again before bringing it to the surface of the liquid, and taking it out. The cork is then tightly fastened down with a piece of string, the bottle washed, and a label put on, with the number of the tun, gyle, and generation of the yeast written on it. For the sake of greater accuracy it is advisable to take samples in duplicate. Such samples may be allowed to stand several days, or be sent some distance away for examination, without deterioration.

When the samples are to be examined they should

be stood on one side for a few hours to settle. The greater part of the clear liquid is then carefully poured away, the sediment shaken up, and a few drops of it poured on to a block of gypsum.

The rest of the sediment is used for a microscopical examination, as already described. As it is sometimes very difficult for inexperienced observers to detect only a slight sarcina infection, it is advisable to take two samples at the same time from the tun and, without pouring away the liquid from the second sample or



FIG. 1. Glass dish, with gypsum block.

loosening the cork, to stand it on one side for a few days ; or to add neutralised yeast water to the sediment of the sample. As a general rule, these bacteria will under such treatment increase in number and be the more easily found.

The cultures on blocks of gypsum (Fig. 1) (plaster of Paris) are set up as follows : A block of gypsum, in shape like a cone with the top cut off, is placed in a round shallow glass dish, fitted with a loose lid. The glass containing the block of gypsum is packed in paper and subjected to a heat of 110° C. (230° F.) for $1\frac{1}{2}$ hours to ensure sterilisation.

When the liquid yeast is to be poured from the bottle on to the block, the outside of the bottle is first carefully cleaned, and then the gas flame of a Bunsen burner passed carefully over it ; next the glass cover is raised partially, and the mouth of the opened bottle

is just put under the cover and a few drops of the liquid yeast poured on to different parts of the surface of the block of gypsum. Then sterilised water is poured round between the block and the glass until the level of the water has risen, say, half way up the block.

The cultures are now placed on one side at a constant temperature of 25° C. (77° F.), or sometimes 15° C. (60° F.), and the yeast cells will multiply, partly by budding and partly by building up spores internally.

When subjected to a temperature of 25° C. (77° F.), the wild yeast will have formed spores after forty hours; the culture yeasts that require a longer period than forty hours to develop their spores can thus, therefore, be distinguished from the wild yeasts. Other types of culture yeasts, that develop their spores as rapidly at 25° C. (77° F.) as the wild yeasts, must be examined after being subjected to a temperature of 15° C. (60° F.); at such a temperature the wild yeasts will have developed spores after three days, whereas culture yeasts will not have done so till the fourth day or even later.

A good method of distinguishing between the two groups of yeast is the *construction of the spores* (Fig. 2). A brewery bottom-fermentation yeast develops large spores; their contents are not uniform, as granulations or vacuoles may be present; they are all surrounded by a distinct thin wall. The wild yeasts produce small spores, which under normal conditions of development (if the sample was taken in the right manner) show the contents of the spores to be clear and uniform, whilst the wall is indistinct. An examination, having

only this object in view, can quite well be carried out after *developing spore cultures under conditions of ordinary room temperature.*

In the examination of a single slide only, prepared for the microscope, thousands of cells present themselves

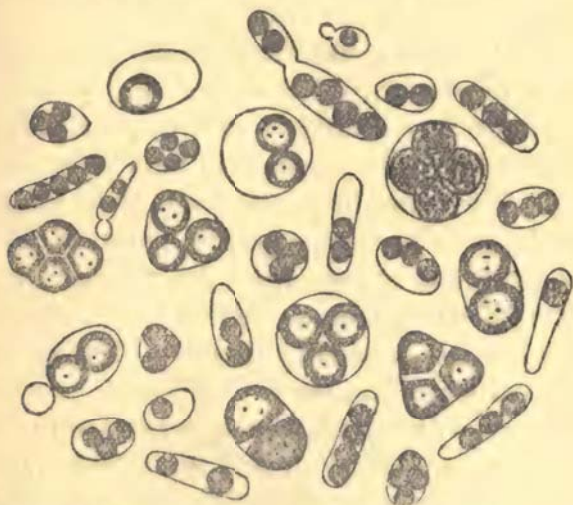


FIG. 2. Cells of brewers' yeast and of wild yeasts containing spores.

to view. An accurate calculation as to the *extent* of the infection, by counting the cells, is plainly impossible. Such an attempt is also quite unnecessary in practice. All that is required is *definite information* as to whether the infection is serious or not.

When analysing *brewery top-fermentation yeast*, a microscopical examination of the yeast mass, or of the beer itself, is carried out for the detection of bacteria, &c., or it is tested for wild yeast infection.

No general rule can be laid down as to *when* the samples should be taken from the fermenting

rounds in order to ascertain the amount of wild yeast infection, as the top fermentations are carried on in such different ways in different countries. The analyst must, after he has become thoroughly familiarised with the method of fermentation practised in the brewery concerned, withdraw several samples from near the surface of the fermenting wort, or from the heads themselves thrown up during the different stages of the fermentation; and by comparing his results ascertain which sample is best suited for this analysis. Here also, however, the following rule may be laid down—the best stage, in point of time, is reached when the change from the vigorous primary fermentation to the quiet after-fermentation takes place: this stage does not, however, always coincide with the racking of the beer.

Analyses for detection of wild yeast are conducted according to the method already described. In some cases it is difficult to distinguish between the two types of spores. When it is required to distinguish the spores by noting the length of time required for the spores to form, it is often necessary to develop the gypsum block cultures at lower temperatures—for example, 10° – 12° C. (50° – 53° F.)—because some types of top yeast develop spores as rapidly as the wild yeasts if higher temperatures are employed.

Since top fermentations are conducted at higher temperatures, acetic acid bacteria and lactic acid bacteria can develop and render the yeast mass impure. It is, therefore, of the greatest importance to *examine top-fermentation yeast for bacterial infection.*

It is not possible to distinguish with the microscope

between the different types of acid-producing bacteria that are found in the yeast, or to distinguish them from other species of bacteria. The acetic acid bacteria do not, as a rule, whilst fermentation is proceeding, become united together in the well-known chain form, and only a few cells exhibit distinct irregular swollen bodies.

If information is required about the proportionate amount of lactic acid bacteria present, recourse must be had to plate cultures, using as a medium a solution of dextrose or sweet wort, and a little precipitated calcium carbonate (CaCO_3) added, mixed with 10 per cent. of gelatine. The solution of dextrose and gelatine is melted at a temperature of about 28°C . (82°F .), and a little of the yeast—an average sample—is mixed carefully in with it when melted. The melted solution is then poured out over a Petri dish. After exposure for some days at a favourable temperature (~~beyond~~ *below* 25°C . — 77°F .—because the gelatine will be melted at higher temperature), the colonies of lactic acid bacteria will have developed sufficiently for recognition. They are distinguished from any colonies of other species of bacteria by being surrounded by a transparent ring. This transparent ring is the result of the particles of chalk, close to the colonies, having been dissolved by the acid secreted by the bacteria. A microscopical examination of some of the other colonies will reveal the presence of acetic acid bacteria, because they will now be found in the typical “chains.” Some colonies of acetic acid bacteria also have a transparent ring, or have a bluish opalescent surface.

When the analysis of the yeast of bottom fermenta-

tion or top fermentation shows that the infection in the tun is really serious, it is well to remember that to throw away the yeast and to introduce pure yeast is only a half-measure; the first thing to be done is to *thoroughly disinfect the tun*. As a rule, a thorough cleaning of such a tun can be effected with bisulphite of lime, which must not be cleaned off when the tun is refilled, but be left on. If the tun shows distinct faults, such as cracks, small depressions, or a rough inside surface, a stronger and more penetrative disinfectant must be employed, such as a solution of salicylic acid (5 to 10 per cent.) in alcohol, and it must be carefully painted on over all the hollows or depressions on the surface of the tun. The tun is then allowed to stand unused for a few days, and the crystals are then washed off with a solution of soda. As the most efficient disinfectant it is recommendable to use a dilution of fluor-ammonium (3-5 per cent.) or of formalin (1.5 per cent. = about 0.5 per cent. of formaldehyde); but the vessel must then be carefully washed with water.

If the contamination is very bad, the tun must then of course be thoroughly cleaned by mechanical means. Such an undertaking is carried out far too seldom in many of the fermentation industries.

(c) ANALYSIS OF THE GROWTH OF BOTTOM-FERMENTATION AND TOP-FERMENTATION YEAST AT RATHER ADVANCED STAGES OF THE FERMENTATION. OLD GROWTHS

Advanced growths of yeast are found, for example, in the sediment developed in the beer left standing in sampling bottles; in the deposit in lager casks;

in the pitching yeast left undisturbed for a short or long time, as the case may be; in yeast pressed for exportation; in samples of yeast sent in filter-papers for examination from breweries, distilleries, &c., at a distance from the laboratories.

A microscopical examination of such samples will often reveal the presence of bacteria and moulds. Both in cask sediment and in thick-fluid yeast, and in pressed yeast, a large amount of fine secreted matter is found present. The analyst must make a most careful examination in order to distinguish the bacteria from these secreted matters.

Under these conditions the yeast will consist either of only exhausted cells, or a mixture of old and new cells.

The analytical examination to be made depends upon the information required.

If some of the cells are *budding*, and it is thought desirable to have some information as to *the nature of these cells*, whether they are culture or wild yeast cells, it is only necessary to develop ordinary spore cultures by transferring some of the yeast at once to blocks of gypsum.

If, on the other hand, information is desired about the *different types of yeast present in the yeast mass generally*—for example, the proportion of culture and wild yeasts present, if one or more races of culture yeast are present, and so on—it is necessary to obtain a development of the whole growth by encouraging the budding of the exhausted cells. The quickest way is to introduce an average sample of the yeast into a Pasteur flask containing sterilised aerated wort,

and let it grow there. It would be of advantage if the development of the yeast cells of the different types took place to an equal extent, so that spore cultures on blocks of gypsum would enable conclusions of a sufficiently practical nature to be formed regarding the ratio of culture yeast to wild yeast in the original yeast mass.

Such information is not, however, reliable. As development takes place in the flasks, the different types may again enter into competition, and the weaker cells in the mixture will be suppressed by the stronger ; also the conditions governing the development of the cells in the flask will be more favourable to some types and less favourable to others than the conditions governing the growth of the yeast mass in the brewery or distillery from which it came.

If the yeast mass is treated in the manner just described, before spore cultures are set up, information is then only obtainable as to the type or types of yeast that will develop most vigorously in the flasks. If, for example, a development of wild yeast is noticed in a sample of yeast taken from deposit in cask, this development will usually continue to a very marked extent in the flask, if the growth of the yeast is allowed to continue to the fullest extent.

Definite information about the *proportion* in which the different types were present in the original sample is consequently only obtainable when it is possible to exclude competition between the types during the development of the vegetation.

If what is wanted is simply a *general approximate estimate* for practical purposes of *the quantities of the*

divers yeasts, the materials must be prepared for such research-work by sowing a comparatively weak dilution of the yeast in wort in a series of Freudenreich flasks (Fig. 3, ii).

For this purpose a drop of the thick-fluid yeast is placed in a Freudenreich flask containing about 7-10 c.c.



FIG. 3. Flasks in which yeast cultures (i) can be sent through the post, or (ii) in which cultures may be developed or (iii) preserved.

of sterilised water, and an estimate made of the number of cells contained in a little drop of the dilute solution. This is determined with the assistance of an ordinary cover slip, with small squares ruled out on the middle of it—twenty-five in number. An average sample of the solution is laid on the cover slip with the aid of a platinum loop, the slip is fastened to a glass ring (moist chamber), and the cells are then easily counted under the microscope, the fine lines being of assistance in the process. The solution is now diluted by adding more sterilised water, until a drop, spread on the

cover slip with the aid of the platinum loop, contains from 10 to 20 cells. Each of the 20 to 40 Freudenreich flasks is next infected with the aid of the platinum loop with a drop of the solution, and the flasks should afterwards be thoroughly shaken for some time, in order to make as sure as possible that the cells are well separated, and that each cell will sink independently to the bottom of the flask. The flasks are then allowed to stand at room temperature, and each of the independent cells lying on the bottom of the flask will develop a colony, appearing like a fleck against the glass. If the flasks are allowed to stand for some few days undisturbed, it will be noticed that each small colony will grow, and remain isolated, so to speak, from other similar small colonies in the flask; the exhausted cells have, therefore, time enough and sufficient space to produce young cells, instead of being suppressed through being in contact with those that are more vigorous; and are thus, in accord with our wishes, able to produce spores if spore cultures are carried out. The flask is thus representative of the ideal: each individual has free scope to grow and develop, and to assert itself without let or hindrance—a noble order of society, where free space is so abundant!

When the flask cultures are from four to five days old, having been kept at room temperature, the development of colonies will be sufficiently advanced. The growth in each flask may then be employed separately for spore cultures, or the contents of several flasks can be mixed up together. If the work up to this point has been carried out with good average samples, the final results of the research will

represent for us the general proportions in which the different types of yeast are present under practical conditions, and reliable conclusions can be drawn with regard to the conditions prevailing in practice.

To ascertain the different species of bacteria to be met with in such old cultures of yeast, the research must be conducted with cultures on gelatine, using as the cultivating medium one specially favourable to bacterial growth.

(d) DETAILED ANALYSIS

If information is required about the *characteristics* of the different *yeast types* found in mixed yeast—for example, about their behaviour in the liquid used in practice, or the temperatures most favourable to their development, and so on—the separation of the cells must be carried out so thoroughly that it is possible to work with vegetations grown from a single cell. The dilution process is carried out to such a degree that only one cell is found in every two or three drops; and these drops are then sown—one drop into each flask—in a large number of flasks containing a suitable medium for the development of the yeast cells. Some of the flasks will develop growths descending from a single cell. The flasks containing yeast colonies can be recognised by the small yeast “spots” settled on the bottom of the flasks, as each cell develops a small well-defined colony.

The methods of analysis described in the last paragraph (weak dilution) will give some idea of the growth of the yeast on a larger scale; the above-described (strong dilution), going more into detail, will

bring out further points of difference. A selection is made of all the flasks, and absolutely pure cultures grown, in moist chambers, from these selected colonies (see paragraph describing Pure Culture). The different cultures are compared botanically and biologically, one with the other (see description of species in the author's "Micro-organisms and Fermentation," 4th edition, published by C. Griffin and Co., London). An important test for the comparison of different yeast types is the film which yeasts spread little by little over the surface of the wort. A microscopical examination can reveal distinct points of difference in the different types in this respect; some develop a distinct branching mycelium, throwing out conidia.

Sometimes yeast cells that will not produce spores can be separated from such old growths, although from the appearance of the contents of the cells one would suppose that one was examining a yeast type that could produce spores. Sometimes, not always, the culture can be forced to develop spores by growing it for several generations in dextrose yeast water. That such old cultures lose the power of producing spores has been proved in the laboratory in the case of pure cultures kept for years. By repeated cultivation in sterile wort, not a few types of yeast have shown a marked falling off in their power of developing spores, and in some cases the power has been lost entirely. In such cases it is not always possible to restore this power, either by cultivation in dextrose solution or in any other way.

When a number of really different types or races of yeast have been secured by the methods just described,

sufficient material has been obtained to consider the problem before us—the importance of these different types for fermentations in practice.

II. ANALYSIS OF THE YEAST IN DISTILLERIES AND YEAST FACTORIES

This analysis is in certain respects not quite so simple, and greater difficulties have to be encountered than when brewery yeast is the subject of analysis, as the process of manufacture is more complicated. This is accounted for by the fact that because the mash and the wort are not boiled, many of the germs present, particularly bacteria, are not killed, but only somewhat crippled; it is, therefore, necessary to be prepared with different means at one's disposal to suppress the growth of these foreign germs, both during mashing and preparation of wort in air-yeast manufactories and also during fermentation. There is, therefore, much to be done in this respect, and a properly conducted microbiological control is of the greatest value.

Temperature plays a very important part in regard to the amount of infection. If sufficient care is not taken to secure certain fixed temperatures throughout the mash or wort, foreign germs can easily gain the upper hand during fermentation.

A microscopical examination of the yeast taken from these rapid vigorous fermentations will be able to give useful conclusions. It is necessary that the yeast, whether it is the yeast grown in the acid wort and to be used for pitching, or the yeast skimmed from the tuns for the yeast press, or the yeast produced in the

air-grown yeast factories, should be uniform and well grown ; that is to say, the yeast must not be a mixture of full-grown ripe cells and cells that have produced only small buds, or contain a high proportion of granulated and exhausted cells.

Whether or not the pitching yeast grown in the acid wash is able vigorously to ferment the wash or wort in the large tun depends partly on the type of yeast selected, and partly on the concentration of the mash or wort and the degree of acidity ; further, upon the pitching temperature, that is, the scale of temperatures at which the fermentation of the acid mash takes place, and also upon the final degree of attenuation. Experiments with the selected race of yeast may give useful indications as to the point to which fermentation in acid mash is to be carried. Valuable information can also be obtained from the phenomena shown by fermentation in principal mash ; further, from the way in which fermentation sets in, or also from the degree of warming and attenuation.

The microscopical appearance of the pressed yeast, which is often used in many factories or distilleries as a pitching yeast, is a useful guide. In a normal yeast of this description the cells contain vacuoles comparatively large, and in the plasma single fine granulations. The presence of cells much granulated, and containing irregular or numerous small vacuoles, is, as a rule, an indication of the yeast being weak.

When a single race of pure selected yeast is employed, wild yeasts will develop only with difficulty during these short vigorous fermentations. If it should be necessary to ascertain if wild yeasts are present, the

same method of analysis is employed as already described with regard to brewery yeasts—namely, to take a sample at the close of the fermentation and make a spore culture on a block of plaster of Paris. With many types of distillery yeast the structure of the spores is not at all, or only very slightly, different from that of wild yeast spores; other tests must therefore be employed—for example, the length of time required for the development of the spores. For this purpose, it is necessary first to ascertain the length of time required for the development of spores of the type of yeast selected for use in practice. The information is obtained by experimenting under absolutely sterile conditions as follows: A few drops of the pure yeast from young flask cultures are placed on several plaster-of-Paris blocks, and cultures obtained at varying temperatures—for example: 10° – 12° C. (50° – 54° F.), 15° C. (60° F.), 25° C. (77° F.), and even 30° C. (86° F.). A comparison with the tables for sporulation of *Ellipsoideus* and *Pastorianus* yeasts, published in "Micro-organisms and Fermentation," will be a preliminary guide in selecting the temperature at which to compare the sporulation of the selected race of yeast and the wild yeast. A temperature should be selected at which the wild yeasts can form spores earlier than the selected culture yeast. As has been already stated, this analysis is only of subordinate value.

Experience has shown that the most dangerous enemies of this branch of the fermentation industries are the *acetic acid bacteria* and *different species of Mycoderma*. As regards *pressed yeast*, moulds are dangerous, as are also *Mycoderma*, putrefactive

bacteria, and wild lactic acid bacteria, which tend to give the yeast a flocky consistence. It is not possible to control the extent of the infection by a simple microscopical examination.

The simplest means for controlling the organisms that easily grow on the surface of the wort, &c., is to prepare plate cultures of wort gelatine. This is done by mixing with the wort gelatine when melted a few drops of the sample, thoroughly well stirred in to ensure good distribution, and then pouring it on to a Petri dish. It is also often necessary to first dilute the sample by mixing a few drops of it in sterile water and then thoroughly shaking it, to ensure the cells being well separated. The cells of *Mycoderma* will develop in the course of a few days dull, greyish, disc-like colonies, whereas acetic acid bacteria develop transparent, slightly opalescent colonies like small specks; and the mould spores develop colonies throwing out many small branches from their edges.

The approximate amount of an infection by *Mycoderma* can be estimated by the following accurate method: On a solid medium (unhopped wort with an addition of 5 per cent. gelatine and $1\frac{1}{2}$ per cent. agar) one prepares a *superficial culture*, that is, one in which all the cells develop on the surface; by this means the peculiarities of all the colonies are caused to manifest themselves very distinctly, and the large colonies of *Mycoderma* can be easily distinguished from those of yeast. By taking small samples from different parts of the yeast mass or of the mash, and transferring them into sterilised water, an average sample is obtained after shaking up well; the latter is

diluted several times, say in some 10 c.c. flasks, with sterilised water, and the last dilution is poured along the surface of some gelatine-agar that has solidified in a Petri dish. Subsequently the water is poured off and the dish, covered with its lid, is placed in a thermostat (1 or 2 days at 25–27° C.). It can then be easily perceived with the naked eye which colonies consist of yeast and which of *Mycoderma*.

A weaker infection of *acetic acid bacteria* in the liquid will often not give distinct growths on plate cultures of wort gelatine. Several Freudenreich flasks containing wort and a little alcohol added must then be infected with the wort sufficiently diluted with sterilised water, and an examination made of the film that will form on the surface. The presence of acetic acid bacteria can always be ascertained by their characteristic growth as well as by the smell of the liquid.

In addition to the culture yeast, cultures of *lactic acid bacteria* are also employed in this branch of the fermentation industry. As undesirable species of bacteria are always likely to establish themselves during this kind of alcoholic fermentation, their suppression is always attempted by encouraging the growth of lactic acid bacteria.*

As it is important to check foreign germs during the early stages of their growth, the development of lactic acid bacteria must be commenced at a very early stage of the fermentation, namely, immediately after saccharification. The growth and activity of the lactic acid bacteria is checked by the lactic acid, and it

* We leave completely out of the question the importance or not of lactic acid bacteria in other directions.

ceases at last altogether, the bacteria being quite crippled. In distilleries and yeast manufactories the lactic acid fermentation is confined to the acid mash after saccharification, and concluded with this stage of the process in order that growing bacteria may not be introduced into the principal mash. The latter must not be allowed to develop considerable quantities of acid (Max. : 0.2 per cent. N.S.).

Experience therefore lays down the following principles for our guidance in carrying out an analysis at the different stages.

An analysis of the acid mash at the first stage, when acid is being formed, should reveal the presence of a healthy, vigorous development of lactic acid bacteria, and only a very slight development (if any at all) of other organisms. In the fermented acid mash, and in the principal mash started with the yeast developed in the acid mash, the lactic acid bacteria will, with the aid of a microscope, be found to be still present, but so crippled that, on making cultures on sweet wort gelatine, there will only be a very weak growth, if any at all.

When making an examination for acid bacteria, plate cultures are made, using either the usual wort or dextrose solution and gelatine; or a small quantity of precipitated calcium carbonate is mixed in with the nutritive gelatine. In the latter case the gelatine has a milky appearance. The colonies developed by the acid bacteria have the power of making the gelatine transparent in their immediate neighbourhood, as has been described, and they appear to be surrounded by a transparent ring, because the acid dissolves the

particles of lime ; thus, partly with the aid of a microscope, partly with the naked eye, it is possible to distinguish whether the colonies are developed by acetic acid or lactic acid bacteria. The former are also sometimes able to produce a transparent zone.

The presence of *wild lactic acid bacteria* is demonstrated by making from the material a plate culture on sweet wort with an addition of agar-gelatine and chalk and leaving it to stand at 25° C. At this temperature the wild lactic bacteria will develop, whereas the culture lactic ferments will be stunted in growth. From the acetic acid bacteria, which may also develop under these conditions, they differ by the appearance of their colonies. Whilst the former group form thin, large, irregular, faintly bluish colonies on the surface, those of the wild lactic bacteria appear as sharply outlined white dots or speckles.

In distilleries and yeast factories, where the yeast is skimmed off the surface of the fermenting liquid in the tuns, the following analyses are made:

1. *Examination of the Acid Mash during Fermentation.*—The necessary biological analysis in this case is to show that a predominating development of lactic acid bacteria has taken place.

2. *Examination of the Acid Mash after Alcoholic Fermentation* (the yeast all ready for pitching).—A growth of lactic acid bacteria should be present, but be unable to develop colonies on gelatine plates ; the yeast should have grown to a definite extent and should be ripe in a certain measure, proved by the cells having a uniform appearance, particularly as regards their size—cells producing small buds should be the exception—and

their contents ; the appearance of the contents of the cell depends to a certain extent on how long fermentation has been allowed to proceed. What is best in this respect cannot be decided by theory, but only, as already stated, by actual careful experiment. Besides the composition of the mash, and the manner in which fermentation is conducted, the type of yeast employed also plays an important part without doubt. Types of yeast are found in distilleries, as in breweries, which suffer if their action is arrested at too early a stage of development. If that is done constantly, they will be gradually reduced to a feeble state.

3. *The Principal Mash or Wort before Fermentation commences* contains only weakened micro-organisms, and should consequently *not show any development* of the same.

4. *The Principal Mash during and after Fermentation*.—Lactic acid bacteria are to be found with the help of the microscope, but they are not capable of development ; they are generally so crippled that they cannot be grown on gelatine plates. The yeast should during the time that fermentation lasts show a uniform development of the contents of the cell. A sample may be taken for spore culture. Samples taken from the surface of the fermenting liquid may be examined to ascertain if *Mycoderma*, acetic acid bacteria, and moulds are present.

5. *The Skimmed Yeast, and Washed and Pressed Yeast*.—Samples should be compared, as usual, to ascertain if the yeast cells maintain a normal and uniform appearance, and if the water has introduced any infection—that is to say, if in washing putrefactive

bacteria have been added and developed. The samples must also be examined with regard to Mycoderma and wild lactic bacteria.

6. *The Yeast used for Pitching.*—This should be examined to ascertain if any undesired development of bacteria, or film-forming organisms, has taken place.

In *air-yeast factories* the following samples are collected :

- (1) A sample from the lactic mash before warming.
- (2) A sample from the lactic wort before the introduction of the yeast.
- (3) The fermented wort containing the yeast that has been grown.
- (4) The (washed and) pressed yeast.

The most important points in the analysis have been given in the previous paragraph.

The process employed in air-yeast factories affords a good example of how a type of yeast can in practice develop a new form. Under the powerful influence of the air that is roused in, a large or small proportion of the cells, sometimes all the cells, will assume an oblong shape.

It is often not possible to distinguish by a simple microscopical examination the cells of Mycoderma from the yeast cells grown in air-yeast factories. They may show the same clear cell contents; or all cells (*Saccharomyces* and *Mycoderma*) may have large vacuoles, and simply a layer of plasma near the walls of the cell. It is therefore always important to make plate cultures as described above, at the same time obtaining information about the bacteria present.

Also, the cells of many types of *Oidium*, *Chalara*,

and Dematium, &c., are difficult to discover in a field under the microscope, and their numbers can only be estimated by taking a good average sample and making a plate culture with it.

In order to be enabled to judge of the quality of a yeast, it is of some consequence to roughly estimate the amount of *glycogen* contained in the cells. For this purpose the preparation is treated with a weak solution of iodine-iodate of potassium (say 0.4 gr. of iodine per 100 c.c.): if glycogen is present, the cells assume a red-brown tinge; if not, they are coloured yellow.

Where, as is frequently the case, particular modifications are introduced in the conduct of distilling operations and in yeast factories, this circumstance must be taken into consideration in selecting samples for analysis.

III. ANALYSIS OF WINE YEAST

In examining the fermented must from grapes, the first point on which information is desired is whether a development of bacteria is going on in it.

Furthermore, information is desired as to whether at any particular advanced stage of the fermentation the yeast cells enter into a state of rest, the liquid thus becoming rather still, or whether the yeast is *still growing*.

When a selected type of *pure yeast* is added to wine must, it should be ascertained whether the type of culture yeast is powerful enough to gain and keep the mastery over types of yeast that have crept into the must.

When the different types of micro-organisms are studied individually, to be used for purposes of experiment, the culture must be submitted to a fractional examination, and absolutely pure cultures must be used for the purpose.

The question of infection, owing to the presence of *bacteria*, can only be determined by a thorough examination with the microscope. There can never be any certainty that the bacteria developed in the wine will grow in plate cultures or in nutrient liquids; with the help of the microscope a real growth of bacteria can be proved to be taking place, whilst plate cultures prepared at the same time give either a negative result or results that are inconclusive and of little value. But many of the bacteria found in wine are very small—the cocci, for example—and it is therefore necessary, when using the microscope, to use a good immersion objective. It stands to reason that, with attentive watching, it can be ascertained if a growth is taking place at the moment or not.

The other question of a practical nature—whether *the yeast in the liquid* is always *growing*, and if *vigorous* cells are to be found, or if development has ceased—can likewise usually best be determined with the aid of the microscope, and not by making plate cultures. Naturally, even very exhausted cells, that are, to a certain extent, of no use in wine, can be resuscitated in fluids that suit them. But no conclusions of service in practice can be drawn from this. The growth must be subjected to a direct examination. Only when no budding cells, no cells with clear plasma, or no uniformly

vacuolated cells are found ; but only cells that are much granulated, and whose contents are very refractive, containing also irregular large and small vacuoles: then only can it be said that the yeast has entered into a resting state. The question should not be answered at once with a single examination ; but the sample must be allowed to remain for some days at a suitable temperature, and then be again examined.

If the wine contains cells of *Mycoderma* capable of germination, they will soon make themselves apparent. If the sample bottles are closed with several sheets of filter paper, and allowed to stand for a very few days only at room temperature, on examination of a drop taken from the surface of the wine budding cells of *Mycoderma* will be found.

If a *selected race of yeast* is employed, the difficulty is to add just sufficient yeast to suppress the germs contained in the must. On the other hand, there must not be so much yeast added that the primary fermentation will be too vigorous, entailing the risk of the yeast becoming too quickly exhausted, and consequently, at the later stages of the fermentation, being so weak that it cannot hinder a secondary development of other yeasts or bacteria. This difficulty is increased by the fact that the quantity of natural yeast appearing on the grapes varies in different years. One solution of the question is to commence with a small quantity of yeast, and to take notes of the rate of growth and the course of the fermentation. After a short time conclusions may be formed, and taken as a guide for future work.

Also the quality of the must has to be borne in mind

when deciding on the quantity of yeast that should be used.

When the vigorous fermentation is over, an examination must be made to ascertain *if the selected type of yeast still has the upper hand*. A microscopical examination will not afford the information. If the yeast examined under the microscope appears very uniform, it is probably the selected culture yeast, because the general appearance of the yeast in spontaneous fermentation is frequently very irregular. But in all cases a closer examination must be made on settled lines. We will content ourselves here with mentioning some of them.

Spore cultures may be made. If the selected race of yeast possesses characteristics that are easily recognisable, for example, in the structure of the spores, and is able under ordinary conditions to build up spores easily, a sample of the yeast taken straight out of the wine during the development of the cells and placed on a gypsum block will afford useful information.

If the selected race of yeast develops spores relatively late or early at a given temperature, it is also possible to determine approximately the extent to which that race of yeast is present in the sample.

If the selected race of yeast has a characteristic appearance when grown on gelatine, and the vegetation contains a sufficient number of cells capable of developing colonies on the gelatine, plate cultures should be also utilised.

Better results are usually obtained when a large number of cells are separated out, and the pure

cultures made from them examined and compared with the preserved sample of the pure yeast species kept in reserve. A portion of the sample diluted is therefore used to infect a large number of flasks, and only those flasks used which contain a single yeast colony at the bottom, after being allowed to stand quiet a few days. The appearance of these pure cultures under the microscope often yields useful information; also the taste and smell of the liquid, the physical nature of the sedimentary yeast, the length of time the liquid takes to clarify, &c.

I must, however, lay stress again on the fact that in not a few cases it is very difficult to obtain a thoroughly conclusive result by analysis. The examination of the fermented liquid itself must then be the means of determining if the desired result has been obtained.

IV. WATER ANALYSIS

A micro-biological analysis of the water used in the fermentation industries is not yet made use of so generally as it should be. It has been proved that information of the greatest value in practice can be thus obtained.

This analysis is based upon the fact that all the micro-organisms that are able to interfere with the fermentations are actually able to live for some time in water. This has been proved by inoculating the different liquids used in fermentation industries with samples of water. Such germs are therefore apt to slip in with the water and grow freely if circumstances are favourable.

On the other hand, very many micro-organisms that can live in water do not flourish in the liquids used in the industries of fermentation. The danger from germ infection through water does not therefore stand in any direct relation to the number of living germs which it contains.

The information obtained from such an analysis is of limited effect, because the germs found in a water, and the extent to which infection is present, vary from time to time, just as the chemical composition of a water is found to vary.

As a rule, a really disastrous development of the germs living in water takes place when liquids are left quite still: for example, if any waste liquid, fermented or unfermented, is not quickly removed from the floors, the walls, or the plant itself; in rinsings which may contain wort or beer; in the water tanks, if these are soiled with malt dust, &c.; in old tuns, if they are allowed to stand empty for some time, and the water soaks into the cracks and holes, where some of the residue of the wort is certain to be, or on coolers that have not been properly cleaned.

When samples are sent to the laboratory, and can only be examined some days after being taken, the growth of the micro-organisms present will have spread. The growth, however, is probably restricted to those germs that flourish in water ("water bacteria" so called, in contradistinction to the germs that flourish in wort).

In many places, special water-tanks are used; it is then very important that the water contained in them should not be contaminated. As a general rule,

samples of the water must be taken for examination before as well as after the water has been put in.

If it is thought that the pipes are infected, it is advisable for purposes of comparison to obtain samples at the same time from the spring, the well, or the stream whence the water supply is obtained.

Absolutely sterile, clear glass bottles should be used for collecting the samples; flasks that have been sterilised by heating at a temperature of 150°C . (292°F .) for two hours should be sent out (packed in white paper) from the laboratory for the purpose.

The samples of water should be taken from the *tanks* at a time when the water is being roused. When the water is still, some of the germs fall to the bottom; a sample that is taken from the upper layers of the water cannot, if it is not roused, throw any light on the real state of affairs. Whoever takes the sample must thoroughly wash his hands just before doing so, dry them, and then rub into the skin a mixture of alcohol and water. Immediately before plunging the sample bottle into the water, and no sooner, the sterilised bottle is taken out of the paper; then it is sunk down as deep as possible under the surface of the water before opening, the stopper loosened, and the bottle allowed to fill. When full, close it again tightly whilst still deep under the surface, take it out and bind down the glass stopper, and *fix a label* to the bottle; and lastly, wrap it up again in the sterilised paper from which it has been removed. If a sample is taken from a well, it should be collected in the same manner, so far as circumstances permit.

When a sample of water is to be taken from a tap

on a *water-pipe*, the possibility of germs growing and flourishing on the inside of the tap itself must not be overlooked. But the problem is to determine the number of organisms present in the total daily amount of water used. It is therefore necessary to let the water run for some time before taking a sample, in order to be sure of obtaining an average sample. The sample is taken when the water is running at full bore, the bottle, with the stopper still in, being thrust into the stream, then opened, and shut again before it is taken out of the stream of water. Samples are taken from a bore tube (artesian well) in the same manner.*

The principle underlying this analysis is, that in the laboratory a few drops of water are used to infect liquids of the same nature as the liquid used in practice, but with this difference—that the liquids used in the laboratory are sterilised. The brewery wort from the filter bags is usually employed for this purpose in analysing water in the brewery; it is then sterilised in Freudenreich flasks. Lager beer, sterilised by steam or in boiling water, is also used: the resulting loss of alcohol is sometimes made up again. In the case of distilleries and air-yeast factories sweet wort is made use of.

The different yeast types, moulds, and bacteria most able to develop during alcoholic fermentation can be grown in these liquids.

If *exceptional* questions require solution—for example, whether the water in a yeast factory or

* The different special kinds of flasks that have been proposed for collecting samples of water are only serviceable when specially trained experts can take them.

distillery contains *acetic acid bacteria*—liquids are usually employed that have been found to be particularly favourable for the development of bacteria of this type ; exhausted germs are thus strengthened and their development assisted. A top-fermentation beer that contains a high extract is usually employed for this purpose ; it is only slightly fermented and lightly hopped, and contains about 2 per cent. alcohol.

If it is desired to ascertain if the sample of water contains a large proportion of yeast types that are capable of rapidly *developing films* on *fermented wort* (e.g. *Mycoderma*), or are particularly prevalent in air-yeast factories and quickly and vigorously develop there, it is useful to make use of wort gelatine plate cultures as described above. After the lapse of a few days any germs that are present will have developed small, grey, film-like colonies, and by counting the number of colonies the number of *Mycoderma* germs in each drop of water can be estimated. Where *acetic acid bacteria* are concerned, plate cultures (wort or dextrose gelatine with an addition of precipitated carbonate of chalk) can also be employed. In the case of *moulds*—when, for example, it is desirable to ascertain if the water is infected with any germs from the malt-house—ordinary wort gelatine plate cultures are used.

For yeasts and some other organisms the gelatine plates are of no use, because the exhausted cells either cannot grow at all on them, or only show slight growth, no colonies being formed that can be identified as yeast colonies. Only nutritive *liquids* can be used. It is known that the wild yeasts are the most

dangerous enemies to all fermentation industries, because they, as a rule, require the same conditions for supporting life as the culture yeasts, and therefore develop when the culture yeasts are growing; consequently, the identification of such organisms is of the greatest importance.

Liquids (wort, beer, neutralised yeast-water, &c.) must also, as a rule, be employed for the identification of *Sarcina* types that are found in breweries.

After a preliminary microscopical examination of the sample of water, to obtain some idea about the infection from moulds, yeast cells, bacteria, or large quantities of decaying organic matter in the water itself, a sample of the water is distributed by drops over several flasks, both as it is, and diluted with sterilised water in varying strengths.

The object is to introduce only *a few germs* into each flask, in order to ensure the growth of the predominating species taking place in a large number of flasks. The types of micro-organisms present in but a slight quantity would commonly not be developed under these conditions.

The flasks containing nutrient liquids (for breweries, hopped wort, beer; for distilleries and air-yeast factories, sweet wort, beer) are sterilised externally with a flame, and placed in a glass case previously washed inside and out with a solution of sublimate (1:1000) or with 50 per cent. alcohol, and having a door that slides up and down (Fig. 4). The bottle containing the water is well shaken and, by means of a sterilised pipette, partially closed at the upper end with sterilised cotton-wool; only a *very few*

drops of the water are taken out after each shaking of the flask and rapidly transferred to the culture flasks, a drop in each flask. The culture flasks are then put

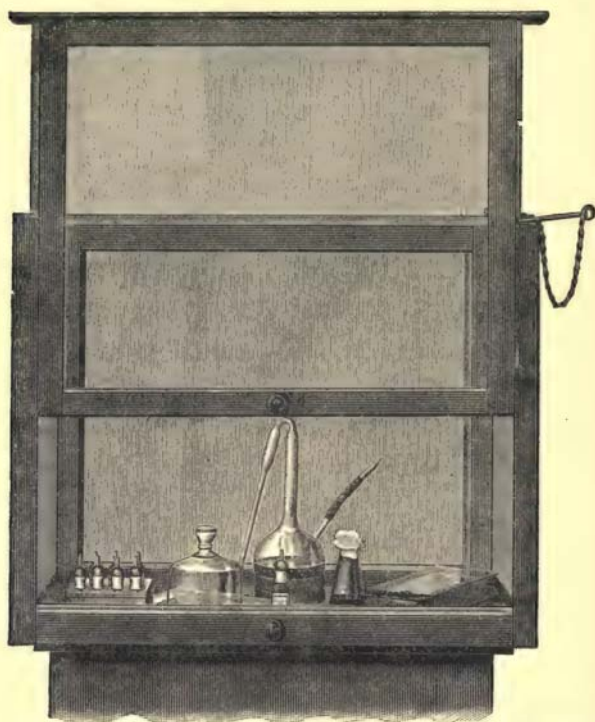


FIG. 4. Sterilised cupboard, in which work is carried on free from risk of infection from the atmosphere.

into a thermostat at 25°C . (77°F .), or kept at room temperature ; or the flasks can be infected in duplicate, and kept under observation—one series at a constant temperature, and the other under varying conditions of temperature.

The research generally extends over a period of *ten* to *fourteen* days.

Organisms found in water develop in varying degrees in the particular liquids produced in breweries, distilleries, &c. Also, during fermentation, they develop with varying power of resistance in the presence of the culture yeast.

A guide in ascertaining how vigorously the different organisms can develop in the liquid concerned is the length of time that elapses before a growth is formed *that is clearly visible to the naked eye.*

The flasks kept at 25° C. (77° F.), or at room temperature, should be looked over every day, and particular attention should be paid to the clearness and colour of the liquid, to the surface-vegetation, and to the bottom-growth.

From time to time the number of flasks that show a change in the condition of their contents are noted. These flasks should be at once examined microscopically, also biologically, as described in the chapter devoted to yeast analysis (Part I, chap. i).

The effect the organisms exercise on the condition of the liquid in the brewery, distillery, &c., when surrounding circumstances are favourable to their growth, stands in direct relation to the vigour with which they are able to develop in the wort, &c., in the small flasks.

In analysing water for fermentation industries, it is often noted that a large number of the wort flasks are infected with a preponderating growth of putrefactive bacteria—at once recognisable by the smell. It is a fact established by experiment that these organisms are not able to assert themselves during *alcoholic fermentation*, therefore from the fermentation

point of view they need not be taken into account when deciding if the water is fit for use. These organisms may be classed as "water bacteria." But still, it must be borne in mind with regard to them that their vigorous development in water betokens that it contains organic matter in a state of decomposition, and there is always the danger that, under unfavourable circumstances—*e.g.* infection of well water brought about by surface water penetrating into it—dangerous organisms may also be able to increase in the water.

For washing yeast in a yeast factory, it is clear that a water containing many putrefactive bacteria should not be used.

It is of particular importance to examine the growth appearing in the flasks *during the last days for which they are left quiet*, because it often happens that the multiplication of *wild yeast* and other organisms which may make their influence felt does not take place till then.

As regards the yeast races employed in factories, it is well known that an inquiry as to their power of resistance towards foreign micro-organisms can be carried out by infecting (with a few drops of the water) flasks containing absolutely sterile wort and the pure yeast type employed. The water should be mixed with the fermenting liquid at different stages—*i.e.* when the yeast is introduced, and at different stages during the fermentation, more particularly during after-fermentation. Fermentation must be carried out at the same temperature as in the factory. It is, however, impossible to work in the laboratory under the same

conditions as in the brewery or distillery; definite conclusions cannot therefore be always obtained. The above method will generally yield more reliable results.

V. AIR ANALYSIS

The number of living germs floating in the air may often amount to several thousand per c.c. The true danger-point for infection, where the fermentation industries are concerned, is rarely in any direct proportion to these high figures. This is accounted for, on the one hand, by the fact that usually only a very small proportion of the micro-organisms present in the air are either able to grow or capable of exerting any influence in the liquids undergoing fermentation; and, on the other hand, that the dangerous organisms are only able to exert any influence when undisturbed, and the occasion is favourable for them to increase in numerous generations, and at the same time to become accustomed to the particular circumstances in which they find themselves.

Such cases occur only, as a rule, when the *thorough* cleaning of the plant is neglected or if an infected yeast is not removed at once without delay.

The germs which have become rooted will then grow to such an extent that they will, under favourable circumstances, be carried about in the air, and an analysis of the air will reveal their presence. They are now no longer air-germs in the ordinary sense of the word.

An expert examination of the state of affairs prevailing in practice will often have exposed the true

nesting-place of the objectionable germs long before it is found out by an air analysis.

A comparative analysis of the number of germs growing in brewery wort at the moment when it leaves the coolers, and twenty-four hours later, after the yeast has been growing in the tun, affords a striking example of the fact that even such micro-organisms as can grow in the wort are only able to assert themselves under certain circumstances. It has been possible, with the help of fractional analysis, to determine the presence of many hundred germs per c.c.—living germs, capable of growing in wort—in the wort on the cooler. Twenty-four hours later, the same wort, in the fermenting tun, showed very slight traces of any growth of these foreign organisms. By far the greater majority had been unable to grow, and thus be recognised in the analysis.

Definite conclusions are therefore only based upon an examination of the fermenting liquid itself, and the surface of all objects in the different rooms.

The analysis of the air may have a very particular object—namely, to ascertain if foreign germs are introduced into the fermenting liquid from any known suspected sources of infection in proximity to the tun room.

Also, in this case, we proceed in a practical manner by making an examination of the suspected sources of infection. For example, if one wishes to know whether the organisms that rapidly make their appearance when the grains are lying exposed to the air, and are often naturally carried up into the air by dry grain particles, are dangerous for the fermentations or not: an answer is best obtained if the wort is infected

with some of the grains, and comparative fermentation experiments carefully carried out—every endeavour being made to approximate to the conditions prevailing in practice.

If it is desired to make an experiment in order to ascertain which of the organisms that are in the air, and are deposited *on the coolers*, are capable of influencing the fermentation, the best plan is to fill cylindrical-shaped glasses with some litres of quite pure wort, put them by the coolers, and leave them open whilst the wort is exposed to the air. Add a suitable proportion of absolutely pure yeast, and carry on the fermentation in these cylinders under conditions identical with those regulating the fermentation in the big tuns. An analysis is made at the end of the primary fermentation and again when the beer drawn off from its sediment has passed through after-fermentation. In many cases we learn from such an examination especially whether the coolers and their immediate surroundings are kept clean or not.

In order that information gained by researches of this kind may be of practical use, a clear understanding of the work in hand must be arrived at between the practical man (brewer, distiller, &c.) and the analytical chemist. The latter must be familiar with the methods of working adopted in practice, and the brewer and distiller must understand the main principles underlying the methods employed in the analytical work.

If the analysis of the samples of air can be carried out on the spot where the samples are taken, no other apparatus is required in practice but the flasks in which the growth of the germs, found in

the air, can proceed. A large number of flasks are placed in the room or locality concerned (the ordinary Freudenreich flasks are very suitable), and they are filled either with sterile wort, distillery mash, or must, as the case may be, varying with the industry concerned. Some of the flasks are only open for one or two minutes, and the remainder are kept open for a quarter of an hour or half an hour. Of course, the man who places the flasks in position, opens them, and later closes them must have on clean clothes and have washed his hands clean. The flasks are then allowed to stand at room temperature, or at 25°C . (77°F .). The examination of the cultures obtained is generally closed after two weeks' observation. If a number of the flasks contain a mixture of organisms, it may be important to separate the types.

If the object is to find out whether particularly strong development of moulds, *Mycoderma*, and acetic acid bacteria are present, ordinary wort gelatine plate cultures can be carried out. The liquefied wort gelatine having been poured out into Petri dishes, the wort gelatine is allowed to set into the closed dish, and the dishes are then exposed on the cooler.

The most important question—whether large numbers of *wild* yeast cells are floating about in the air—can only be answered by resorting to the use of liquids, as many of these cells, when they settle out of the air on the gelatine plates, are not so vigorous that they can develop colonies, and therefore they escape observation. An analysis with the aid of liquid media is, therefore, always the most important, as with the aid of liquid media alone is it possible to discover the most

dangerous germs. The reason why *yeast* infection is the most dangerous is that these organisms, as remarked already, flourish well under the same conditions as the culture yeasts, whereas the majority of other organisms are suppressed under the conditions of fermentation that usually prevail.

When it is not possible to carry out an analysis of the air on the spot, the germs must be *collected and*



FIG. 5. Diagram of bottle used in air analysis.

despatched to the laboratory. The following is the most sure method of carrying this out :

Several bottles (Fig. 5), containing about 25-50 c.c., are sterilised at the laboratory. They are closed with a conical-shaped india-rubber cork, which only enters the bottle to the extent of one-third of its depth. Some of these bottles contain sterilised water ; the remainder, the liquid that is to be fermented, wort, distillery mash, or whatever it may be. The bottles are placed at different levels in the place where it is desired to make an examination of the air ; the corks are withdrawn with clean hands, taking care not to touch the rim of the bottles, and are laid down close

to the bottles with their inner surface turned up, where they should remain undisturbed. After an interval of between a quarter of or half an hour, the corks are put into the bottles again and tightly fastened down. The bottles are then packed up in clean paper and sent back to the laboratory.

During transit, the germs will increase particularly in the bottles filled with a nutritive medium ; some of the predominating types present will therefore be the more easily identified.

On reaching the laboratory, the contents of the bottles are transferred, directly or after being first diluted with sterile water, to flasks containing the nutritive liquid employed in practice, a drop in each flask. A larger quantity of the water is put into some flasks filled with the requisite nutritive liquid ; here the different types will be mixed up together and struggling for survival.

The flasks are allowed to stand at a temperature of 25° C. (77° F.), or at room temperature, and submitted, after ten to fourteen days, to the same methods of examination as employed in yeast analysis (Part I, chap. i).

VI. NUTRITIVE LIQUIDS AND GELATINE

Only a very small number of nutritive liquids are used for the cultivation of alcohol yeasts and fermentation bacteria in a laboratory, where the work chiefly follows practical lines. As the characteristics of different yeast types are relative, and to a certain extent dependent upon the nutritive medium in which

they are grown, it follows that attention must be paid to the point—from which branch of the fermentation industry the particular yeast type under examination is obtained. Experience has proved that, at any rate, certain types of yeast employed in the fermentation of molasses from beet sugar should be grown in the laboratory in a solution of molasses and nitrogenous matter—for example, a decoction of malt rootlets. They otherwise require a long time to accustom themselves to carrying out a *rapid* fermentation of molasses in large tuns in the factory. On the other hand, the ordinary yeast types of the distillery and yeast factory can be grown in the laboratory in sterilised hopped brewery wort without any risk of deterioration. To use sterilised distillery wash is to run the risk of great difficulties, because it undergoes a marked change on being raised to boiling-point.

The most valuable nutritive liquid is certainly *hopped brewery wort*. The majority of yeast types grow freely in this medium. The best plan is to use wort from the coolers that has been filtered (wort from the filter bags), as it only throws down a slight deposit during boiling in the flasks. The wort is first boiled in large copper cans and placed on one side to clarify before it is used in the small flasks. When the *Pasteur flasks* are sterilised on a sand-bath, they are first allowed to continue boiling for half an hour with the side tube open; the tube is then closed with a glass stopper, and boiling is continued for twenty minutes longer, the steam escaping through the bent tube. Experience has proved it to be quite superfluous to close the bent tube, after the boiling of the

wort is finished, with an asbestos stopper ; or to use asbestos stoppers later on when working with the flask—in fact, they are found to be a hindrance, because they collect the dust. Often some of this dust is scattered in the air before it is all destroyed by playing on the asbestos stopper with the flame of a burner. If the sterilised flasks are allowed to stand in a closed cupboard and not exposed to any sudden change of temperature, there is no risk of living germs being drawn into the liquid. The flasks should be kept for some time before being used ; partly in order to make sure that they are sterile, and partly in order that a slow oxidation may take place from the surface of the liquid. *Freudenreich flasks* and other flasks of the same sort are best sterilised by being steamed in a closed cylindrical-shaped vessel, in which they can be placed on metal perforated trays, one tray above another. They are boiled for an hour and allowed to remain in this vessel until they have cooled again. *Pasteur flasks* are also often sterilised in a steam cylinder ; the india-rubber connexions are first sterilised by boiling and then closed with a glass stopper that has been sterilised in a flame.

When one is obliged to use flasks that have only quite recently been sterilised, then, in order that their unsuitability may be less pronounced, the liquid should be well shaken for a few minutes, so that it may take up free oxygen. When Pasteur flasks are being shaken, the bent tubes must be kept heated in a flame.

The large copper cans (*Carlsberg cans*), Fig. 8, are like the Pasteur flasks, but they are fitted with an out-

let pipe at the bottom and a cotton-wool filter fits over the bent tube. These large copper cans are used for growing large quantities of a particular culture, and it is best to sterilise them over a naked flame, both upper tubes being allowed to remain open for an hour, boiling being then continued for another quarter of an hour with the india-rubber connexion on the short upper outlet pipe closed. In order to ensure complete sterilisation of the lower india-rubber connexion, a little wort is allowed to run out through it whilst boiling is going on, and then the glass stopper, sterilised in a flame, is again pushed firmly in. Before the cans are removed from off the burners, the cotton-wool filter must be fixed very firmly on to the long bent tube. When the wort in these cans is to be used for the cultivation of brewery yeasts—able to come out after a short time either as sedimentary yeast, or yeast floating on the surface of the liquid, and able to settle out in flakes (a “break” in the liquid) while the liquid is clarifying—it cannot be made use of immediately after boiling, but must be allowed to stand for at least a week, in order that a gradual oxidation may take place.

When *distillery mash* or *wort* from the *yeast factory* is to be used, neither can be used as it is, as in the case just described, but they must first be filtered. An ordinary sterilisation is attended by the difficulty that these liquids undergo marked changes, because a large quantity of nitrogenous matter is precipitated out of solution during boiling. Experience has proved sterilised brewery wort to be a suitable medium for growing cultures of distillery yeast in the flasks. If distillery wash is to be used, in order that it may

preserve its nutritive properties after having been sterilised, peptone must be added, or a fractional sterilisation must be carried out. This is done by repeatedly raising the liquid to a temperature of 70° – 75° C. (158° – 167° F.) at intervals of twenty-four hours. It is, however, an operation attended by considerable risk, because there is no certainty of securing thorough sterilisation. Experience has also taught us here that germs, which have been so severely crippled that they could not develop so long as the flasks remained undisturbed (thus leading the operator to think that the liquid was sterile), have developed after the introduction of a pure culture of yeast. This may perhaps be explained to a certain extent by the fact that the germs which are resting on the bottom are brought to the surface with the motion set up in the liquid by fermentation, and there find the conditions favourable to their development, or perhaps only motion is necessary for them in order to start development.

Wine yeasts can in many cases be grown in wort without suffering any deterioration. A little tartaric acid may be added. When *wine must* is required for use and ordinary wine must is not at hand, concentrated (*in vacuo*) must can be purchased from the trade, and then be used diluted with distilled water. A suitable nutritive liquid to grow wine yeasts in can be obtained by using a decoction of raisins, to which a small quantity of tartaric acid or citric acid together with tannic acid has been added. Malt extract or a solution of cane sugar, to which citric acid and acid phosphate of ammonia have been added, can also be used.

Yeast water may be used for testing the yeast in a pure culture machine to ascertain if bacteria are present. One litre (1 l. = about 1.75 pints) of well-washed yeast and two litres of water should be boiled for a couple of hours. The solution after being made up to its original volume (3 litres) is then filtered off clear and rendered either slightly alkaline or neutral by adding sodium or calcium carbonate.

A solution of *dextrose and yeast water* is employed to obtain a stronger growth of the yeasts that do not ferment maltose, as well as for the purpose of resuscitating yeast types that are likely to lose their power of producing spores owing to their constant repeated culture in hopped wort. Ten to fifteen per cent. dextrose is added to the non-neutralised yeast water.

A 10 per cent. solution of *saccharose*, usually clear crystallised sugar and distilled water, is used for preserving pure yeast cultures. After boiling for a short time the solution is filtered off quite bright and sterilised in the flasks.

Hopped wort, distillery mash, must, dextrose, yeast water, and dextrose and yeast water together, are all used with nutritive *gelatine*. Ten per cent. by weight of gelatine mixed in a little lukewarm water is added to the wort or liquid required. The mixture is warmed, boiled with white of egg, allowed to clear, and filtered through flannel; the nutritive gelatine is then sterilised in the flasks by being submitted to boiling in a steam bath for half an hour. It must be remembered that either too long boiling or repeated boiling may prevent the mixture from gelatinising properly.

Agar-agar is sometimes used instead of gelatine.

It is cut into small strips, and allowed to soak in a 1-2 per cent. solution of ordinary salt and water for twenty-four hours before it is required for use. The solution of salt is then poured away, and the agar-agar washed with cold water; it is then dissolved in the nutritive liquid selected for the purpose, being added to it in the proportion of $1-1\frac{1}{2}$ per cent., and then boiled. A mixture of $1\frac{1}{2}$ per cent. of agar and 5 per cent. of gelatine is often used.

The advantage of operating with solid media is that, both when sowing a culture and when taking out a sample, the opening or mouth of the flask can be turned down, and thus any direct infection from germs that are in the air be prevented.

II
THE PURE CULTURE OF ALCOHOL-
YEASTS

II

THE PURE CULTURE OF ALCOHOL- YEASTS

GENERAL REMARKS. COLLECTION OF SAMPLES

THE pure culture is raised from a single cell. Such a culture is thus free from all infection, such as bacteria and wild yeasts, and it also possesses the condition that makes it possible for the yeast to maintain its character unchanged because the cells are all of one and the same species ; so far as we know at present, it is absolutely impossible, by mixing several species of yeast together, to secure a reliable culture capable of keeping unaltered for a length of time. After such a mixture has been used in some fermentations, it is impossible to know in what proportions the different species exist together ; and careful research has furthermore shown that one or another of the component types will sooner or later gain the mastery and become the predominating species. Such a mixture has therefore again and again to be prepared afresh.

In some isolated cases brewers have expressed the opinion that beer fermented with a single species of yeast does not possess its former brilliancy. Whenever this objection has been raised, the question has been very carefully inquired into, and it has always been found that the fault has nothing to do with the selected

species of yeast, but has been traced back to the wort. In such cases a faulty conversion of the starch has been often noticeable, but it could not be detected in the course of the ordinary brewing operations; it can only be found out by careful microscopic examination of preparations mounted in iodine solution.

✓✓ At the same time that these small irregular starch
✓ particles were found to be present, it was sometimes noticed that it became impossible for the yeast to increase sufficiently during fermentation. Whether or not there lies a causal relation between these two facts is not yet known for certain, but it is probable.

Formerly the complaint was raised on behalf of bottom fermentation, as well as on behalf of top fermentation, that the employment of a single race of yeast did not allow of a satisfactory secondary fermentation being carried through. Practice has shown it to be possible to find, by careful selection, a type of yeast that will, whether the fermentation be "bottom" or "top," when proper attention is given to the fermentations, carry through the secondary fermentation also.

Certain *Torula* species are sometimes added, especially to top-fermentation beer, for the purpose of imparting a particular flavour to it. In most cases, however, such an addition of foreign organisms may be considered as superfluous, and at any rate it must be effected with the utmost caution.

As a rule, the type of yeast selected from the mixed yeast mass to form the starting-point for the pure culture in mass will be, if the samples are taken in the proper manner, the predominating type in the mixed

yeast and the type that gives the mixed yeast its particular character. But the single cells of this type are not found to be identical. One would not achieve what *can* be achieved, if one employed a yeast raised from a single cell of the predominating type, selected only by means of botanical and other characteristics. As, on the one hand, it is not an easy matter to define the limits of the species, conjectures more or less accurate can alone be made; and, on the other hand, in individual cultures of a group which may be regarded as belonging to one and the same type differences are to be noticed which can be traced back to peculiarities innate in the cells from which these cultures were originally raised. It is therefore necessary to make a choice between these different *varieties*, and to seek to find out the particular variety that in practice will be able to produce the proper taste, smell, degree of attenuation of the beer or wine, and proper yield of alcohol in the case of distilleries, the highest yield of yeast in yeast factories, &c.

This selection must in any case be first carried out in the laboratory. The preparation of pure cultures thus demands not only skill in carrying out trials, but also practical insight into the particular branch of fermentation industry concerned. Certainly, experience can alone guide one in selecting the particular type from which the pure culture is to be grown.

The procedure adopted in taking the sample from which the pure culture will be raised is a very important matter.

With regard to *brewery bottom-fermentation yeasts*,

we know that at the end of the primary fermentation wild yeasts can develop strongly in the beer, that at this stage the culture yeast will soon cease increasing, and that the culture yeast cells are to a certain extent exhausted. During the first stages of the fermentation the culture yeast is the predominant type, and in a state of lively and vigorous development. And, as it is from this yeast that one wishes to raise the pure culture, either a sample of the fermenting wort can be taken during the first stages and the cells floating in it made use of; or, what is often done, after the fermentation is finished and the beer has been racked off, a sample can be taken from the *middle* layers of the sedimentary yeast left in the tun. A few drops of this thick-fluid yeast are collected either on sterilised filter paper or in sterilised bottles sent from the laboratory for that particular purpose.

The samples of *top-fermentation yeast* are taken from the strong yeasty heads thrown up in the tun, or sometimes in cask, as the case may be. When a yeast that works very slowly over a long period is required, the right thing is to wait to take the sample until the quieter fermentation has set in, as it is highly probable that the races of yeast that work quietly are then predominating.

The samples of *distillery yeast* are taken from the yeasty head in the fermenting rounds. In the air-yeast factories pressed yeast is made use of.

Wine yeast samples are taken at different times during the fermentation, bearing in mind that the types that play an important part in the process do not always develop during the earliest stage of the

fermentation. Samples are taken from the liquid itself, and also from the sedimentary yeast, using a pipette—a thin pointed glass tube—for the purpose.

I. THE PREPARATION OF A PURE CULTURE

Before making use of the samples sent to the laboratory, an examination should be made to see if the cells are vigorous or exhausted.

As a rule, one type of yeast predominates in brewery yeast. If all that is desired is to obtain a pure culture of this type, and the cells in the sample are vigorous, they can be introduced directly into the moist chamber; but if they appear exhausted after the journey, the culture is sown in a nutritive liquid. The preponderating type will be the first to grow, and the young culture is used directly the first signs of fermentation are visible.

In distilleries and wine factories, on the other hand, several types are often found all growing freely at the same time. It is then necessary to make a pure culture of them all in order to make a comparison between them. In order to carry this out accurately, the yeast sample is used to infect a number of flasks, after first diluting it considerably with water, so that each drop of water may contain only one cell, or at the most only a few cells. Fifty or more flasks are then sown, one drop only of the sample being sown in each flask. The several more or less predominating species have then been separated from one another, and a selection among the flasks is now proceeded with, some of which are used for

the preparation of single cell cultures in a moist chamber.

The *moist chamber* (Fig. 6) is made by fixing a cover slip, ruled out with numbered squares, to a glass ring with wax, and it is then mounted on an ordinary glass slide with vaseline. About fifty colonies can be grown on this cover slip, all well separated from each other.

After the yeast has been sufficiently diluted with



FIG. 6. Moist chamber.

wort or water, one or more drops of the sample are passed over to a small flask containing wort gelatine, the wort gelatine having been kept for a considerable length of time in a water bath at 28° C. (82° F.). The flask containing the yeast and gelatine is well shaken for a long while—frothing must, however, be avoided—and well stirred with a sterilised glass rod; a drop of the gelatine is then placed upon an ordinary glass slide, covered with a cover slip, and the preparation searched over under the microscope, using a low power (100 magnification) and little illumination. If cells are found here and there without any difficulty, the mixture of wort gelatine and cells which has been standing in the water bath during this examination is fit for use.

If there are too many or too few cells, it stands to reason that the sample must be either diluted with more nutritive gelatine or more yeast must be added, as the case may be. Shake the flask again, stir it vigorously with the rod, and place a drop of the gelatine on the under side of the cover slip of the moist chamber, turned face up, cover over the moist chamber, lying face upwards, with a sterilised glass plate, and leave the gelatine to set. The glass slide is then ringed with vaseline according to the size of the moist chamber, and the ring is pressed tightly down on to the vaseline. The position of a number of isolated single cells is noted down under the microscope. The squares and their numbers are drawn out on a piece of paper, and the corresponding positions of these single cells are noted down on the paper by little dots. Then a drop of sterilised water is placed on the bottom of the chamber. This plan of marking down the position of cells with the aid of figures in the large squares of the cover was introduced by the author in 1883 in order to do away with object markers.

The growth of the marked cells is now kept under observation.—It is always possible when searching for single cells to overlook small budding cells, or bacteria that will be able to grow in the gelatine. When such small cells or bacteria lie close to the marked cells, and possibly develop small colonies, they may eventually be absorbed by the larger yeast colonies. When, however, the growth of the yeast cells is kept carefully under observation, the small colonies whose growth in the neighbourhood of the

large colonies may render the latter useless are quickly discovered.

As a rule, three days' cultivation at room temperature is necessary to produce colonies large enough to be taken out of the chamber and placed in flasks.

The Pasteur flasks previously sterilised on the surface by passing them through a flame are placed in the glass case (Fig. 4), also the moist chamber and a small metal or glass box or tray containing platinum or copper needles that have been heated red hot and allowed to cool, and a pair of sterilised forceps are placed in the case in front of the operator. The glass ring with the cover slip on top is carefully lifted from the glass slide, and a needle held firmly with the forceps is put in from underneath; and one of the marked colonies, whose position is easily located with the help of the numbered squares, taken off on the point of the needle. The needle, with colony attached, is dropped into the flask, and the glass ring is placed back again on the slide.

When a sufficient number of flasks have been sown, each with one colony, they are gently shaken to disperse the yeast cells, and placed on one side at room temperature or 25° C. (77° F.).

When it is necessary to grow large quantities of yeast for use in breweries, distilleries, &c., the colonies can be introduced into $\frac{1}{8}$ litre ($\frac{1}{4}$ pt.) Pasteur flasks; otherwise Freudenreich flasks can be used. The propagation of the yeast in larger flasks is not proceeded with until the cultures have been developed to their *fullest extent* in the Pasteur flasks—that is, until the greater part of the yeast has settled at the bottom. Opportunities for making observations of a practical

nature already present themselves in the course of this first fermentation—the time that elapses before the yeast is deposited, the nature of the foam on the surface, and particularly the nature and colour of the sedimentary yeast, &c. (whether compact or loose, white, yellow, &c.), should all be taken note of.

When work is being carried on with brewery yeasts, it must be borne in mind that not only certain types of wild yeasts, but also certain types of culture yeasts, settle down or rise to the surface with difficulty, and form a very loose-lying sediment in the flask of sterile wort.

There are both top-fermentation and bottom-fermentation yeasts amongst the types suitable for carrying on the fermentations in distilleries, and both kinds of yeast are in use in practice.

Also with wine yeast, differences are noticed in these flasks according to the appearance of the fermentations.

The yeast developed in the first series of flasks should be used to obtain young cultures in a second series; and from this second series samples are taken for microscopical examination and for spore-cultures. The information thus obtained enables one to arrange the pure cultures according to any particular characteristics they may present biologically and botanically.

Some idea is thus obtained as to which types predominate under these conditions.

Valuable information is obtainable by careful examination of the structure of the spores. Distinct differences are observable if the spore-formation is allowed to proceed slowly on the gypsum blocks at low temperature.

After the cultures have been carried through a few fermentations in the Pasteur flasks, comparisons of growth are made by introducing the yeast into cylindrical glasses, containing one or two litres ($1\frac{3}{4}$ – $3\frac{1}{2}$ pts.) of sterile wort, and closed with two or three layers of sterilised filter paper. A little of the yeast in the small flasks is grown on in larger Pasteur flasks for this purpose, the yeast grown in these larger flasks is transferred to the cylindrical glasses, and the yeast that remains over in the small flasks is put on one side for use later on, if necessary, or some of it can be used at once for obtaining fresh fermentations.

The cylindrical glasses are thoroughly cleaned and dried, a little alcohol is then poured into them and set alight to sterilise them, and filter paper is then placed over the open ends. The wort is drawn from a Carlsberg copper flask (Fig. 8). As these experiments are made to obtain comparisons between the yeasts, it is necessary to supply all the cylinder glasses with wort from the same copper flask: when the wort from two or more flasks is used, it must then be mixed up in the same proportion in each cylinder glass.

There is something in the fermentation in the Pasteur flasks that is like the features of wine fermentation; such flasks can therefore be used when experimenting with wine yeasts. The fermentations in the cylinder glasses, on the contrary, offer a greater analogy to those carried out in breweries and distilleries; but the glasses must be closed over. These fermentations are carried on at ordinary room temperature, or on a large water bath, and all the different features of the fermentation are carefully noted—*i.e.* when fermentation commences;

the appearance of the head ; the colour of the head, which can be very different according to the amount of coagulable matter thrown out of solution as the fermentation proceeds ; whether the fermentation is a top or bottom one ; the appearance of the liquid during fermentation (the colour can vary from bright yellow to dark brown) ; the length of time that elapses before the fermented liquid is quite clear. The attenuation should be recorded, but only conclusions of a limited nature can of course be drawn from these figures. Of particular importance when comparing beer yeasts is a thorough comparison, on practical lines, of *the taste and smell* of the fermented liquid. Experience has taught us that fairly reliable conclusions can be drawn from these small experiments as to the behaviour of the types on a large scale in practice. Some experience and taste for the work are required to carry out these practical tests properly.

The earliest researches of the author with regard to *top-fermentation brewery yeasts* (1884-85) have already shown that, by means of such a series of comparative experiments, it is possible to differentiate between culture yeasts that quickly cease to carry on fermentation and, when no particular measures are taken to assist it, only produce a very feeble after-fermentation, and, on the other hand, other types, that carry on their work over a longer period of time and, under the usual conditions prevailing in practice, are able to carry through an after-fermentation as well as a primary fermentation.

After the choice of a yeast has been made, return again to the flask containing the original pure culture

of that type, transfer it to another flask of the same size, or freshen up the yeast in the same flask with fresh wort, and distribute the young yeast after it is grown, as already explained, into three or five $\frac{1}{2}$ litre ($\frac{3}{4}$ pt.) flasks.*

The propagation of the yeast in the Pasteur flasks is carried on as follows :

After the outside surface of the flask has been cleansed with the flame of a Bunsen burner, grasp the flask in the right hand, heat the bent tube of the flask red hot, pull out the glass stopper with left hand, and then place the burner so that the flame plays upon the bent tube. Now pour away the greater part of the liquid, close the flask again with the glass stopper, previously heated in the flame, and shake up the sediment, taking care that the bent tube is kept in the flame all the time.

The work is carried on on a table about 3 feet 6 inches high. The table must be washed immediately before commencing operations. A small tin plate that has been cleansed with the flame is placed in front of the burner. On the left of the plate is placed the small flask that contains the yeast ; on the right, the larger flask of wort that is to receive the yeast. The side tubes of the flasks are opposite to each other. The outer surfaces of both flasks are again cleansed all over with the flame, and the bent tube of the small flask slowly warmed until the lower part of the tube begins to glow. The tin plate is again cleansed with the flame. The side tube of the small flask is now pushed

* A small trace of the young culture is also placed in a flask of saccharose solution and preserved.

into the india-rubber tubing of the larger flask. In order that there may be less danger of infection from the atmosphere whilst the connexion is being made, the side tube of the small flask is first put into the flame of the burner, the india-rubber tubing and glass stopper being pulled off together in the flame and

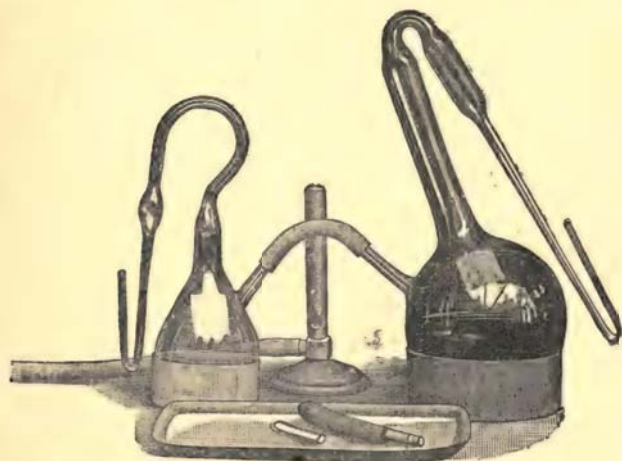


FIG. 7. Two Pasteur flasks connected together.

dropped on to the tin plate placed to catch them; the mouth of the side tube is still kept in the flame, whilst the glass stopper is pulled out of the india-rubber tubing of the side tube of the other flask and dropped on to the plate, and *at the same moment* the side tube of the small flask is pushed into the rubber tube of the other flask. The connected flasks (Fig. 7) are now allowed to stand until the side tube of the small flask is quite cool. The under part of the bent tube of the small flask is next warmed in the gas flame, and the body of the flask is grasped in the right hand, whilst

the Bunsen burner is placed in position against the bent tube with the left. A portion of the yeast is now poured into the larger flask, the air that is drawn in being sterilised as it passes up the heated bent tube.

It is now necessary to close both flasks without letting the germs floating in the air get into them. Take up the glass stopper lying on the tin plate and heat it very hot in the flame; whilst holding this stopper between two fingers of the right hand, hold the india-rubber tube connecting the flasks tightly with the remaining fingers of that hand, and with the left hand draw the side tube of the small flask from the rubber tubing of the large one. Quickly pass the opening of this side tube into the flame of the Bunsen burner, and at the same moment quickly push the glass stopper into the open tubing of the large flask on the right hand, and, lastly, fix the rubber tubing and glass stopper attached on to the side tube of the small flask, keeping it in the flame of the burner whilst doing so.

The contents of a $\frac{1}{8}$ litre ($\frac{1}{4}$ pt.) flask may be distributed over three to five $\frac{1}{2}$ litre ($\frac{3}{4}$ pt.) flasks, according to the type of yeast being developed. The next step is to use the yeast grown in the Pasteur flasks to infect an equal number of Carlsberg cans (Fig. 8), each holding about 10 litres (2 gals.). The yeast is introduced through the tube at the top, and the fermented liquid, and the yeast that has been grown, are withdrawn through the outlet pipe at the bottom. A culture for commercial use is usually grown on in three or four, or even five, such cans, according to the type of yeast.

The Carlsberg cans are used in the following

manner : After making a microscopical examination of the wort in the can, withdrawing for the purpose a small sample through the pipe at the bottom, the side tube of the Pasteur flask is connected with the india-rubber tubing of the small pipe on the sloping top of the Carlsberg can just in the same way as the glass



FIG. 8. Carlsberg can.

flasks are connected up, and the yeast is poured in, the bent tube of the glass flask being kept heated very hot whilst doing so. The tightly fitting cotton-wool filter must be removed from the long pipe of the Carlsberg can before pouring in the yeast, a loose fitting stopper of sterile cotton-wool being put into the pipe in place of the filter, after the introduction of the yeast. The can is then placed on one side in any convenient place where the temperature is suitable.

After the yeast has settled down in these large cans,

usually after five to seven days, the liquid is withdrawn, the bent pipe being kept very hot whilst doing so. After the tubing has been closed again with the clips, the exposed piece of the tubing in front is dried inside with a spill of filter paper; and the glass stopper, after being well heated, is pushed in again to dry the tubing and clean it. Sterilised water is then poured into the can through the side pipe at the top from a Pasteur flask. The yeast is shaken up and a sample drawn out for microscopical examination, and the contents of the three or five copper cans transferred to a travelling can connected up with the lower pipe of the copper can.

The travelling can (Fig. 10) is closed with a cotton-wool filter, if the culture is to be introduced into a pure-culture machine.

Such a pure culture can be preserved for a long time. If there is no use for it directly it arrives, it can be left in the box in which it is packed for the journey, and which must be enclosed in ice.

Such cultures were sent out from the author's laboratory in 1885 for the first time to breweries in tropical countries, and the experiment was completely successful. It is therefore possible to supply breweries, distilleries, &c., in all parts of the world with absolutely pure cultures. It stands to reason that the yeast will be more or less exhausted after a long journey, therefore the first culture in the brewery or distillery must be grown in a very small quantity of nutritive liquid at the favourable temperature of 20° – 25° C. (68° – 77° F.).

If cultures are to stand a long transport by land,

where nothing like the ice-chamber of a steamer is at disposal, they are *dried* in a state of absolute purity.

For laboratory purposes the cultures are sent out on sterilised *cotton-wool* in Hansen flasks (Fig. 3, left). The Hansen flask is attached to the Pasteur flask containing the pure culture, and a few drops of the yeast are poured on to the cotton-wool. On arrival at the laboratory the little flask is connected with a Pasteur flask containing aerated sterilised nutritive liquid, and the yeast on the cotton-wool is rinsed into it.

II. EMPLOYMENT OF PURE CULTURES IN PRACTICE

(a) THE DEVELOPMENT OF THE CULTURE IN A SMALL TUN

The introduction of pure cultures in alcoholic fermentations has obtained recognition as an important advance in all branches of the industry. Attention has sometimes been drawn to the fact that a certain time must elapse before the pure culture works in the same way as the ordinary commercial yeast, and this has been stated to be a weak point in the pure-culture system. The pure cultures require a certain time to accustom themselves to the conditions obtaining in fermentation industries; they must be gradually *acclimatised*. But this is the case with every yeast mass, pure or impure, that is introduced in the brewery or distillery; it is not a special characteristic of the pure cultivated yeast.

The pure culture does not always work during the first fermentations, as regards *outward observable conditions*, in full accord with the usual rules laid down for the conduct of the fermentations in the factory where it is introduced. This is often, however, also the case where only an ordinary change of yeast is introduced in breweries and in distilleries, and one is accustomed in the latter case only to regard the fact as something quite natural, which it certainly is. But in the case of the pure culture, there is this further difference that it must be developed in a *small* tun before introducing it into the ordinary large tuns, and it is a well-known fact that fermentation conducted on such a small scale always exhibits different outward phenomena from those observed in the big tuns. When, however, the race of pure yeast has been properly selected and suits the local requirements, the variations noticeable in the external appearance of the first few fermentations will have no real importance, and by carefully considered management the proper working of the yeast in the large tuns can be secured.

It is self-evident that the new type of yeast must be proved to be satisfactory for the establishment concerned in all essential features; that is to say, it must give to the resulting product the proper character and conditions. In distilleries the required amount of alcohol must be produced during fermentation, &c. It is worth while, therefore, to make a distinction between the features that are important and those that are of less importance, or of no importance at all.

In the case of *brewers' bottom-fermentation* yeast, one cannot as a rule during the early stages of fermentation,

when the yeast culture is developing, judge with perfect certainty to what extent the selected race is capable of accommodating itself to given circumstances.

A small tun is needed for the first fermentations. The culture is grown for a few hours in, say, 5 litres (1 gal.) of wort at 12° – 15° C. (54° – 60° F.),* and then passed on into 80 litres (18 gals.) of wort at 10° C. (50° F.) placed ready in a tun that can hold about 6 hectolitres (4 brls.). Directly fermentation has set in, about 80 litres (18 gals.) of wort, of the same temperature as the fermenting wort, are added, and when the head has been formed, 2 hectolitres ($1\frac{1}{4}$ brls.) of wort at the same temperature as the fermenting wort are added. When the whole contents of the tun are in a vigorous state of fermentation, the fermenting wort and yeast are all run together into an ordinary tun, and roused up with 4 to 6 hectolitres ($2\frac{1}{2}$ to $3\frac{3}{4}$ brls.) of wort at 10° C. (50° F.). After wort has once more been added to it, a gyle of usual length is in fermentation. This fermentation is carried through in the ordinary way.

If, on the other hand, the fermentation is allowed to run its course in the small tun, in order to subject the yeast to a preliminary test, or to enable the yeast to come to rest, if necessary, at this stage of its growth, it must, then, be borne in mind that the appearance of the fermentation will not be quite the same as in the large tuns: the head has often a different appearance, the attenuation may be greater than in the large tuns, a distinct "break" is not always seen in the liquid, taste

* If the culture has undergone a long journey—for instance, a long sea voyage—the first preparation should be in 5 litres (1 gal.) of wort at 20° – 25° C. (68° – 77° F.).

and smell of the liquid may turn out somewhat different, and so on.

Slight differences that are perceptible in these small fermentations are, as a rule, of no importance. Only when serious variations from the rules laid down for guidance in practice are noticed, more particularly in the *smell* and *taste* of the liquid in the tun, has one cause to think that the yeast mass is seriously at fault.

When no signs of developing a "head" are visible, when the attenuation is markedly higher or lower than is desirable, or if an extraordinarily long time passes before any signs are visible of the liquid "breaking" or clarifying, then must care be exercised. If such is the case, it may be advisable simply to remove the yeast from the tun, manipulate it in the ordinary way, put again a suitable quantity of it in the *same small tun*, and repeat the fermentation to see whether the marked differences are transient and disappear or not.

It is without doubt quite possible, as experience has shown, that the operator is often to blame for such great variations making their appearance in the fermentations in the small tuns, when he neglects to exercise the care that must be taken *to keep the yeast pure* during its growth and *to secure a proper growth* of the yeast. In some cases the irregularities that appear are not in any way attributable to the type of yeast employed, but to foreign germs, bacteria, or wild yeasts, that have crept in with faulty manipulation; in other cases he has forgotten to bear in mind that so small a fermentation must be carried through with greater care and attention than the fermentations conducted on a larger scale.

Before all things it is necessary to take great care that the pure culture, on being introduced for the first time into the ordinary wort, should at once *rapidly increase*. Arrangements must be made to ensure the production of a large number of young cells in a few hours. It is most important to attend very particularly to this when the culture is sent off to some distance in a closed can, and is perhaps many days on the journey. This is the reason why the culture is first passed into the small quantity of wort as described, and a strong fermentation is usually obtained after a couple of hours; the rejuvenated culture will then be able to make *a more rapid start* in the larger quantity of liquid, which is of the greatest importance *for the preservation of the culture in a state of purity*. The ordinary wort used in practice for developing cultures always contains foreign germs. When the culture cannot, after being sown in this ordinary wort, bring it rapidly into *pronounced* fermentation, there is every opportunity for the foreign germs to get a good start, and it is well known that these latter have the greatest power of resistance when they are able to develop at the commencement of the fermentation. It is not enough that the yeast cells should show signs of budding a few hours after being sown in the liquid; they must rapidly bring about a strong and distinct fermentation.

There is, however, still another standing rule of equal value, but one that is often neglected. It is—to be careful of *the temperature during fermentation in the small tun*. If this point is not attended to, one not only runs the risk of the yeast being checked in its

growth, and consequently the chance of foreign germs gaining a footing is made easier, but also *the character of the culture yeast itself suffers harm* in consequence. This is a fact that holds good in the case of, at any rate, a large number of yeast types, and it must never be overlooked.

The small tun, in which the pure culture is to be developed, is generally placed in the tun room, the best place for it, as it is not possible in most breweries to find space elsewhere where the temperature is suitable. As mentioned above, the fermentation is started in the small tuns at a little higher temperature than that at which fermentation is started in the large tuns. The temperature rises during a normal fermentation. When the tun room is cold, the lower temperature will have a much greater influence on the small volume than on the larger volume of liquid. Consequently during the growth of the culture the temperature will either not rise at all or else fall back. Such a mishap can be very easily avoided. Instead of using the ordinary means of isolation in the small tun that may easily carry infection if not kept scrupulously clean, all that is necessary is to raise the tun on to a couple of blocks so that it stands off the floor, and place a small spirit lamp or a candle under it. Experience has proved such a small flame to be quite sufficient to enable the yeast to bring about the necessary rise of temperature. A small cylinder may be placed round the light to keep off the draught.

There are finally about 4 hectolitres ($2\frac{1}{2}$ brls.) of wort undergoing fermentation in the small tun.

If the yeast should be subjected to a preliminary test at this stage, or if, on account of its specific peculiarity, it should be preferred to let it rest, the fermentation should be carried through before developing it further and the yeast settle down, as it will in the ordinary course of things. If the taste and smell differ to a marked extent from what is usual in that respect, see if these differences disappear after carrying through another fermentation in the small tun, starting the fermentation with the proper proportion of yeast.

If everything has proceeded satisfactorily, the yeast that has been grown is now added in the usual proportions to wort in one of the large tuns, and when a head has been formed, the tun is filled up either at once or gradually with wort of the same temperature as that of the wort that is fermenting.

Even if the attenuation of the first fermentation in the large tun varies somewhat from the usual limits, and this sometimes may well happen, it will not make a great difference in the end, as the beer when racked is distributed over several casks.

With regard to the so-called "break" in the liquid, it should be here pointed out that there are races of yeast that work excellently, but do not effect a true distinct break at the end of primary fermentation.

The rapid growth of the culture in the manner first described secures the advantage of its uninterrupted development ; thus danger of infection is lessened, and the yeast can be transferred all the sooner to the large tuns. There are, however, a few types of yeast that suffer during the first fermentations on a large scale as

the result of so rapid a growth—for example, in respect of power of clarification—and they must consequently be allowed to carry the fermentation through to the end in the small tun.

In a properly managed business only one race of yeast is employed, and experience will show how to manipulate the pure culture during its period of growth—whether the slow or the rapid development should be preferred.

Often the brewer does not select the pure culture from his own ordinary yeast, but allows the laboratory to select a type that is already well known in practice, giving him at the same time some hints about the best method of treating the culture.

Brewery top-fermentation yeast will always grow rapidly, owing to the higher temperatures employed, and fermentations can be started with a smaller quantity of yeast than when fermenting with bottom-fermentation yeast. It is advisable to be particularly careful when types of yeast that clarify quickly are employed, and in any case to allow the fermentation of, say, 5 to 10 hectolitres (3 to 6 brls.) to continue until a distinct clarification is noticeable before making use of the yeast produced. Only intimate acquaintance with the race of yeast itself will enable one to decide whether it will stand being rapidly developed. At any rate the development must take place at higher temperatures than in the case of bottom-fermentation yeast. No general rule can be laid down, because top fermentation is carried out at very different temperatures in different countries.

A pure culture of *distillery yeast* does not, when

introduced, actually present greater difficulties in acclimatising it than ordinary yeast introduced from another distillery. The culture may be propagated in the saccharified sweet mash of the distillery, which should have a concentration of about 15 per cent. (Balling) and by addition of sulphuric acid be brought to an acidity of 0.6–0.7 N.S. But it is preferable to employ the *Lactic Mash*. The mash must first be warmed. By this means a good deal of the foreign germs are checked, if not completely stifled; the same effect is also brought about by the high temperature of acidification and by the lactic acid produced.

The initial quantity of mash, 5 litres, is increased to the requisite amount by successive additions, each five times as much as before.

Seeing that the acidified—lactic—mash is after acidification always warmed to 75° C. or thereabouts, it follows that the mash in which to grow the yeast culture need not be warmed again.

After the 5 litres of mash have been pitched with the yeast culture, the temperature should be about 25° C. This small portion of mash is now covered up and left to undergo fermentation in a warm place; the temperature of the mash, however, must not rise above 30° C. After the completion of the principal fermentation the acidified wort is, then, gradually increased to the requisite amount by adding fresh mash.

If the yeast developed is to be used for distilling purposes, the pitching temperature must be by slow degrees lowered until the usual level is arrived at.

As for the propagation of yeast cultures in air-yeast

manufacture, a similar procedure must be adopted, only with this difference, that the mash is replaced by concentrated lactic wort (original wort). Before pitching the principal fermenting vessel, the yeast is slightly aerated.

Pure cultures of *wine yeast* are employed partly to ferment grape must, and partly to produce a normal fermentation of different kinds of fruit must.

When fermenting *grape must* with a pure culture of wine yeast, the object is to develop the selected type at the earliest stage possible, in order that it may at once hold in check the development of the many and various germs in the must. Amongst these germs the small lemon-shaped yeast is one well-known form that commences to grow at once, and this type of yeast is commonly supposed to be a disease-producing yeast in wine. The selected race of wine yeast will therefore be enabled to assert itself more actively if introduced during the mashing or pressing of the grapes, the yeast being first strongly diluted and then poured out on them. It may be necessary to add some more yeast after the lapse of a few days.

No general rule can be laid down stating the proper proportion of yeast to add to the must. The proper proportion to add in each case depends, as already stated, upon the amount of yeast found on the grapes, which varies very much each year.

The quality of the grape must has to be taken into consideration when determining the amount of pure yeast to be used. With light wines about $\frac{1}{4}$ – $\frac{1}{2}$ ‰ of vigorously fermenting must can be employed; with heavier wines up to 1‰.

But the importance of a proper after-fermentation also limits the amount. If too great a quantity is added at first and the primary fermentation is too strong, the yeast will work out too quickly, and the wild germs can then again take up the work at a later stage.

The proper rule would certainly appear to be not to add too much yeast, but to employ it rather sparingly.

It has been found that the proper employment of a pure culture of wine yeast at advanced stages of the fermentation is of great value—namely, where a micro-biological examination shows that wild ferments are multiplying. But no general rule can be laid down even here. The matter must be settled in each particular case according to previous inquiry. All that can be said is that it is often important to add larger quantities of fermenting grape must than stated above, up to, say, 2–3%.

Pure wine yeasts have proved themselves of great use in producing a pure fermentation of the *juice of fruit and berries*.* During the spontaneous fermentation of these juices strong growths of bacteria and yeasts, having a most undesirable effect, are often the first to develop. It would therefore be advisable to pasteurise the juice before adding the pure culture yeast.

Pure cultures of wine yeast—Rhine wine yeast, port wine yeast, &c.—must not here be used in too small quantities to suppress the wild germs; and as the

* A methodical use of the yeasts found on the fruits themselves would probably give us still better results.

after-fermentation is generally of short duration, it does not matter if the primary fermentation is vigorous. A fresh culture of the yeast can be afterwards added at a later stage, if it is desired to maintain a vigorous after-fermentation continuing over a long period.

The absolutely pure culture, say 1 litre ($1\frac{3}{4}$ pts.) of very diluted yeast, is added to 5 litres (1 gal.) of juice that have been previously boiled, and allowed to ferment at 20° – 25° C. (68° – 77° F.). When in a state of vigorous fermentation, the whole is added to, say, 1 hectolitre ($\frac{1}{2}$ brl.) of juice, and this juice, after fermentation has set in, is employed as a ferment in the proportion of 5 to 10 litres (1 to 2 gals.) per hectolitre (about 22 gals.). The stronger this fermentation is, the smaller will be the amount of fermenting liquid required.

Without doubt the best results are obtained by adding the yeast, after development, directly to the *mashed or pressed* fruit. The fermentations in bulk proceed most satisfactorily at 12° – 15° C. (54° – 60° F.).

(b) DEVELOPMENT OF A YEAST CULTURE IN A PROPAGATING MACHINE

Special plant has been constructed in order that large quantities of absolutely pure yeast may be ready at hand; it is so arranged that if the instructions are closely followed the pure culture introduced can be kept pure for an unlimited time. It is also necessary to provide for the stock being so preserved that it will not change its properties during continued development in these nearly closed cylinders.

If the rules of instruction are properly followed out,

the first requirement can be satisfied without any difficulty. The second requirement sometimes gives rise to difficulties, and particular attention is always required in the case of special types of yeast.

The two types of pure-culture machines in use are HANSEN AND KÜHLE'S (Fig. 9, large model) and BERGH AND JÖRGENSEN'S (Fig. 11, small model).

INSTRUCTIONS IN THE MANAGEMENT OF PURE-CULTURE MACHINE

1. When using the large model proceed as follows :

STERILISATION

Both the cylinders and the pipes connected with them are *sterilised* with *steam*. The *air filters* are sterilised for two hours at 150° C. (300° F.)

The fermentation cylinder.—The steam is brought in through the upper tap on the pipe *kk*. All the taps connected with the cylinder are alternately opened and shut, so that the steam can pass through them all, first one and then the other, also through the glass tube *f*. This blowing through with steam should be kept up for half an hour. The filter is screwed into place directly the steam has been shut off for the last time from the pipe with which it is connected. All the taps are closed with the exception of *c*, and cooling commences, cold air being let in through the filter *g* and the cock *h*. The steam is gradually shut off, and the cylinder will be cool in about two hours' time. It is necessary to keep up a certain excess of pressure in the cylinder whilst this work is going on. In order to control this pressure with certainty, the bent end of

the tube *c* is placed in a small pail of water. If there were allowed to be a vacuum for only a moment in the

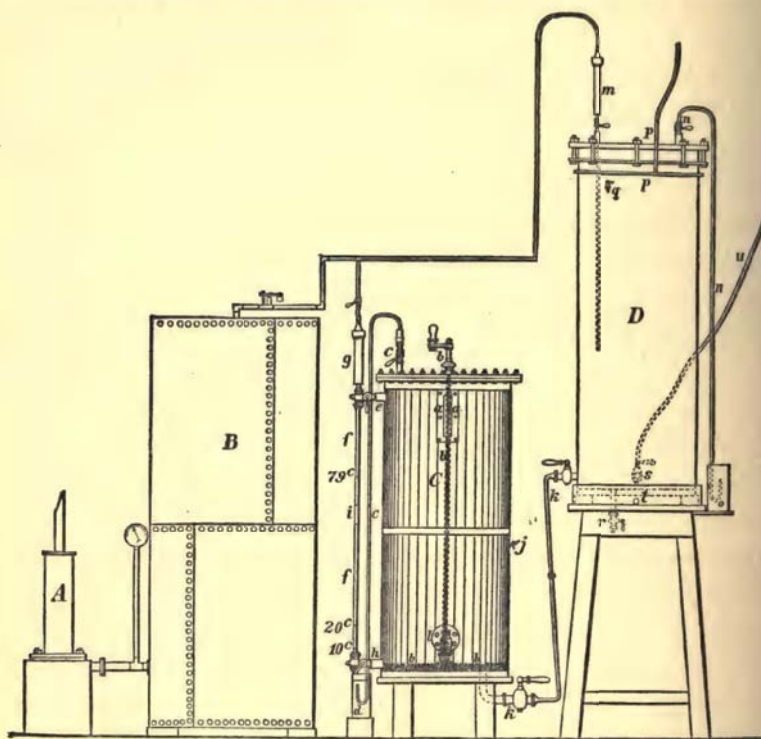


FIG. 9. Pure yeast propagating machine (Hansen-Kühle).

A. Air pump. *B.* Air reservoir. *C.* Fermentation cylinder. *a.* Window. *bbb.* Rousers. *cc.* A pipe with both ends curved. *d.* A bowl containing water. *l.* Outlet tap. *ff.* Glass tube connected with the cylinder by the pipes *e* and *h*, and marked off in order that the volumes of wort contained in the cylinder can be measured. *g.* Filter. *i.* An india-rubber connexion in the centre of the glass tube. *j.* Pipe and india-rubber end, through which the pure culture is passed into the cylinder. *kk.* Pipe connecting with *D*, the wort cylinder. *m.* A filter. *nn.* A tube with both ends curved. *o.* A bowl of water. *pp.* A pipe for spraying down the sides of the cylinder. *u.* Wort pipe, connecting with the cock *s*. *z.* Waste pipe for the cooling water. *q.* A tap (the cylinder is filled up to this level). *r.* An outlet pipe.

cylinder, impure air would be sucked in. If the steam were suddenly shut off, the resulting vacuum might be so great that the cylinder would be pressed in.

The wort cylinder.—The steam is conducted in through the lowest cock of the pipe *kk*. The pipe *su* is sterilised at the same time as the cylinder, so that the steam rushes freely through the main wort pipe that brings the wort from the hop-back. In other respects this cylinder is sterilised in exactly the same way as the fermentation cylinder. Of course this cylinder should not be cooled down.

ARRANGEMENT OF THE WORT CYLINDER

The wort must be ready for use about ten minutes before steaming out is completed. At the same moment that steaming out is stopped, the filtered air, and afterwards the wort, which must be as hot as possible, are allowed to enter. The first small quantity of wort has become a good deal diluted with the condensed steam on flowing into the cylinder, and consequently contracted an unpleasant taste; this should be drawn off through the tap *r*.

It is possible that under certain circumstances the wort may have cooled down to such an extent before entering the cylinder that suction through the tap *q* can take place. Such suction must be guarded against by allowing the cylinder to cool down to, say, 90° – 85° C. (195° – 185° F.) before letting the wort flow in. The pipe *u* must in such cases be isolated. If any suction has taken place, it is necessary to boil the wort again

by means of the steam coils placed in the cylinder for this purpose.

The wort flows in until it has risen to the level of the tap *q* and begins to pour out of it; the tap *s* is then closed.

The air and the steam that enter the cylinder with the wort escape out partly through *q* and partly through *n*.

AERATION AND COOLING OF THE WORT

The tap *q* having been closed, sterile air is now passed through the hot wort, passing into the cylinder through the filter *m* and the long pipe in connexion with it. Aeration is kept up for *one to two* hours before commencing to cool down the wort. Aeration is also kept up uninterruptedly whilst the wort is being cooled down.

A pressure of 1 to 2 atmospheres is sufficient in the air reservoir. There should always be a slight excess of pressure in the wort cylinder to ensure the wort taking up sufficient oxygen, and no unfiltered air being drawn in.

After one to two hours' aeration of the wort, the cylinder is cooled down, water being sprayed down the sides, outside, through the ring *p*, until the temperature of the wort is reduced to about 10° C. (50° F.) in the case of bottom fermentation. This occupies about one hour. Any further cooling, if necessary, must be carried on with ice-water. After cooling down to, say, 11° C. (52° F.), froth passes through the pipe. It is not advisable to aerate too vigorously, as too much wort may be lost in so doing.

The froth is less troublesome if the receptacle *o* is filled with warm water. In case of top fermentation the wort is cooled to, say, 20° C.

TRANSFER OF THE WORT TO THE FERMENTING CYLINDER

The long pipe from the filter is closed and the short pipe opened. The cock *n* is closed, and then the cocks on the pipe *kk* are opened, and the necessary amount of wort is allowed to flow in.

If, as in the drawing, the feed pipe *j* is situated on the side of the cylinder, the pure yeast must be passed in before the wort comes up to its level. In some cylinders this pipe is placed in the top of the cylinder. This arrangement has the advantage that the operator introducing the pure culture can place the lamp in front of him, and complete the work without further assistance, whereas in the first case the assistance of another man is required to hold the lamp.

The culture is passed into the cylinder from a Pasteur flask or from a travelling can* (Fig. 10), constructed on the same principle as the Pasteur flasks, but with the addition of a cotton-wool filter to the pipe through which the air is drawn in whilst the yeast is being poured out. The can is washed with alcohol, and shaken for a few minutes to loosen the yeast and distribute it through the water in the can, and the side tube of the can or flask is connected up, in a small flame, with the small rubber connexion on

* This holds, say, 1 litre, and receives the yeast grown in a number of flasks.

the side or the top of the fermenting cylinder. This rubber connexion is closed with a glass stopper. A spring clip is fixed on the rubber, or this is fixed on an ordinary tap. After the pipe on the side of the can has been connected with the cylinder, open



FIG. 10. Travelling can for the despatch of pure cultures of yeast.

first the tap on the cylinder and then the tap on the side pipe of the can ; a large proportion of the contents of the can will be at once discharged into the cylinder owing to the pressure of the carbonic acid gas : then only is the tap situated on the other pipe connected with the can and below the cotton-wool filter opened, and the remainder of the yeast allowed to flow out of the can into the cylinder, or forced out by blowing through the filter. The spring clip or the tap on the

cylinder is then closed, the glass stopper is heated in a flame, the side pipe of the can is withdrawn from the rubber tube of the cylinder, and the latter is closed with the glass stopper whilst still hot. The glass stopper is taken out of the rubber tube, at the same time squeezing the tube in order to drive out any liquid remaining in it, again heated, and finally fixed tightly into the rubber tube again.

It is advisable for breweries that obtain their cultures from a distance not to fill their cylinders full at once, but only to run in about 15 litres (3 gals.), and let the yeast develop in that first, at a somewhat higher temperature, say 15° – 20° C., and when a vigorous fermentation has set in to add what wort is still required to fill it.

The *withdrawal of the yeast* from the large size cylinder is usually carried out as follows: The fermented liquid is first withdrawn under slight pressure; a little cooled and aerated wort is then run in from the wort cylinder, and yeast and wort roused up with the rouser in the fermenting cylinder and the diluted yeast then drawn off. Some of the yeast is left behind to start a fresh fermentation. What amount is necessary must be ascertained, as it may vary with the type of yeast. The following may serve as a guide:

The gauge tube or the cylinder is marked off with three marks at 80, 20, and 10 cm. (32, 8, 4 inches) from the bottom of the cylinder respectively. When the fermentation is completed, the beer is drawn off under pressure through the tap *L* until frothing commences. Wort is then run in from the wort cylinder until the next lowest mark is reached, roused

up, and drawn off into a clean tub until the lowest mark is reached ; then wort is again introduced, until the next lowest is again reached, roused up, and again drawn off until the lowest mark is reached. The new fermentation is started with the yeast now left behind, wort being run in up to the top mark on the gauge tube.

With the *small model* of the propagating machine (Fig. 11), consisting of a small cylinder (A), in which yeast is kept, above the large cylinder that serves as a wort or fermentation cylinder as required, operations are carried on as follows : While the pure culture is being introduced into the upper cylinder, a little wort is raised by pressure from the lower cylinder into the upper, and the yeast is then washed down with it into the lower cylinder. When a strong fermentation has set in in this lower cylinder, the liquid is carefully roused and some of it is forced up into the upper cylinder, where it remains until the next fermentation. The cylinder B is surrounded with a cast-iron jacket divided into two compartments, the lower half serving as a steam jacket, whilst the upper one is used to cool the wort and control the fermentation.

The task, therefore, which we have to solve is not only to obtain a sufficient quantity of yeast within a reasonable time with the help of these machines, but *also to obtain a yeast that works normally.*

If possible, the brewery wort must be used in the cylinders in the state it is in as it passes from the hop-back to the coolers, being carried to the cylinders by a pipe connected with the main wort pipes delivering the wort on to the coolers from the hop-back.

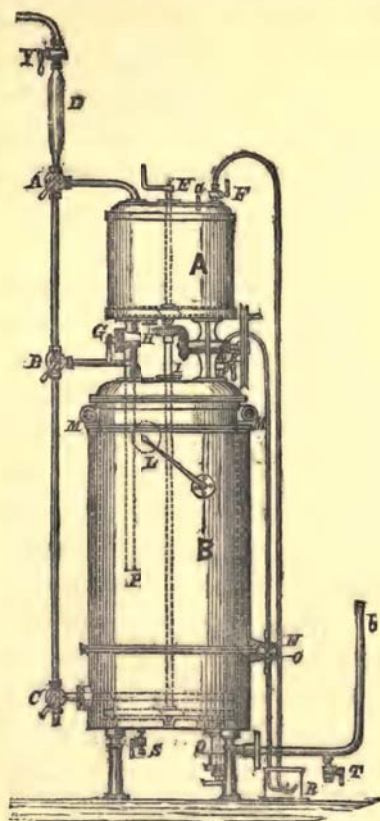


FIG. 11. Pure yeast propagating machine (Bergh and Jørgensen's model).

A. Yeast cylinder. **B.** Wort and fermentation cylinder. **A, B, C.** Three way cocks. **E.** Rouser. **a.** A pipe for introducing the yeast and for withdrawal of samples. **F.** A bent tube for the escape of CO_2 . **GP.** Connecting pipe between the two cylinders. **G.** The tap. **H.** Outlet pipe for the water used for rinsing. **O.** A tap for letting steam into the steam jacket. **S.** A tap for letting out the steam. **M.** A pipe for letting out water for cooling down the cylinder. **N.** A tap for carrying off the cooling water. **I.** A rouser. **L.** Indicator and float to register the height of the liquid in the tun. **K.** A pipe curved at both ends. **Q.** A tap in connexion with the pipe **b** (with tap **T** attached). **R.** A vessel to contain water.

It is known that if the wort is re-boiled in the cylinder, a considerable amount of matter is thrown out of solution, thus giving it a new composition, and there is no doubt that if the yeast is grown for a length of time in such wort, different from the wort used in the

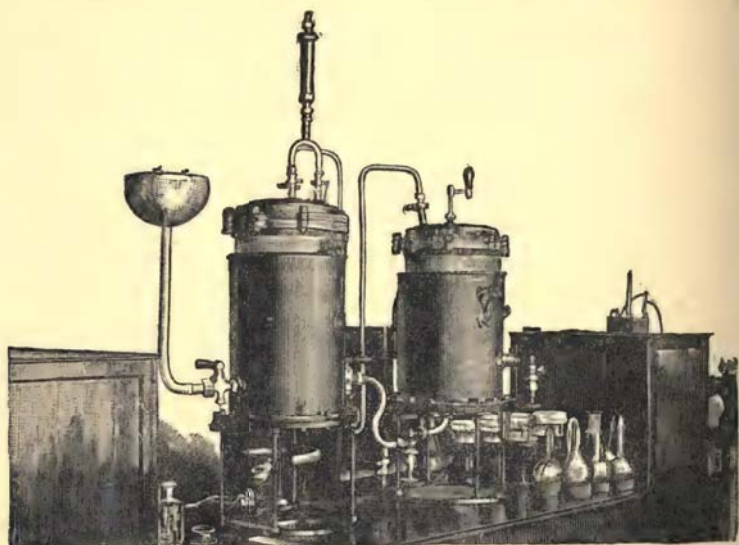


FIG. 12. Model of pure culture apparatus, set up in the author's laboratory.

brewery, its character may be altered little by little. When circumstances render it necessary to boil the wort in the cylinder, one often is obliged to introduce fresh cultures of yeast at intervals of a few months.

If one adds malt substitutes to the wort, there is always the danger that the yeast in the cylinder will degenerate in a proportionately short time.

The hot wort in the cylinder must be cooled down slowly, and must be well aerated both at high and at

low temperatures. When the selected race of yeast appears to work irregularly in any way, and particularly if clarification proceeds too slowly, experiments should be carefully carried out in different directions to ascertain the cause, the length of time already given for cooling being taken as a guide. One valuable remedy to be recommended in such cases is to prolong the length of time of aeration at the higher temperatures by running hot water into the jacket surrounding the cylinder.

It is, however, as a rule easier to master the clarification and attenuation when close attention is paid to *the fermentation itself*. Not only *the amount of yeast* used, but *also the temperature, duration of the fermentation*, and *aeration* during fermentation, all play a part when a good yeast is to be continually produced in the cylinder.

Fermentation proceeds under different conditions in the cylinder from those which exist in the open vessels, and one cannot therefore draw a very close comparison between the two classes of fermentations.

It is necessary to commence with a suitable quantity of yeast in order to develop the required quantity within a reasonable time under ordinary conditions of fermentation. It would hardly be correct to set up exactly the same proportions as in the large tuns, in order that the *course of the fermentation* should be the same, because the carbon dioxide given off during the fermentation can only escape through the very narrow bent tube, so there will naturally be pressure on the surface of the liquid, even when the carbon dioxide is removed by air being pumped in; consequently the

fermentation will proceed more slowly. Therefore some yeast types at any rate require to be used in rather larger quantities than in the ordinary tuns.

Temperature, however, is of more importance. It is certainly inadvisable to work continually at temperatures that differ considerably from those employed in practice. Higher temperatures may be used experimentally for certain exceptional fermentations. Cases have arisen in practice where it has been advantageous to do this: for example, a brewery top-fermentation yeast, cultivated *once* at a temperature only just lower than the temperature in which it could live, gave a better clarification than before. On the other hand, it is not at all advisable to let the yeast in the cylinder be acclimatised to temperatures quite different from those maintained in the tuns. The fermentation should be carried on one or two degrees higher than in the tuns; cooling down can be carried out at the close of the fermentation just as well in the case of top yeasts as of bottom yeasts, in order to ensure a better settling out of the yeast.

The *duration* of the fermentation also plays a part. To start with, the rule must be laid down that the yeast in the cylinders must pass through a *complete primary fermentation*. To take the yeast when a vigorous fermentation has set in—that is to say, when the yeast is still suspended in the fermenting liquid—certainly offers the advantage that the yeast can be renewed in the large tuns more frequently; but to carry out this principle as a regular thing may be detrimental to the particular type. Normal working is to take the yeast after it has settled out of solution

on the surface or at the bottom: to take it when fermentation is at its height is an exception to this rule.

Aeration in the fermentation cylinder is of the greatest importance. The view has been expressed that as the yeast in the closed-in cylinder is cut off from the influence of the air, fermentation must be assisted by pumping air through the liquid. But experience has shown that in this respect many yeast types, both top and bottom yeasts, cannot stand such vigorous aeration, and perhaps particularly the strong motion set up; if so treated, such yeasts may undergo a change both in respect to clarification and also powers of attenuation. The rule as regards aeration in the fermentation cylinder must, under ordinary circumstances, be as follows: Immediately the fermentation is started, a slight current of air is passed through the liquid, partly to secure a thorough distribution of the yeast cells, partly to replace the free oxygen taken up by the cells during the first hours. The bottom air valve is then closed, and the liquid is allowed to remain undisturbed. Subsequently during the whole course of the fermentation a very slight current of air is passed *over the surface* of the liquid to gradually drive off the carbon dioxide produced during fermentation.

The above-mentioned rules are given as *general directions* for the proper working of a pure yeast culture machine. If any change appears in its quality, the special requirements of the type of yeast in use must be carefully studied and its treatment in the cylinder regulated accordingly.

The pure-culture yeast cylinders have been found

useful not only in breweries but also in *distilleries working with molasses*, and in other *distilleries and yeast factories*. No difficulty is found in the molass distilleries in working the cylinders under the same conditions as those of the large fermentations, as the liquid employed does not appear to undergo any important change in respect of its nutritive value for the yeast when it is boiled in the cylinder. A sufficient supply of nitrogenous compounds is sometimes added to the molasses in the cylinder to secure a more vigorous growth of the yeast. On the other hand, in ordinary distilleries and yeast factories the liquid undergoes considerable changes owing to the liquid being boiled to secure sterilisation—a considerable quantity of nutritive matter being thereby thrown out of solution. This loss must be made good, but it still is not possible to restore to the liquid the same composition that it usually has. If the yeast undergoes too great a change under these conditions, there is nothing for it but to pass it several times through a lactic acid mash before it is used on a large scale.

The normal way of withdrawing samples from the cylinder for analysis is through the pipe by which the pure culture is introduced. A Pasteur flask or a flask specially constructed for the purpose of forwarding yeast in it (Fig. 3, left) is connected up with the cylinder in the same way as flasks containing pure cultures are connected. The sample is taken a few days before the close of the fermentation. The greatest possible care must be taken in collecting these samples, as the most searching methods of analysis are employed to ascertain if

the culture in the cylinder is absolutely pure. It sometimes happens that slight traces of the liquid have been left behind round the mouth of the pipe or the rubber tube on the cylinder. In order to be quite safe, the tube and stopper are first washed with a little alcohol; the glass stopper is then heated in the flame and replaced in the rubber tube and allowed to remain there until quite cool, and then the sample is taken.

A portion of the sample is examined to detect wild yeasts, *Torula* and *Mycoderma*, and a portion to detect bacteria.

In order to detect the slightest trace of wild yeast, a portion of the sample is placed in a 10% solution of cane sugar, with tartaric acid (1-4%) added. An average sample of the yeast developed in this solution is taken, and again treated in the same manner. A little of the growth is then passed into a flask containing hopped wort, and re-invigorated. The least trace of wild yeast or *Mycoderma* originally present will have become so strongly developed by this treatment that the infection will be at once evident on examining spore cultures of the growth developed on blocks of gypsum.

The rest of the sample is next examined in order to detect bacteria. If actual bacterial *development* has taken place, the microscope will reveal it. In order to discover even a *trace* of bacteria capable of developing, a few drops of the liquid are introduced into a flask containing neutral or slightly alkaline yeast water, and kept at 25°-35° C. (77°-95° F.). The bacteria will then develop in this solution. It is not all bacteria that will, when grown in this way, make the liquid

cloudy ; after allowing sufficient time for their development, the contents of the flask must therefore be examined under the microscope.

A general microscopical examination of the yeast in the cylinder is undertaken to find out whether the yeast has grown *uniformly*. It sometimes happens that the inside tinning of the cylinder wears away in places, the liquid takes up salts of copper, and consequently the growth of the yeast is sickly. This is ascertained by a microscopical examination, because a proportionately larger number of exhausted cells will be found—that is, cells whose contents have a very different appearance from that of normal cells.

It is important to empty out the fermentation cylinder at least once a year, to examine its interior carefully, and inspect all the smaller parts of the machine.

III. DEGENERATION OF THE YEAST

VARIETIES DEVELOPED WHILST YEAST IS IN USE

The pure cultivated alcohol yeast is not only free from all disease-producing germs, but consists of *one single species* only.

Only when employing one single species is it possible, as already stated, to carry on the fermentation with regularity and certainty. Every attempt to secure constant and uniform results will be wrecked if the yeast is a mixture of several different types, because such a mixture cannot preserve its original composition unchanged after repeated use.

Only when the yeast is composed entirely of one species is it possible to obtain a real knowledge of its nature.

In practice it is clearly impossible to ascertain by a *systematic selection* the yeast most suitable for one particular method of work, when in every fermentation more than one type is to be found.

But practical work with *pure cultures* of yeast has shown that *there are very great race differences* between the cells of the same yeast *type* in daily use. In the days when unpurified yeast was used, there was no idea that such was the case, and that these differences might be turned to great advantage.

Accurate conclusions as to the great difference between yeast *types*, particularly with regard to the effect of their respective characters in practice, can only be drawn after carrying out *accurate comparative experiments* in a laboratory especially fitted out for the work in view.* In carrying out such accurate comparative experiments new discoveries may be made and marked industrial advances consequently secured.

When using the different types of yeast in breweries, distilleries, the wine industry, &c., it must be clearly understood that *there are definite limits to the possibility of a particular type bringing all its qualities to the front under industrial conditions.*

Certain characteristics of yeast may make their influence felt in varying degree under very different conditions in practical life; whilst there are others that only exert their influence when certain definite external conditions exist.

* Refer to the chapter dealing with the production of pure yeast.

To take an example of the first state of affairs. A yeast that imparts a particular pronounced flavour in hopped wort, or in wine must, will always impart the same flavour to the same type of liquid, even when the composition of the latter is changed not a little. The *strength*, however, in which this flavour is present will vary with the composition of the liquid and with the whole course of the fermentation.

Yeast types which set up a vigorous alcoholic fermentation are so able, even when ordinary wort or when must shows varying compositions, to make their influence felt that the final attenuation shows the character of the race. But not only the real limit of attenuation, but also the stage when the fermentation is seen to be working particularly vigorously when compared with that produced by other types, can vary in the case of the same type, because they depend upon outside conditions.

As an example of the last-named characteristic, it may be said that weak fermenting brewery yeasts often only show this characteristic feature when the wort has a composition favourable to it and the fermentation is conducted in a particular manner.

Every yeast, even the most typical, is accordingly to a certain extent dependent upon the conditions under which work is carried on. The result of the fermentation is a product of the yeast and liquid; a selection must therefore be made between the types and also *between the varieties of the same type* in order to select the particular race that will secure the desired result *under the particular circumstances that present themselves.*

When this has been found, it is noticed that the slight variations that make their appearance in every method of work have no influence on the result of the fermentation as a whole. In other words, under so-called normal conditions of work, the selected type of yeast will work normally in respect of the essential features. It is actually in this respect that the great advance brought about by the employment of pure yeast lies.

But, just as the yeast is always dependent upon the nutritive liquid and external conditions, so also the *long-continued influences of any particular kind* will be apt to cause radical changes in the character of the yeast race, and it may under such circumstances almost entirely lose, for example, qualities that are appreciated in practice. Such influences may, for instance, be introduced into a brewery, when new plant is put up; when the composition of the water is changed; when the aeration of the wort is conducted in a different way, and so on. The fact that under such conditions changes in the character of the yeast mass itself can actually take place is borne out by special experiments made in practice, and, consequently, the unsatisfactory issue of fermentation is not attributable solely to the altered composition of the fermented liquid.

When ordinary unpurified brewery yeast was used, one was accustomed to the fact that the fermentations showed variations during their course, and one came to look upon it as quite unavoidable. But the use of selected races of yeast has led to more rational methods of work and the demands for uniformity in fermentations have increased.

If the yeast mass has manifestly changed its character, find out first of all if wild yeasts or bacteria are developing.

Only when the yeast is found to be sufficiently pure for commercial purposes are such deviations to be accounted for by a *degeneration of the yeast* itself. This is only natural; but the difference deserves to be brought to notice, because the two things are often confused one with the other. It must here be borne in mind that a pure primary fermentation is no absolute security for the beer being pure at the end of the after-fermentation. Practice has shown often enough that it is necessary to control the after-fermentation also, because certain impurities which are found in the casks are able to exert an influence in the last stages of the fermentation.

The degeneration of the yeast manifests itself, according to the author's observations, by the fermented liquid developing a different *flavour* or the *attenuation* or *clarification* being different from the normal state of affairs.

The comprehensive researches carried out in the author's laboratory in regard to the yeast used in daily practice have disclosed the important fact that it is *not all the cells that degenerate* in such a case. If a sample of this pure yeast is taken and a dilution made, the individual cells being well separated from one another, a comparative series of fermentations will show that there are differences between these cells. Some of the new growths will show the newly developed disagreeable character to a marked degree, others to a less extent; but it has always been

possible on taking a sample from the tun to find cells of the same race that had not changed their character.

Based on the foregoing remarks is the following method of regenerating pure yeast. Single cell cultures are prepared from it as described in a previous paragraph, and a selection is then made by means of comparative fermentation experiments among the isolated single cell cultures to obtain a culture similar to the original. This culture therefore starts from a particularly resistant cell.

The pure cultures should be preserved in the laboratory in the manner described in the following chapter. Therefore when the yeast degenerates, one has first to fall back upon the culture preserved in the laboratory, and develop a pure supply of yeast from it for practical use. In this manner the yeast is more quickly regenerated. The method first described solves the difficulty if the yeast preserved in the flasks has undergone any change.

The foregoing statement that some cells, having varying characteristics, will in the course of time appear in the yeast as a consequence of the changed composition of the nutritive liquid, is not to be confused with that change in the attenuation which may *suddenly* make its appearance when a malt of very different composition from that previously used is taken into use. When this happens, and the usual general known rules do not help, one must, for a time at any rate, use a yeast of a different type.

It is evident that the afore-mentioned system of selection between individual cells can be utilised to *improve still further*, in certain respects, a yeast that

is giving satisfaction. Yeast samples can be taken from fermentations in the factory that show the desired characteristics to a marked extent, and from this yeast pure cultures may be systematically developed. As an example, it may be mentioned that brewery bottom yeasts which develop a very strong fermentation, as a rule clarify out slowly and with difficulty. In this case the object of *a systematic selection* has been to separate out the individual cells that will produce the strong fermentation characteristic of the type, but will at the same time clarify out with less difficulty during after-fermentation.

IV. PRESERVATION OF YEAST

Many races of yeast may be preserved in the laboratory for a longer or shorter time without changing their specific qualities, which make them valuable in practice. One can therefore always renovate such a yeast by growing a very little of the culture that is being preserved, and gradually increasing the quantity by passing the young growth to larger and larger flasks.

The best-known medium for preserving yeast is a clear solution of ordinary commercial cane sugar and distilled water. The solution—10 per cent.—is filtered bright and sterilised in the flasks. The flask used for these cultures is one designed by the author; it takes up less room than the Pasteur flask, and evaporation of the liquid during long storage is checked (Fig. 3, right).

A small quantity of the selected culture is intro-

duced into the cane-sugar solution, and the flasks are kept in a dry place. If the cotton-wool filter in the cap becomes damp and consequently mouldy, mould and bacteria can easily grow through it and infect the culture.

The culture must be kept in the dark and undisturbed. If it is often shaken up it will be weakened, and may change its character.

With careful attention many brewery, distillery, and wine yeasts can be preserved in the foregoing manner. There are exceptions among top-fermentation brewery yeasts, for example, when it sometimes happens that after about six months' preservation changes have set in in the character of the race; consequently one has to fall back upon the yeast used in the brewery in order to secure a normal culture. Also a few of the bottom-fermentation yeasts, that ferment only slightly, undergo changes after being kept for a comparatively short time.

Variations in temperature that are not too marked do not seem to have any effect on this method of yeast preservation; it is, however, clearly impossible to lay down any general rule that is suitable for so many widely different races.

When the culture is required for use, the stock flask is connected on to a Pasteur flask containing the liquid, well sterilised and aerated, that is used in practice. The flask with the cane-sugar solution in it is shaken most carefully, only just enough to render the liquid slightly clouded. A few drops are then transferred to the Pasteur flask. The first cultures are allowed to proceed quietly, the fermentation being

allowed to finish before passing the yeast to a fresh flask. Only when one has ascertained that the dormant yeast will stand being rapidly developed can one push on the propagation after the yeast has shown the first signs of fermentation in the flask.

In the case of yeast that would not stand being repeatedly shaken up when a drop of it is passed into

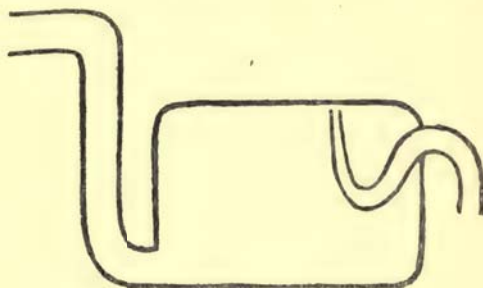


FIG. 13.

the Pasteur flask containing the wort in which to develop it further, the flask shown in Fig. 13 has been found suitable. The outmost part of the small bent tube to the right is plugged with cotton-wool, through which the air penetrating into the flask is filtered. The wide tube to the left hand is closed with an asbestos stopper, and its undermost bend is on a level with the bottom of the flask. If this tube is put, in a flame, into connexion with the lateral tube of the Pasteur flask, a little of the yeast which has settled to the bottom of the small flask can, by suction through the bent tube of the Pasteur flask, be drawn into the latter without the remaining part of the sediment yeast in the small flask being disturbed.

In practice, distillery yeast is preserved in a pressed

state. Brewery yeast may also be preserved in the same way, if it is desired to keep the yeast when no brewing is going on, in order to commence a new season with the same yeast, and use it until a pure culture has been worked up. After the yeast has been washed, it is pressed in double-fold or three-fold bags and hung up in a dry, airy place. If any mould appears on the outer bag, this is taken away and the inner bag is washed with alcohol that contains a slight trace of salicylic acid, or with a solution of water and boracic acid. The washed yeast may also be pressed and stored in newly pitched or perfectly clean barrels, which are then closed and stored in ice.

It has often been found more suitable to preserve the brewery yeast in a liquid state. The yeast can be washed several times in water that has been well boiled and then aerated. The *thin solution* of yeast is then poured into a cask that has been freshly pitched; the cask is then tightly bunged down and covered over with ice. Or the yeast can be passed into the cask after being *simply strained off*. A yeast carefully handled in this way has been known to keep all right for two months. It naturally follows that some of the cells have died; therefore, when using the yeast again for the first time, a larger quantity than usual should be used; also, the fermentation should be pitched at a somewhat higher temperature. The visible phenomena of the fermentation will be rather different the first time from what they usually are, and the course of the fermentation will also proceed somewhat differently. This yeast is only used, however, a few times until the new culture has been worked up.

Hardly sufficient emphasis can be laid upon the fact that such methods of work are very much to be preferred to the introduction of a *strange unknown pitching yeast*, that may in one moment introduce an infection into the brewery or distillery, whose removal may only be accomplished after many months' effort.

