The student's manual of yeast culture / by Grove Johnson.

THE STUDENT'S MANUAL OF YEAST CULTURE

GROVE JOHNSON

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By GROVE JOHNSON

BY THE SAME AUTHOR :

SACCHAROMYCES THERMANTITONUM

(A paper read before the Institute of Brewing, June, 1905)

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PREFACE.

IN presenting this manual on the important subject of practical yeast culture it may be as well to give certain reasons which collectively have drawn me to the conclusion that such a work would be welcomed by the majority of those connected with the fermentation industries, whether as masters, brewers, or students.

During 25 years of active practical experience the fact above all others that has impressed me most is the lack of what (for a better term) may be called an adequate connecting link between the mature work of our greatest investigators and the

immature minds of those whom their researches have been designed to benefit.

Whilst contemplating the works of Pasteur, Hansen, or Lindner—to mention but a few investigators—one must confess amazement at the infinity of detail contained therein and the colossal proportions of the tasks they have accomplished.

In the majority of the works, however, at present available it cannot be gainsaid that they are, if one may use the expression, written "above the head" of all but specialists. This is to be regretted, for, with a fund of information so vast and valuable at hand for our improvement, it seems wasted energy should it not be fully understood, or that it should be regarded in the light of something too difficult for daily practice.

It is the object of this work to lay, step by step, such a substratum of knowledge in the mind of the student that books which beforehand conveyed little or nothing to his intelligence may in future be read with understanding.

I have endeavoured also to arrange facts in such a manner that, reading in his chair and without the necessity of continually consulting text books, a brewer or distiller, worn out by his multifarious duties, may gain the information that others more fortunately situated have acquired from practice in the laboratory.

Furthermore, I have endeavoured to make my work suggestive, leading the student into such a train of thought that he may become persuaded to weigh and prove facts for himself; above all, to instil into him the desire to work with enthusiasm.

Imbued with the spirit of research and equipped with a full sense of the advantages of applying the knowledge won with difficulty by his predecessors—and not until then—the student will find himself possessed of the paramount factor controlling the sum of success. Work will no longer be laborious, but engrossing, as deeper knowledge is gained and facility acquired in the performance of the various operations.

A very little honest work will be of the greatest assistance also towards the realisation of the points of view held by opposing factions, and the better understanding of facts deduced from the labours of master minds.

I desire to avoid the impression that my work should be regarded as an exhaustive treatise or study of yeast in all of its divergent qualities and habits. That has already been ably done by others. I have been compelled, however, to touch upon clearly established facts, respecting the differentiation of types, in order to assist the student to form his own judgment in respect of the results of his personal researches.

The somewhat lengthy details I have furnished regarding the actual manipulation of implements, flasks, and other objects incidental to practice I have thought to be really necessary. As the student becomes more enlightened he will doubtless devise simple means appropriate to his requirements, and vary from time to time any little

"tricks" of manipulation I have thought it desirable that he should be acquainted with. A guiding hand in the first instance from one who has accomplished much detail cannot but be helpful, and I recommend my suggestions to be followed until such facility in operation has been acquired that the directions given may with safety be neglected.

I have thought it better to assume that some at least among my readers have no knowledge whatever of the practical rules and methods guiding the bacteriologist. I make no apology, therefore, for including details and facts already well known to many.

In the general descriptions relative to actual practice I have not thought it necessary to state that such and such a device is due to a particular Professor, or that credit should be given, here or there, to one or another individual. I express, however, generally my deep sense of obligation to all who have preceded me, both in their writings and investigations.

Although desirous of avoiding controversy, I do not think I shall be considered biased should I express the opinion that too little energy hitherto has been directed (so far as everyday practice is concerned) to the primary cause of effects.

Have we not, for example, been rather content to confine our bacteriological researches to a few forcing tray experiments?

May we not ask ourselves whether we are not liable to the charge of resembling the ostler who locked the stable door after the horse had gone?

Forcing tray experiences have their value without doubt, but they should not, and cannot, be regarded as anything more important than a check on previous operations.

Surely, the operations themselves should have had the preference respecting care and diligence? There cannot be any doubt that this fact merely requires recognition to be fully accepted, and it is in the hope of causing others to see things in this light that these sentences have been written.

PREFACE.

It is inevitable that a book on yeast culture from my pen must bear the impress of my work in connection with the discovery of *Saccharomyces Thermantitonum*.

It was this discovery that brought me in contact with the Professors at all the principal bacteriological laboratories of Europe, and I owe I cannot say how much to their courtesy and their appreciation of my work.

At intervals during the space of five years I have visited Paris, Vienna, Berlin, Brussels, Copenhagen, New York, Washington, the Brewing School of Ghent, and several other centres of research. In exchange for any knowledge I had to impart I was overwhelmed with assistance willingly bestowed, and I am glad of this opportunity to express my deep and abiding gratitude to all who have made my path easy.

How much I learned during those visits it would be difficult to estimate; I can only hope that some of the impressions gained under such happy

auspices have illuminated the text of these pages.

How startling my discovery in fermentation at high temperatures proved to be is evidenced by the mass of controversial articles in my possession. To none, however, could it have been more astonishing than to myself; for, whilst I was actually possessed of *Saccharomyces Thermantitonum* for weeks before its real presence dawned upon me, it was not because I was unobservant, but for the reason that all evidence available at that time precluded the chances of such a possibility.

And in this conjunction it may not be inappropriate to utter a word of warning respecting the rashness of jumping to conclusions until such a time that suppositions shall have passed into the region of fact. Even the greatest have been too hasty in framing rules circumscribing limits to future discovery, and the finding of *Saccharomyces Thermantitonum,* whose properties have been acknowledged

to lie far outside previously admitted possibilities, furnishes an instance of the necessity of being very circumspect in laying down hard and fast rules for to-day which may have to be modified to-morrow.

I was much impressed whilst working with Professor Lindner during my visit to Berlin in 1904 by the mass of evidence he had collected and the means whereby he had acquired it. Upon asking him how he found time to give his opinion on the numerous matters in respect of which his advice was sought, in addition to his already arduous duties, he replied: "That is simple; I require, for example, a thousand experiences. Here under my charge I have a thousand students. I wish to ascertain a fact. I set each student the same task. I take the mean of their results—presumably it should be correct. I then satisfy myself that it is so by performing the same task personally in my own laboratory."

Another remark that he made has assisted in

guiding me in the general idea and construction of this work.

Speaking to me one day he said: "I never attempt to teach a student anything until I have arranged a plan in my mind in such a manner that the knowledge I wish to impart must be immediately obvious."

His wonderful power of imparting information is easily explained by the foregoing plan, and I think that there are none of us who would not have been glad to have received tuition from him during our student days. How many times a lad has had to say to himself: "Yes, I am told to do so and so, but I have not been told, and I do not know, why.

It is not sufficient for me to be told this fact, or to have that method explained. In order to make everything clear to me I want to know the reason for the fact—the end to be kept in view, should a particular method be adopted."

PREFACE.

Following Professor Lindner's example I have endeavoured in this manual to furnish a reason wherever one seemed to be necessary.

Recently I have been re-reading the remarks with which Prof. Hansen has prefaced his "Practical Studies in Fermentation." He describes the opposition he had to meet when first he announced his scheme of single cell culture—not only from the outside world, but from Herr Jacobsen himself, at once his patron and the principal of the Old Carlsberg Brewery, Copenhagen.

But to-day the fame of the breweries of Denmark, which include Old and New Carlsberg and Tuborg, is world-wide, and their success is based on the truth Hansen worked so hard to establish. He recites further the bitter controversy emanating from Prof. Delbrück and his followers in Berlin, which, eventually subsiding, ended by the adoption of his methods in 1889, and placed him in the position of being considered worthy of the receipt of their diploma as honorary member.

The tide in time returned in Hansen's favour, and Herr Jacobsen, originally a sceptic, became one of his most enthusiastic supporters. He was not merely content to remain a passive admirer, but endowed the Carlsberg Laboratories, celebrated throughout the world as the "Mecca" of bacteriologists, which may be looked upon not only as a tribute paid to Hansen's diligence, but will be regarded for all time as a monument to his memory.

I hope to be acquitted of any idea suggestive of a comparison between the value of my work and that of Hansen, if I say that, in reading his confessions of his difficulties, perplexities, and the unreasoned opposition he had to meet, I see reflected something of the story of my own life.

I have, in common with him, had to realise that there are some minds so constituted that they regard it as an offence—the daring to be original. I have so dared, and, like other pioneers, have paid the penalty for my temerity.

There are not wanting signs, however, of giving way. Already the result of my work in the tropics, accomplished with but a few hundred pounds and the sweat of my brow, has been instrumental in forming two companies—each with £10,000 capital—one for India and one for the Far East, and I cannot doubt that it will be but a question of time for others to follow suit, and that eventually my work in connection with tropical fermentation will be judged in its true perspective.

The greatest encouragement extended to me during the period of my most strenuous endeavours to obtain recognition of my claims emanated from Tuborg, Herr Dessau having written to me in July, 1905, in the following terms:—

> "In October last year we heard about your discovery of the *Saccharomyces Thermantitonum* and its fermenting effect in wort. It was in Berlin, and the Professor, Doctor Lindner, accompanied his

lecture by showing a fermenting liquor with the *Saccharomyces Thermantitonum* in work.

"We beg leave to bring you our most sincere compliments for this highly interesting investigation, that proves existence of undreamt kinds of Saccharomycetes with effects far above any hitherto known suggestion."

The commercial value of fermentation at high temperature having been so far established as to have reached a recognised position above controversy, I feel somewhat like a man tired out at the end of a race, yet, having won the prize, is only pausing to gain breath before entering into another competition.

I cannot conclude this short preface in a better manner than by quoting a few of Hansen's actual words, which, under the circumstances, might have been written verbatim by myself:—

"The new ideas which I brought forward in my practical studies in fermentation were

at first favourably received by a few only of my colleagues, but were, on the contrary, opposed by most of them. I am glad to be able to state here that some of my former opponents may now be counted amongst the most active supporters of my work. I regard this as the greatest tribute which could be paid to it. Notwithstanding the success which in different countries attended my efforts at reform, I had in reality to fight an unbroken battle for its progress; every step had to be gained by a struggle."

Were I to write volumes in addition I could not add a thought more intimate than that involved by the foregoing quotation.

GROVE JOHNSON.

Savoy Court, London,
September, 1908.

CHAPTER I.

ADVANTAGES TO BE GAINED BY THE USE OF PURE CULTURES.

IN order that the fullest advantage may be taken of facts established by scientific workers it becomes necessary to learn something of the nature of the facts before making an attempt to profit by them.

By applying this axiom to the study and practice of yeast culture we shall become acquainted with the fundamental principles which have guided the bacteriologist in his methods of research, as well as some of the truths he has been enabled to deduce therefrom.

If one were asked to give a reason for cultivating

pure yeast, the answer, at first sight, might seem simple enough, and, speaking broadly, without giving much thought to the subject, one might reply that the special object to be kept in view in the cultivation of pure yeast is to obtain alcoholic fermentation.

Let us consider the subject a little further.

It is true that the distiller has this consideration brought to bear upon him with greater emphasis than the brewer, for in his case the production of the greatest quantity of alcohol from a given percentage of saccharine matter in solution is a matter of vital necessity. Therefore the obtainment of a type of yeast capable of reducing the specific gravity of his washes to the lowest degree possible becomes a consideration above all others in importance.

The brewer, however, approaches the subject from a different standpoint. His object is not merely to split up saccharine matter into alcohol and carbon dioxide—other weighty considerations

guide him—malt liquors have to be full or dry; they must fine with facility; they must be capable of a good secondary fermentation, in order that cask condition may be obtained; they must be agreeable in flavour; they must, in short, possess innumerable qualities, according to the respective requirements of districts.

In the case of the brewer, therefore, we have seen that his requirements are not confined solely to alcoholic fermentation.

A wide view of the brewer's requirements and of the forces opposing him might be thus briefly summarised:—

(1) The fermentation of saccharine matter by the means of suitable yeast, at the same time keeping under control attenuation limits by the selection of types whose attenuative powers have been accurately determined.

(2) The avoidance of bacteria and types of yeasts unsuitable to the ends in view.

Evidence is at hand that certain species of yeast

produce definite results, and that such results may be controlled should essential primary conditions be observed.

In order, therefore, to achieve a specific object we are bound to select and propagate under favourable conditions a type or types of yeast suitable to that purpose.

The advantages of applying the foregoing principle, being self-evident, need not be enlarged upon.

Our desire, then, being to achieve a definite object, all that scientific investigators have made clear to us is at our disposal to assist us in obtaining it—that is to say, a definite instead of an indefinite result.

We have facts at hand (thanks to the unremitting labours of enthusiasts) that issues exceedingly wide and variable may be controlled, should correct means be employed to attain them, that it is difficult for us to believe at the present time that the world in general received Pasteur's great truths—now

regarded as axiomatic—with incredulity at the time of their announcement.

A recapitulation of his claims will assist us in forming a correct attitude of mind towards the subject of yeast culture in its entirety.

"Every unhealthy change," he said, "in the quality of beer coincides with a development of microscopic germs, which are alien to the pure fermentation of beer."

"The absence of change in wort and beer coincides with the absence of foreign organisms."

To many of us the repetition of the above stated facts may seem to be unnecessary, but we must keep in remembrance that Herculean labours had to be performed before their acceptance became general, and, further, that much of the work accomplished by others following in Pasteur's footsteps would have been impossible unless so great a leader had shown the way.

The present ceaseless activity towards the pursuit of further knowledge is fraught with

endless possibilities, scientific men having realised not merely the necessities of commerce, but also the value of their investigations towards its assistance.

The advantages to be gained by the cultivation of pure yeast being now clear, then, in our minds, we may consider whether or not we have any special object in view that we are desirous of attaining.

Should we wish, for example, greater or less attenuation, fermented worts presenting greater facilities for clarification, variations in flavours, good secondary fermentations, instances might be multiplied indefinitely; should we wish any of these we may select such types whose properties have been proved to be appropriate to the particular end in view.

But in order that work such as has been indicated may be conducted upon a reliable basis it becomes necessary to learn a few facts regarding yeast itself, which we will endeavour to do before attempting to make experiments.

CHAPTER II.

YEAST: ITS RELATION TO ORGANIC CHANGE.

SO far we have merely considered yeast in relation to its commercial aspect. Let us go a little further and try to make clear in our minds:

(1) Its origin or place in the general scheme of created things, and

(2) Its connection with those organic changes which take place as a result due to its presence in saccharine solutions.

Whilst considering its place in creation let us first ask ourselves to which kingdom does it belong—animal or vegetable?

For argument's sake let us say animal.

Now, two essential conditions to animal life involve orifices for the introduction of food and the discharge of unassimilable products.

Do yeast cells exhibit such orifices? We conclude that they do not, for the most powerful microscope so far available has revealed nothing to draw us to such conclusions.

Does it belong, then, to the vegetable kingdom? The answer in the affirmative has been established by Pasteur in such a manner that no trace of doubt of its truth may remain in our minds, as the following facts will show:—

Plant life is divided generally into two great divisions:

(1) Phanerogamia—Flowering plants possessing sexual organs.

(2) Cryptogamia—Those plants bearing no flowers.

It is obvious that if yeast be truly a plant it must belong to Class 2, since no one has observed

a yeast blossom. Yet it is so different from our ordinary conception of one that we may naturally ask ourselves to what class of plant does it belong?

The flowerless plants are sub-divided into other divisions, one of which includes the fungi, which, besides being flowerless, are also leafless plants, which, containing no chlorophyl, are, therefore, incapable of decomposing carbonic acid gas and assimilating carbon for the purpose of sustenance.

How, then, do plants, such as fungi, subsist if incapable of decomposing carbon dioxide?

They obtain their nutriment by the assimilation of complex nitrogenous bodies, to which the name proteins has been given.

But, if there are no orifices for the introduction of nutriment, how are yeast cells fed?

It is generally accepted that every cell is self-contained, that is to say that its digestive apparatus, together with its organs for reproduction, are enclosed within a cellulose tissue (just as an egg is surrounded by a shell), which if ruptured determines its life.

It is assumed that this cellulose tissue is porous to a certain degree, and that nutriment is absorbed by it and digested within the interior of the cell. The technical expression "nutrition by osmosis" is generally employed to describe the assimilation of food by absorption.

Now the function of absorbing nutriment in the manner described surely has little or nothing in common with anything that we know respecting the nutrition of animal life?

Truly we may reply, but animals also subsist on proteins; here, at any rate, we find some similarity.

Yes, but here also we find the point of divergence. The only reliable method by which we may determine the kingdom to which minute life belongs is to test its ability to build up its protein —that is, be it understood, an organic compound out of mineral matter.

In no case has it been found that any species of the animal kingdom has been able to do this. Yet it is possible in all plant life.

Pasteur proved that yeast cells were capable of development and increase by immersing them in a fluid containing water, sugar, tartrate of ammonia, potassium phosphate, calcium phosphate, and magnesium sulphate.

Thus we see that yeast conforms to the essential condition by which we test animal and plant life.

There being no longer any doubt in our minds that yeast is truly a plant, that it subsists upon protein, whether ready formed or manufactured by itself as required, should the necessary ingredients be available, we may ask ourselves how this protein, this necessary organic substance, is to be provided for its sustenance, in practice.

The malt wort of the brewer and the wash of the distiller provide the protein for the yeast's sustenance in greater or less degree, and in succeeding chapters will be found passages in reference to the subject of alimentation or feeding matter suitable to yeast, gained by much experience under a great variety of circumstances.

But, before giving it further attention at the moment, let us consider the subject of yeast's relation to those organic changes which take place as a result of its presence in saccharine solutions.

It is as well whilst speaking of organic changes affecting the brewers' and distillers' operations to remember they are principally of two kinds :—

1.—That due to enzymes or soluble ferments, as illustrated by the hydration of starch by diastase during mashing operations; and

2.—The splitting up of saccharine bodies in solution by the agency of yeast.

Although the two cases of organic change above quoted appear at first sight to have little or nothing in common, we find that there is a substratum of resemblance or analogy between them if we probe the subject a little deeper.

It was generally held by investigators who first studied the subject that the conversion of saccharine matters to alcohol and carbon dioxide was dependent absolutely upon the reproduction of the

yeast cell—in fact, that, unless actual life was in progress, fermentation could not take place.

This we know now to be but partially true.

It is a fact that unless yeast cells are active in reproduction fermentation becomes sluggish and ceases altogether should their increase come to a halt, but fermentation itself is not occasioned because a yeast cell buds, but for the reason that it contains within itself an active principle called zymase, which is capable of effecting the change between saccharine matter and alcohol and carbon dioxide, just as diastase is capable of converting gelatinized starch to saccharine matter.

This zymase if separated from yeast is capable of causing fermentation up to a certain point, fermentation ceasing as it becomes exhausted.

Zymase cannot apparently increase or reproduce of itself, its reproduction being dependent upon the growth and multiplication of the yeast cells themselves, just as the increase of diastase seems to be dependent upon the growth of the radicle of a barleycorn during the period of germination.

Theoretically, then, the action of yeast in saccharine solutions appears to be as follows :—

Individual cells probably absorb saccharine matter through their cellulose sac or covering, whereupon the active zymase acting upon it or digesting it produces alcohol and carbon dioxide, to be eventually exuded as waste or bye-products.

The soluble nitrogenous substances of the wort absorbed are probably entirely assimilated, and assist in the building up of the new cells, which when fully developed repeat the functions of the parents which gave them birth.

We have learned, then, that zymase is the active principle causing the organic change we know by the name of fermentation, and that for its energy to be exerted to the full the active reproduction of yeast cells becomes a matter of the utmost necessity.

It seems scarcely probable that yeast cells exude zymase as a bye-product after the digestion of their nutriment, and that actual fermentation

occurs, therefore, outside as opposed to the inside of the cell, though there are those who have inclined to this latter theory.

The investigations of Büchner certainly point to the contrary, and amongst other things seem to establish the fact that zymase splits up sugar into lactic acid, when another enzyme named lactacidase present in the yeast cell converts it (lactic acid) to alcohol and carbon dioxide.

As reference has been made in a recent paragraph to the budding of a yeast cell, it may be as well to give this matter some attention, for herein we have one of the means of differentiating between the two sub-divisions of plant life known by the names of yeast and bacteria.

In order to observe what actually happens when yeast cells increase by budding recourse may be had to the simple experiment of sowing a few cells of yeast in some brewer's wort and mounting a drop of this mixture on a cover glass of a microscopic slide.

The slide used should be one containing a cavity instead of the flat sort, so that when the cover glass (to which a drop of wort containing yeast cells is suspended) is placed in position the cells are for all practical purposes contained in a small air chamber.

Under these conditions they will increase with greater facility than if pressed between a cover and flat slip glass.

Microscopic observation taken from time to time will show that the yeast cells alter their appearance. At first a slight swelling or protuberance in some position on the outside of the circumference of a cell will become apparent—the swelling will increase—gradually it will assume the appearance of a perfect cell—in time it will become detached—by then it is a complete cell, able to repeat the process of budding in the same way as the parent cell which gave it birth.

Bacteria, on the other hand, do not increase by budding; they are known by the name of fission

fungi, which term is used to describe a transverse separation of cells, which when parted are capable of further sub-divisions.

Bacteria can also reproduce themselves by the formation of spores.

Spore formation or sporulation is a subject of sufficient importance to consider in some detail, and in the chapters devoted to the differentiation of types will be found particulars setting forth the ways and means by which it may be recognised and the methods to be employed for its accomplishment.

Yeasts incapable of spore formation are known by the name of Torulæ, but, again, this term as used by Pasteur included micro-organisms, which are not yeasts. Although he included yeasts in the term Torulæ he did not call all Torulæ yeasts.

From time to time endeavours have been made to obtain a universal recognition of nomenclature, but unfortunately this has not yet been accomplished. However, the general acceptance of the

term Saccharomyces is taken to mean yeasts capable of spore formation.

Alcoholic fermentation is not confined solely to the action of yeasts, certain bacteria and moulds are able to effect alcoholic fermentation.

By low fermentation or low yeasts we understand those types which work from the bottom of the wort, or, at any rate, settle there at the close of fermentation.

By top yeast or high fermentation we understand those types which rise to the surface at the close of fermentation.

An infinity of further details might be entered into, but for the moment we will content ourselves with the few facts which have enabled us to grasp a bare outline of the life's history of yeast, together with something of the manner in which it affects our operations.

In order to make clear in our minds the usually accepted meanings of the terms employed to denote the various types of micro-organisms with

which the brewer and distiller may have to deal, the subjoined list, together with a few details under each heading, may be found useful for guidance.

True Sporulating Saccharomyces

causing alcoholic fermentation.

SACCH. CEREVISIÆ (*High fermentation*)	The type commonly employed by brewers. It rises to the surface at the close of fermentation.
SACCH. PASTORIANUS I. (*Low fermentation*)	Ferments from the bottom of the tun and imparts a disagreeable bitter taste to beer.
SACCH. PASTORIANUS II. (*High fermentation*)	Incites a feeble high fermentation, but does not impart any disagreeable flavour.
SACCH. PASTORIANUS III. (*High fermentation*)	A yeast which separates from wort with difficulty and therefore causes cloudiness in beer.
SACCH. ELLIPSOIDEUS I. (*Low fermentation*)	A bottom fermentation yeast found on fruits and constitutes the ordinary alcoholic ferment of wine.
SACCH. ELLIPSOIDEUS II. (*Low fermentation*)	A bottom fermentation yeast associated with cloudiness and turbidity in beer.
SACCH. THERMANTITONUM (*Low fermentation*)	A bottom yeast whose working temperature 110 F. is the highest yet discovered.

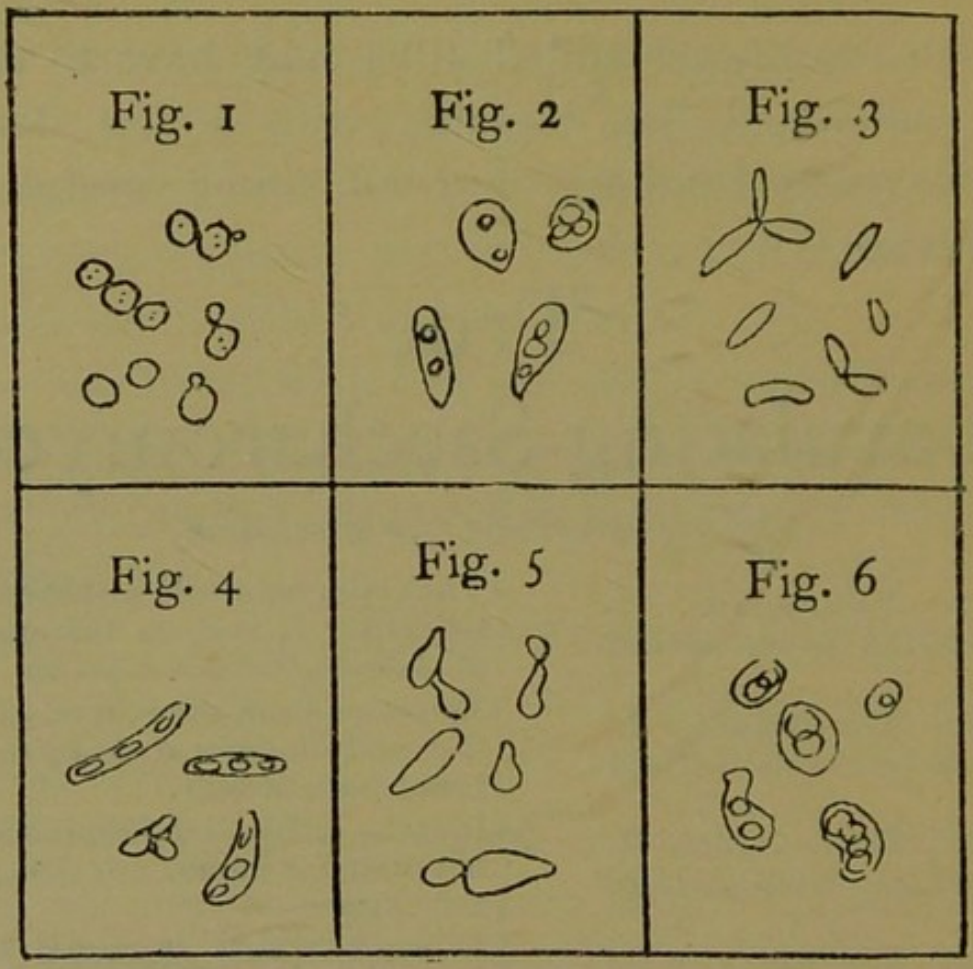


Fig. 1. SACCHAROMYCES CEREVISIÆ.
,, 2. Do. do. Spore formation
,, 3. Do. PASTORIANUS.
,, 4. Do. do. Spore formation.
,, 5. Do. ELLIPSOIDEUS.
,, 6. Do. do. Spore formation.

Torulæ

Those yeasts inciting alcoholic fermentation but are yet incapable of Spore formation.

APICULATUS - - - - Is a widely distributed form and may be found on the skins of many fruits.

BRETTANOMYCES - - - A type isolated by Claussen and said to give rise to the aromatic flavour in English bottled beer.

Bacteria

SARCINA - - - - - Small egg-shaped cells, usually in pairs or fours, forming a square —generally associated with dirty brewing plant, especially wort mains and the like.

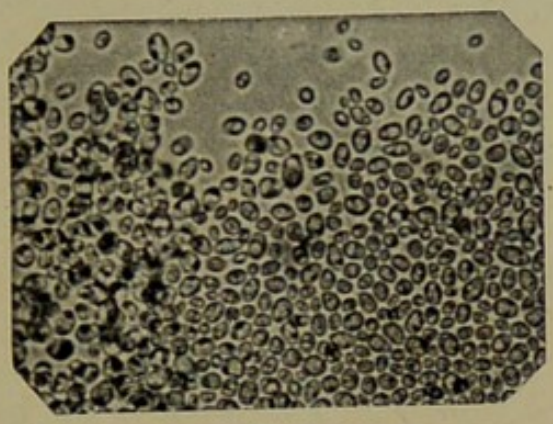
BACTERIUM TERMO - - Short oblong cells, invariably to be found in decomposing yeast, and able to cause a nauseating flavour in fermenting wort.

BACTERIUM SUBTILIS - - Cylindrical rod formation. An organism capable of living without oxygen, and able to withstand excessive temperature. Its spores resist boiling point 212 F.

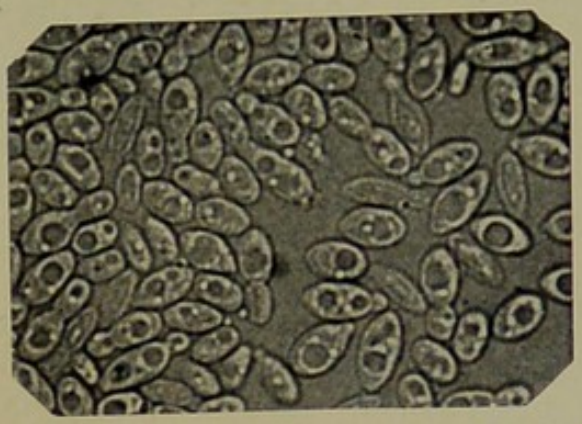
BACTERIU ACETIM - - The organism causing acetic fermentation, commonly used in making vinegar.

Bacteria—*Continued.*

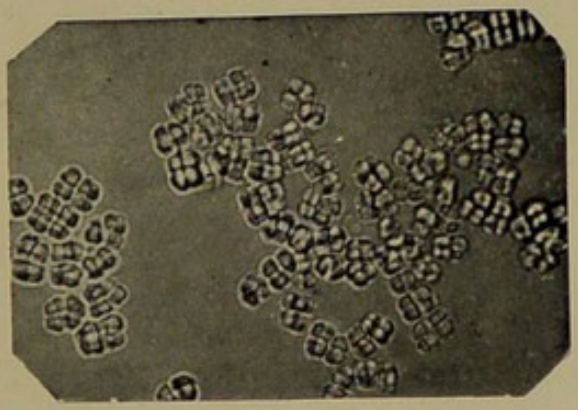
Bacillus Acidi Lactici	Cells giving rise to the formation of lactic acid in milk and brewer's wort.
Bacillus Butyricus	Similar in appearance to Bacterium Subtilis. It is widely distributed in nature and is the cause of the maturing of cheese, the flavour of pineapples, etc. It may be isolated from the green top of a pineapple. The flavour of rum is partially due to butyric ether.
Leuconostoc Mesenteroides	Cells generally in chaplets enveloped in slime and giving rise to the "frog's spawn" of the sugar factory.



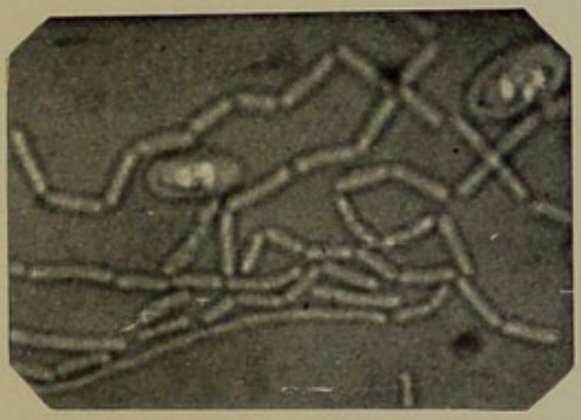
Wild Yeast, similar in appearance to Saccharomyces Thermantitonum.



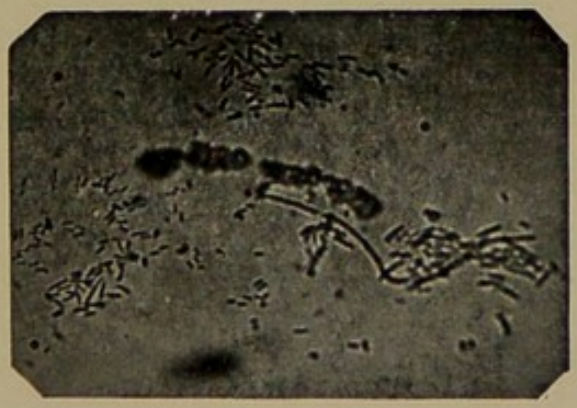
Apiculatus from an eight days' old culture.



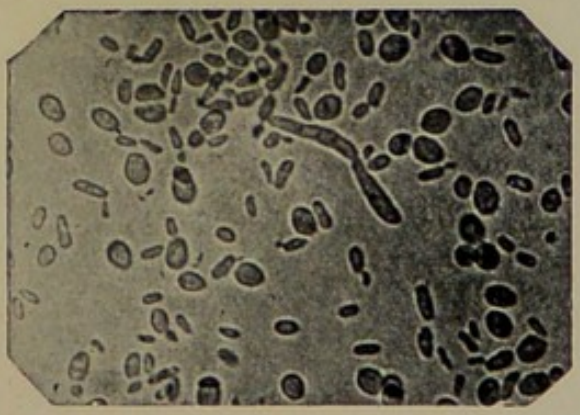
Sarcina from beer. Adhesion culture.



Lactic Acid Bacteria from a baker's yeast. A three weeks' old adhesion culture.



Bacteria often occurring in wort.



Wild Yeast from a filter press.

Illustrations reproduced by the permission of Professor Lindner from his
"Atlas der Mikroskopischen Grundlagen der Gärungskunde."

CHAPTER III.

PRIMARY CONDITIONS NECESSARY OF OBSERVATION.

HAVING learned a few facts respecting the principles involved by the use of pure cultures, and having considered superficially some of the habits of yeast, as well as its ability to effect organic change, we may discuss the means whereby types may be cultivated in such a manner that no doubt may remain in our minds that the results obtained by our experiments may be considered reliable—that is to say, due alone to the specific type or types occasioning the cause.

The primary condition essential to obtaining accuracy in result is the complete sterilisation of everything connected with the experiment, and this regarding both nutritive media and objects incidental to its performance.

Let us consider for a moment the significance of the word *sterilisation*. It is a word we have constantly to employ; it is as well that its actual meaning should not escape us.

When we speak of sterilising an object we mean that by the process of sterilisation all organisms capable of effecting organic change have been destroyed.

How are we, then, to achieve such a condition? What means shall we employ to attain it?

Complete sterilisation of objects may be effected by heat—direct heat or steam—the action of certain acids and chemical compounds, alcohol, antiseptics, and so forth.

Objects such as glass culture boxes (Petrie's dishes) liable to fracture by the direct application

of heat are usually washed with dilute sulphuric acid and rinsed with sterilised water, dried at a temperature they are able to withstand, and then rinsed with alcohol before being put to use.

In general heat is applied either by steam or flame. Objects such as cotton wool, bottles, flasks, tubes, and the like are baked in a suitable oven before being put to use. Instruments such as forceps, platinum wire, glass rods, or in fact any object capable of being burned without injury, are passed through the flame of a Bunsen burner or spirit lamp. Submitted to the latter drastic course, no fear need be entertained of any living organism remaining upon an instrument so treated. The steaming of objects is generally performed by first arranging them in wire baskets. In the case of bottles, tubes and flasks they should be placed neck upright, care being taken to pack them closely to prevent their tumbling against one another or falling over, and so becoming full, or partially full, of water during the steaming process.

The wire basket being suitably filled with objects is then ready to be placed in a steaming kettle.

As a rule it is better that the basket should be supported by legs, so that the objects contained therein should not come in actual contact with the water in the kettle.

The kettle should contain water to the depth of two inches, for example. The basket (on its legs) should then be put inside and the whole placed on a stove or Bunsen burner in order that the water may be boiled.

It is advisable to employ a kettle of such a depth that the wire basket when placed inside it does not reach within six or eight inches of the top. This allows a sufficient atmosphere of steam at a high temperature to come into contact with the whole surface of the objects before there is a chance of their becoming cooled by the surrounding air.

Upon removing flasks or bottles from the steaming kettle their necks should be immediately plugged with well-baked cotton wool, whereupon

they should be placed in a dust-proof chamber until required for use.

Aseptic and *antiseptic* are words we have constantly to employ in relation to the practice of bacteriology; let us consider the exact value of these terms before proceeding further.

By *antiseptic* (as applied to a solution, for example) we mean that something is contained therein inimical to the growth and development of micro-organisms. By the term *aseptic* we understand a condition not prone to change, such as, for example, a sterilised object.

The significance of the terms sterilisation, sterilised plant—sterilised media—aseptic conditions, and so forth, being clear in our minds, there are two further words, *inoculate* and *infect*, that are worthy of a few moments' consideration. By the use of the term inoculate—in such a sentence as "inoculate that flask," for example—we mean to convey the idea of deliberate intention of introducing into a sterile medium an organism which

we are desirous of propagating, whereupon it, and it alone, may develop.

When we speak of an object being infected we may either mean that it has been inoculated by deliberate intention or that it has become so by chance infection by any micro-organism.

In ordinary conversation the use of such a sentence as the following, "Do not inoculate that flask, it is infected," would be taken to mean freely "that flask contains bacteria," the inference from such interpretation, therefore, being that it is useless for propagating a pure culture.

We understand, then, that the most important condition that has to be taken into account in the preparation of a pure culture is sterility of everything in connection with the experiment. We may pass on, therefore, to the consideration of the objects and instruments that will be required to carry out our designs.

The following list may be found useful. In some cases the descriptions given may be sufficient

indication to the student to devise means of his own for the fulfilment of his requirements. For example, a biscuit box will answer the purpose of a water bath; a wire basket may be home woven; a water bath controlled in temperature by a Bunsen burner will in many cases be as useful as a thermostat; a double saucepan would make a suitable laboratory mash tun—and so forth.

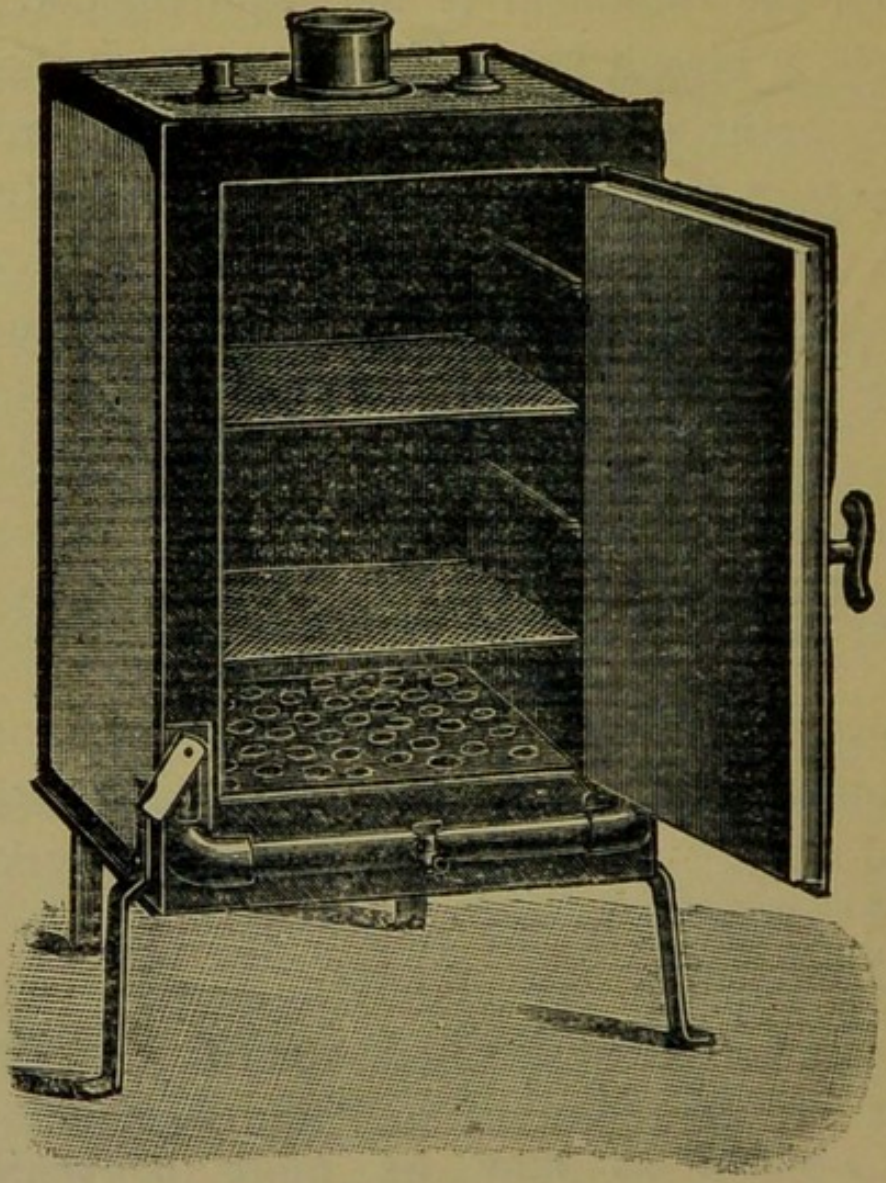
A List of Objects and Instruments Required by the Bacteriologist.

(*Illustrations kindly lent by Messrs. Townson & Mercer.*)

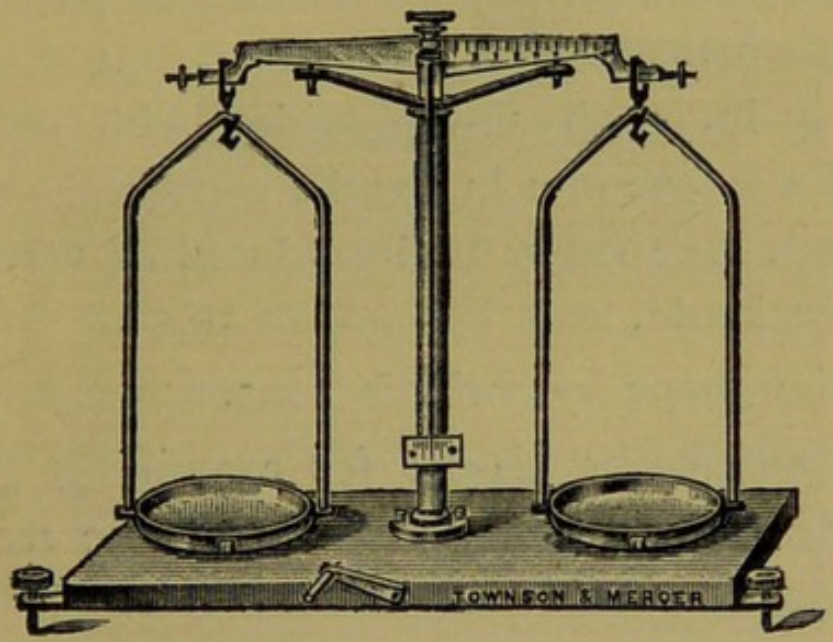
A dust-proof chamber.

A thermostat.

An oven for the purpose of baking objects such as cotton wool, blotting or filter paper, or such glass objects as may be more conveniently sterilised by baking as opposed to steaming.



Balance and Weights.



A steaming kettle. A cylindrical or rectangular vessel will be found most suitable. Anything with curved sides, such as, for example, a gipsy kettle, is not to be recommended. Respecting size, should the cylindrical variety be selected a depth of about 12 in. and a diameter of about 8 in. (a flat bottom is understood) will be found convenient. Rectangular varieties might be in proportion.

A wire basket appropriate to the kettle to slip in easily, without any possibility of jerking the objects which may be placed in it. It should be about 4 in. deep and provided with stiff wire legs of a length of 2 in., and a convenient handle, so that it may easily be introduced or withdrawn from the kettle.

A microscope by Swift & Sons.

Slip and cover glasses for examining objects. Slip glasses containing cavities will be required, as well as some without cavities. The advantages of the cavity glass will be found to be fully explained in the chapter devoted to the methods whereby single cells may be isolated.

A tube of vaseline.

A camel's-hair brush for painting edges of cover glasses with vaseline before placing them in position on the slip glasses.

An etching pen to make a droplet on a cover glass (also explained further on).

A brush made of platinum wire for the purpose of painting stripes on nutritive gelatine.

A glass rod about 6 in. long and $\frac{1}{8}$ in. diameter, in which is inserted a piece of platinum wire about 2 in. long.

Several glass rods of the same dimensions as the latter with ends rounded, also a few such with ends flattened out, which will be found convenient for removing a mass of pure yeast from the surface of a gelatine culture.

Files.

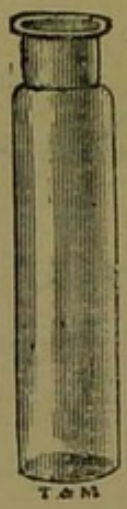
Some glass tubing of various sizes.

Aseptic cotton wool for plugging necks of bottles and flasks. It is well to rebake "aseptic" wool on every occasion before use, in case of chance infection. The usual custom also is to fold or screw the wool into a cork-shaped stopper, then hold it by the forceps or crucible tongs in a Bunsen flame to set it on fire, and whilst still ablaze to plunge it into the neck of the flask or bottle for which it is intended.

Forceps for placing cover glasses in position, and for plugging necks of flasks with cotton wool ablaze.

Crucible tongs for holding such objects as are to be sterilised by flame, and for the purpose of packing necks of large flasks with cotton wool ablaze and other convenient purposes.

Small bottles of about the following capacities: 5 c.c., 10 c.c., 25 c.c., 50 c.c., 100 c.c., 200 c.c.



Bohemian flasks of capacities ranging from 5 c.c. to 5 litres; these need not be graduated,

but they should be measured before use, in order that their approximate capacities are known.

Half a dozen round glass culture boxes (Petrie's dishes) with rubber bands.

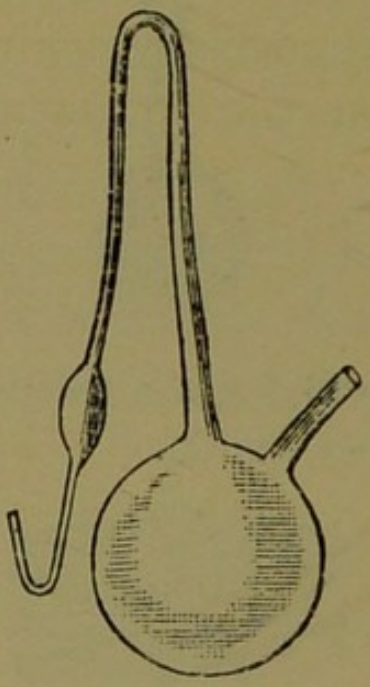
Rectangular bottles of about 3 in. high, and sides $1\frac{1}{2}$ in. wide by $\frac{3}{4}$ in. wide, which may be used in the place of round glass boxes.

Test tubes, which will be found useful for a variety of purposes.

Test tube racks.

Beakers.

Pasteur flasks of ½ litre, 1 litre, and 2 litres capacity.



A sterile cover.

A laboratory mash tun.

A water bath.

A spatula, some glass bell covers, some white tiles.

Funnels and funnel stands, filter papers, burettes and burette stand, Bunsen burners and spirit

lamps, tripods with circular and triangular tops, wire gauze, pipeclay triangles, pipettes, a graduated litre measure, thermometers, saccharometers and weighing cylinders for taking specific gravities, agar, gelatine, alcohol, mercuric perchloride.

CHAPTER IV.

THE PREPARATION OF NUTRITIVE MEDIA

THE preparation of nutritive media—that is food in an assimilable state—is the first operation necessary to be performed for the development of pure cultures or colonies from single cells.

Should brewery wort be available it will answer the purpose admirably, that is to say, assuming that it is composed of malt extract or malt extract plus carbohydrates from raw grain and inverted sugar.

Preferably nutritive solutions should be composed entirely of malt wort of a specific gravity of

1·050 degrees; but wort derived from a mixture of malt and sugar, or malt and raw grain, or malt raw grain and sugar, may be employed provided that of the total gravity of such worts 75 per cent. at least is derived solely from malt extract.

It must be kept in mind that worts of great density, 1·080 degrees, for example, are not to be recommended for the preparation of pure cultures, as the following reason will show. As fermentation proceeds alcohol is produced, and alcohol is inimical to fermentation. An extreme case will illustrate this. Organic matter, whether animal or vegetable, is frequently preserved by immersion in alcohol. Fruits or vegetables for the table, objects of interest to the surgeon, and so forth, are kept in this manner for future use or observation.

Alcohol, then, being a preservative, is also an antiseptic; it is clear, therefore, that in the case of fermentation yeast cells must diminish in their ability to develop as the percentage of alcohol is increased. The greater the density of the wort

the higher may become the percentage of alcohol as fermentation progresses, with a corresponding reduction in the vitality of the yeast.

As it is not to be expected that malt wort from a brewery may be available upon all occasions, it becomes necessary to consider how to prepare it in the laboratory.

Upon reference to the tables at the end of the book a scale will be found setting forth the quantity of malt necessary to be mashed in order to produce a given volume of wort at a specific gravity of 1·050 degrees.

We will take, for example, one kilo of malt, which, according to the table, is capable of yielding 5·38 litres of wort—we will say 5 litres in round figures.

The malt is first ground and then mashed with 3 litres of water at 165F. The temperature of the mash is then taken, it should be as nearly as possible 152F.

The mash may be made in any suitable can

controlled in temperature by a water bath maintained at 152F.

After the mash has stood for 90 minutes at 152F. it may be filtered through blotting paper.

When the wort has run through the filter, water at 170F. may be poured on to the grains in the funnel, allowing the filtrate to mingle with the first wort until a volume of 5 to 5½ litres is collected.

The wort must thereupon be boiled for 90 minutes with hops.

The amount of hops required may be calculated on the basis of two per cent. of the weight of malt taken. Thus in the present case, one kilo of malt having been mashed, 20 grammes of hops will be necessary.

After boiling the wort thoroughly it must be again filtered, this time from the hops. Should the gravity be found in excess of 1·050 degrees water should be added; if below, the wort should be boiled until it is reached.

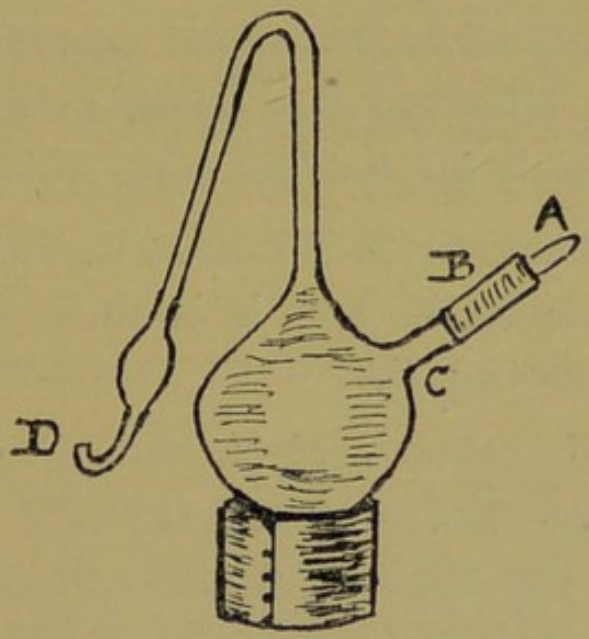
Assuming that we have made 5 litres of wort, it might be conveniently distributed as follows :—

	Litres.
Two 1-litre flasks to be used as stock flasks to be drawn from as required	2·000
Ten cylindrical bottles of 50 c.c. capacity, each containing 25 c.c.	0.250
Four Pasteur flasks of 1 litre capacity, each containing 500 c.c.	2·000
Three Pasteur flasks of ½ litre capacity, each containing 250 c.c.	0·750
litres	5·000

The flasks and bottles should be immediately plugged with cotton wool, whereupon they should be reboiled for 30 minutes on three successive days, and then set aside in a dust-proof chamber until required.

The following short description of a Pasteur

flask may be found useful to inexperienced students :—



A is a piece of solid glass rod rounded at each end to form a stopper. *B* is a piece of rubber tubing 2 to 3 in. long. *C* is the spout. *D* is the neck. To introduce wort into the flask the stopper *A* is removed, whereupon a glass funnel is inserted into the rubber tube *B*. Clear wort is then poured into about half the capacity of the flask. Never

on any account fill a flask above the level of the spout.

The wort having been introduced, the flask is then set on a circular-topped tripod and boiled thoroughly, allowing the steam to escape at the spout and neck for half an hour.

The glass stopper *A* is passed through a Bunsen flame to sterilise it, and it is then replaced into the rubber tubing *B*.

A piece of cotton wool is then plugged into *D*, for the reason that (a partial vacuum having been created) as the flask cools air will be drawn in, which, unless filtered by passing through the wool, might carry infection into the flask.

Flasks so prepared must be boiled on three successive days, the stopper *A* being removed as boiling point is reached. After boiling for 10 to 15 minutes the glass stopper *A* is replaced, and a new piece of cotton wool ablaze is plugged in at *D*.

Useful sizes are flasks of 500 c.c. 1 litre and 2 litre capacity.

When removing flasks from the iron tripod, after boiling them, they should be supported by stiff cardboard cylinders, as shown in the sketch. They may also be slung by passing the neck through a ring suspended by a chain; but this plan is not to be recommended, as the weight of the flask is sufficient to oftentimes cause the neck to fracture.

It is not sufficient for the purpose of the bacteriologist to be provided with nutriment in a liquid form alone. Nutritive media must be of two kinds, solid and liquid. So far we have confined our attention to its preparation in the liquid form, let us now consider what steps we may take to solidify it.

In some cases gelatine is used for the purpose, in this instance we speak of wort gelatine—in others a gelatinous sea moss named agar is employed—in this case we speak of wort agar. Wort gelatine

is generally made in the proportion of 10 grammes of gelatine to 90 c.c. of malt wort. The mixture should be collected in a beaker and then placed in a hot water bath and frequently stirred by a glass rod until all the gelatine is dissolved.

Wort agar is prepared in precisely the same manner as wort gelatine, the proportions only being different.

Two grammes of agar to 98 c.c. of wort will be found suitable for most purposes, but where cultures are liable to exposure to high temperature, such as in the case of being despatched to the tropics, for example, it may be necessary to increase the percentage of agar at the expense of the wort.

Nutritive media prepared according to these methods serve also another useful purpose. They provide a very important indication of the type of yeast with which they may be inoculated.

For example, some yeasts are capable of completely liquefying wort gelatine, but are unable to

do this in the case of wort agar. We will merely note the fact while passing without entering into further details, as another chapter is devoted to the consideration of the differentiation of types.

Wort agar being more generally useful, we will consider its preparation in preference to wort gelatine. Half a litre of wort is taken in a suitable beaker, whereupon 10 grammes of agar are dissolved in it according to the manner described.

When thoroughly dissolved, the mixture should be boiled well, and whilst at boiling point be distributed amongst such various flasks, tubes, or plates as may probably be useful.

For example :—	Litre c.c.
Twelve test tubes about 6 inches long and half an inch in diameter, each containing 10 c.c.	=0·120
Six rectangular bottles 3 inches high and sides 1½ inches by ¾ inch wide, each containing 30 c.c.	=0·180
Four culture boxes each about 3 inches diameter, each containing 50 c.c.	=0·200
litre	0·500

The bottles and test tubes containing the respective volumes of wort indicated above should be arranged in a steaming basket and be steamed for at least half an hour.

Upon removing them from the steaming kettle their necks should immediately be plugged by aseptic cotton wool. Care should be taken to roll up the wool into "stoppers" that will fit the necks tightly. The stoppers should then be held by forceps or crucible tongs and plunged into a Bunsen flame, and whilst still ablaze introduced into the necks. The bottles and tubes should then be placed in a dust-proof chamber and allowed to cool and the contents to solidify.

Even with the minute observation of the foregoing conditions media must not be considered sterile. For three successive days the bottles and tubes should be removed from the dust-proof chamber and steamed for half an hour, for boiling, although annihilating most organisms, does not destroy all, neither does it kill spores, micro-organisms in their embryonic state.

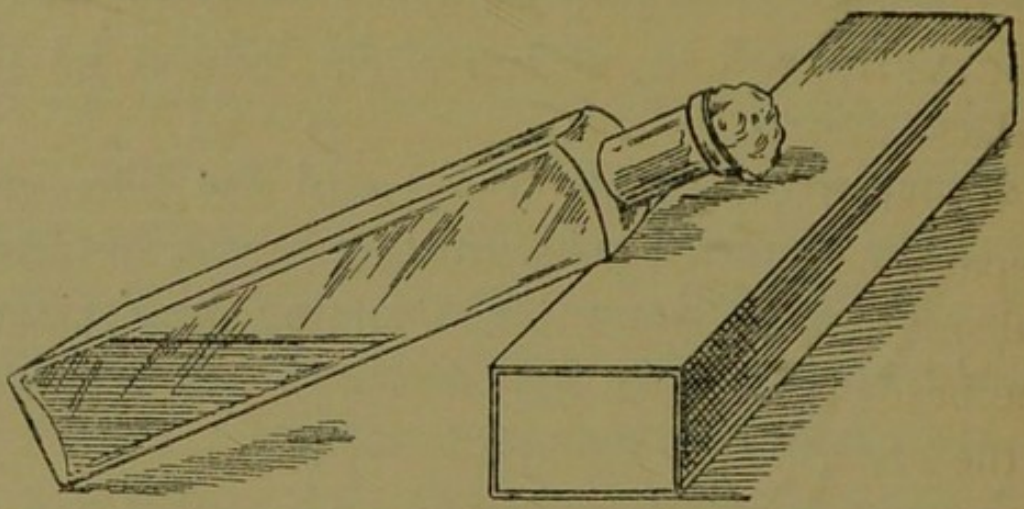
A space of three days is sufficient for spores to develop into mature cells, and these, in common with heat-resisting bacteria weakened by successive operations, will be destroyed by boiling on the third occasion.

When steaming test tubes and the like, care should be exercised to keep them upright, but upon removing them from the steaming basket they should no longer be kept upright, but arranged upon a table in the manner following.

A piece of wood about one inch square and of any length suitable should provide a "pillow" upon which the upper ends of the test tubes (plugged with cotton wool) may rest. The lower ends of the test tubes should rest upon the table. By the foregoing means the wort agar will solidify in the tubes so as to present a long diagonal surface instead of a small circular surface, which would be the result should the solution solidify whilst the tubes remained upright.

What has been said respecting tubes also applies

to bottles, but an illustration may be helpful to make all clear.



In the case of glass boxes the wort agar should be allowed to solidify whilst they are kept perfectly flat. Directly the liquid agar is poured in (care must be taken not to introduce it too hot or the boxes may crack) the lids or covers should be placed in position, the rubber bands previously washed in a solution of mercuric perchloride should protect the intervals between boxes and covers, when they should at once be removed to the dust-proof chamber until the solution solidifies.

Glass boxes will be found most useful for the development of cultures in the laboratory, but for the purpose of sending samples of pure yeast by post the bottles and test tubes containing diagonal surfaces of wort agar are to be preferred.

Although directions have been given for the preparation of nutritive media in a variety of forms and sizes, it is inadvisable to hold too large a stock. The best plan is to think over carefully the nature of the experiments we propose to perform, as well as our requirements during the progress of their development, and prepare media but slightly in excess of our requirements.

CHAPTER V.

METHODS FOR THE PREPARATION OF PURE CULTURES

THE nutritive media in solid and liquid form having been prepared according to the directions given in the last chapter, we have at hand the necessary means whereby pure cultures may be developed.

We are now in the position, therefore, to discuss details necessary to be observed during the actual operation of inoculation.

The first point of all for consideration is the separation of a single cell of yeast from the pasty mass with which it is associated.

Individual cells being so minute as to be incapable of recognition without the aid of a powerful microscope, it follows that a speck of yeast visible to the naked eye may contain an incalculable number of cells, not only of yeast, but of other varieties of micro-organisms intermixed with them.

How, then, shall we devise means for their separation? How may we be sure that cultures developed on the surface of wort agar or wort gelatine, or propagated in a liquid nutritive medium, result from one single cell of yeast and that alone?

We have the choice of at least two well-established methods, each of which depends on the dilution of a mass of cells until the separation of one of them becomes simple.

The methods are generally known by the following descriptions.

The plate or film culture obtained by surface inoculation.

The plate or film culture obtained by inoculating wort gelatine or wort agar whilst in a molten state.

The droplet system.

The Plate Culture obtained by Surface Inoculation.

A glass culture box containing a film of solidified wort agar prepared according to the directions given is taken from the dust-proof chamber and examined to see there are no traces of mould upon it or any specks indicating colonies of micro-organisms.

In the event of a superficial observation by the naked eye being satisfactory (that is to say no traces of infection being visible) it is placed on the bench ready at hand, care being taken that the rubber band and cover be not removed for the moment.

We will now consider its inoculation by a single cell of yeast, or rather several cells, but each

separated in such a manner that colonies growing from either of them eventually may be safely considered as being the offspring of a single parent cell.

With this end in view we proceed to the dilution of some yeast according to the following method:

The tip of a glass rod is allowed to come into contact with a mass of yeast, it is then stirred into about 10 c.c. of sterilised water contained in a beaker or some other suitable vessel.

When thoroughly mixed a cursory observation should be taken of a drop of the mixture by the aid of a microscope in order to gain an idea as to whether there be few or many yeast cells contained in it.

An ideal result of such a preliminary observation would be the finding of but two or three cells in the microscopic field, but, as this is unlikely to happen, more water should be added to the mixture in the beaker if there be cells in excess of requirements, or more yeast should there be too few.

Assuming that our microscopic observation has revealed but two or three cells in a field, we may make up the volume of 10 c.c. contained in the beaker, with sterilised water until it measures approximately 100 c.c.

The volume should be then thoroughly agitated in order to distribute the cells as evenly as possible, and we are then ready to commence the inoculation of the culture box waiting ready at hand upon the bench.

A platinum wire brush is then taken and held in the right hand and plunged into a Bunsen flame. It is then dipped into the water containing the yeast cells and stirred for a moment. With the left hand the lid or the cover of the culture box is raised sufficiently to allow a stripe to be painted on the wort agar with the platinum wire brush. The stripe should be painted at one side of the film's surface, leaving space for two more stripes.

Upon the surface of the film a slight depression,

due to the water conveyed by the brush, will be visible after passing it over.

Immediately the first stripe has been painted, the cover belonging to the culture box must be replaced.

Always lift the cover in such a way that the surface of the film is not exposed unnecessarily. Practice will make perfect, and after a little experience it will be found possible to paint the stripe with the lid raised but about half an inch above the culture box.

Further dilution of the water containing the cells now becomes necessary.

10 c.c. of the volume from which our first stripe has been painted should be diluted with sterilised water to 100 c.c.

The platinum wire brush is again sterilised by flame as before, it is then dipped into the last-mentioned solution, whereupon a second stripe is painted upon the film, this time in the middle of it.

Again 10 c.c. of the last-mentioned volume should be diluted to 100 c.c., and from this solution a third stripe should be painted upon the film.

To summarise, we have by now painted three stripes on the surface of the film, the second of which can contain but 10 per cent. of the number of cells contained in the first stripe.

The third stripe can contain but 10 per cent. of the number of the cells in the second stripe.

The special object of the foregoing dilutions is that, should there be so many cells develop in the first stripe as to form colonies overlapping one another, they are less likely to do so in the second stripe, and still less likely to do so in the third stripe.

When painting the stripes never allow the brush to pass over a space previously touched, neither allow drops to fall from it. A culture box of 3 inches diameter allows plenty of space for the

painting of three stripes without the necessity of their overlapping one another.

The film having been inoculated as described, the rubber band is washed in a solution of mercuric perchloride and replaced in position, whereupon the box is conveyed to the dust-proof chamber, or thermostat, should a particular temperature be important.

Observations should be taken within 12 to 18 hours to see whether there be signs of yeast colonies growing in either of the stripes.

Little cream-coloured specks should be looked for, which once visible to the eye speedily increase in diameter.

It has been urged upon many occasions that the practice of propagating single cell colonies on films is open to objection on the ground that a bacterium may have been introduced at the moment of inoculation and may have developed in the midst of what we had supposed to be pure yeast colonies.

Admitting this possibility, it is conceivable that

any nutritive solution becoming inoculated from such a culture might also be infected with the bacteria.

The danger is, however, slight, and depends to a great extent upon the operator.

Careful microscopic examination of a few cells taken from a colony by the means of a sterilised platinum wire would at once disclose the presence of any organism other than yeast.

The Plate Culture obtained by Inoculating Wort Agar whilst in a Molten State.

Sterilised wort agar is warmed until melting point is reached. Cells of yeast are introduced whilst the media is still warm, whereupon it is thoroughly shaken in order that the cells may be distributed as evenly as possible. It is then cast into a culture box and allowed to cool and solidify.

The details of the operation are as follows :—

A flask of 100 c.c. capacity containing 50 c.c. of

wort agar is taken and placed in a water bath until the melting point is reached, care being observed that the wool stopper be not removed, neither that it becomes wet.

Should the temperature rise above 120F. before liquefaction the flask should be stood on one side until the temperature falls to 95 or 100F.

It will not be disposed to solidify again for some minutes at this latter temperature.

Sterilised water containing a few yeast cells should be ready at hand. The usual precautions being observed to obtain excessive dilution, one cell in 1 c.c. of water suffices.

The flask containing the wort agar should be taken in the left hand and a Bunsen flame be allowed to play around its neck for a moment, at the same time setting the wool on fire. The wool should then be removed by forceps and a drop or two of the water containing the yeast cells be immediately introduced, whereupon the wool stopper (still ablaze) should be replaced.

Care must be exercised to perform the operation with all haste, for the woollen stopper may be entirely consumed.

It is as well to place upon the bench another woollen stopper before removing the one from the flask, in order that in the event of such a contingency arising as has been indicated an immediate remedy is available.

The flask being again stoppered is then agitated in order that the yeast cells recently introduced may be evenly distributed.

An empty glass culture box having been first sterilised is now taken and the contents of the flask are poured into it, the lid and rubber band are at once placed in position, whereupon it is conveyed to the dust-proof chamber and there allowed to cool and the contents to solidify. In 12 to 18 hours creamy specks will become visible here and there, indicating colonies which have been developed from single cells.

The same objection urged against cultures obtained by surface painting applies also to this latter method, but here also success again depends to a great extent upon the operator.

The Droplet Culture.

A droplet is obtained by dipping a sterilised etching pen into wort containing yeast cells and then marking a sterilised cover glass with a minute drop of the liquid so conveyed, the object being to obtain but one cell of yeast in one droplet.

Before commencing operations the following objects which will be required should be arranged conveniently upon the bench :

A slide glass with a cavity.
An etching pen.
A camel's hair brush.
A tube of vaseline.
A glass or beaker.
A circular cover glass.

A microscope.

$\frac{1}{4}$ and $\frac{1}{8}$ in. objectives.

A Bunsen burner.

A pair of forceps.

A pipeclay triangle.

A tripod.

A sterile flask of about 100 c.c. capacity containing about 10 c.c. of sterile wort.

Now to proceed. Introduce a few yeast cells into the 10 c.c. of wort contained in the 100 c.c. flask and shake well. We will assume that a droplet taken from this mixture demonstrates by the aid of a one inch objective the presence of 10 cells. It will be obvious that if the 100 c.c. flask be now filled with wort a droplet will contain but one cell, that is approximately. Such exact distribution is not probable or to be expected.

Now take a cavity glass by a pair of forceps and hold it for a moment in the Bunsen flame, and then face downwards place it on a pipeclay triangle supported by a tripod. Next take a circular cover

glass by a pair of forceps, pass it through the flame, and when cool mark upon it a droplet with the etching pen that has been dipped in the wort containing yeast cells. On the same side of the glass that has been marked with the droplet, paint the edge with vaseline. Now take the cavity glass, reverse it (it has been upside down until now), now place the cover glass in position, lift it a moment, breathe into the cavity so that the air be not too dry—the breath contains moisture—and now examine.

If the operation has been successful the whole "droplet" will contain but a single yeast cell.

Should the droplet contain more than one cell the operation must be repeated.

The one inch objective having revealed but one cell in the droplet, it should next be examined by a one-eighth inch objective in order that a better idea of the species of the cell isolated may be determined.

It will be remembered that the media is "nutri-

tive," we have employed wort not water, so that the cell now has a chance of budding and developing. It should be placed in a thermostat at the temperature most favourable for the type operated upon, and the next day the droplet should contain a whole colony of cells from our single cell of the day before.

Assuming that upon the day following, microscopic examination of the droplet reveals the fact that the colony has not developed materially, it should be allowed to remain for another period of 12 to 24 hours.

When fully developed we may proceed to inoculate small bottles of wort from the colony.

But before considering details in connection with the inoculation of flasks by cells taken from a pure culture growing either in a droplet or on a film it becomes necessary to discuss the means whereby we intend to carry out our design.

It needs but little intelligence to grasp the fact that should a platinum wire, glass rod, or other

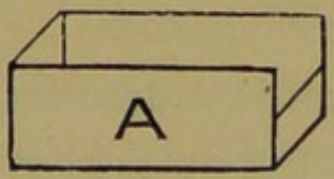
instrument be introduced hot into a colony the colony may conceivably be destroyed by the heat.

Again, should an instrument be introduced cold it might carry infection with it.

How are we to get over these embarrassing difficulties?

Simple means have been devised and special objects may be purchased, but wherever trifles are to be found in an ordinary household, which answer the purpose, they are to be preferred to unnecessary expenditure.

We will take an oblong tin with a removable lid, about 6 in. long by 2 in. wide by 2 in. deep. From one end of the tin we will cut away a piece 1 in. deep and the whole of its width



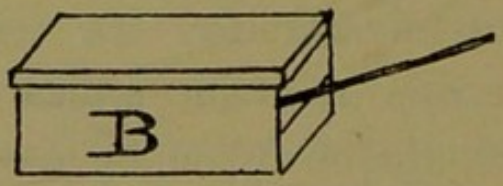
(see Figure A), thus leaving an open space at the end of the box when the lid is placed in position.

We place this tin on the bench, and, taking a

Bunsen burner in the hand, allow the flame to play around the inside of the box. We now treat the inside of the lid in the same manner and place it in its position.

Now the box may be considered sterile for a few moments.

Next we take the instrument by which we intend to convey yeast cells from a colony to a flask (*e.g.*, a glass rod either flattened at the end or tipped with a platinum wire according to the amount of yeast we desire to remove), plunge it in a flame, introduce it into the sterile box, leaving an inch or two of its end protruding (see Figure B).



In this position an instrument may safely remain for a few moments, chance infection having been reduced to a minimum, the cover of the box preventing air-borne micro-organisms from falling upon an object placed as described.

CHAPTER VI.

THE INOCULATION OF SMALL BOTTLES OF WORT.

THE successful propagation of yeast cells from a pure culture is dependent upon a series of operations, the essential condition being that the volume of sterilised wort intended to be fermented should be in proportion to the quantity of yeast available.

To illustrate this point more clearly, an amount of yeast that could be taken from a plate culture by the aid of a platinum wire should not be sown in a volume of wort exceeding 25 c.c.

Again, assuming that we are possessed of a flask containing 25 c.c. of wort in active fermentation, we should not introduce this volume into another

flask containing more than half a litre of sterilised wort.

It must be remembered that during transference of pure cultures from a colony to a flask or from one flask to another it is conceivable that infection by bacteria may be possible at the same moment, so that unless we can depend upon the survival of the fittest—that is to say, unless yeast cells are sufficient in number, or rather greatly in excess of requirements to start immediate fermentation, with consequent further increase of cells—the bacteria may obtain a more favourable chance of development.

Before proceeding to inoculate a flask from a colony, microscopical examination of a few cells having grown therein should always be undertaken.

A glass rod tipped with the platinum wire is sterilised by the flame and then placed under the sterile cover as described until cool, and then introduced into the colony, quickly withdrawn and

dipped into a small bottle of sterile water. A drop of the water is then examined, and if bacteria are found to be present the whole colony from which the cells were taken must be rejected.

Should no bacteria be disclosed by microscopical observation, the colony may be considered pure, and the operation of inoculation be proceeded with.

The chances of finding a bacterium in a droplet culture are very remote, and the same may be said of a plate culture, though (as has already been indicated) it is certainly conceivable that a bacterium, together with a yeast cell, may have developed in the same colony.

The dilutions at the time of inoculating a film render this contingency improbable. It is, however, better to take cells from a colony growing in the third stripe, the chances of infection by bacteria being less than in colonies growing in the first or second stripe, owing to the fact of there being fewer cells in it as a result of the excessive dilutions.

Assuming that microscopical examination has been satisfactory, we are ready to discuss the transference of cells from a pure colony. We will consider the various methods for which we have preparations at hand under separate headings.

Inoculation from a Droplet.

About 25 c.c. of wort is taken in an ordinary Bohemian flask of about 50 c.c. capacity.

A temperature of anything between 60F. and 70F. is suitable in the generality of cases, but in such instances as lager beer yeasts requiring low temperatures, or tropical yeasts requiring high temperatures, the actual degree of the wort at the time of inoculation should be taken into account.

Although directions have been given respecting the care and attention necessary during the preparation of nutritive media it may be as well to summarise them generally.

Flasks having been carefully sterilised are filled to about half their capacity with malt wort previously filtered and sterilised.

Cotton wool stoppers are then plunged into their necks (care being taken not to pack them so tightly as to endanger their breakage), whereupon they are immediately boiled.

To effect perfect sterilisation flasks containing wort should be boiled on three successive days.

If upon any occasion flasks such as may be required are not ready at hand, empty ones may be taken and maintained at 160C. in an oven for three hours before use.

They may then have sterile wort poured into them from the stock flask kept in the dust-proof chamber, whereupon they must be boiled and allowed to cool.

Although the latter directions are given in order to provide against emergency, they are not to be recommended, everything should be prepared in advance.

Stress having been laid upon the necessity of limiting the quantity of wort contained in a flask to an amount not exceeding half of its capacity, it may be as well to cite the reason.

As fermentation commences wort is liable to form a frothy head, which may possibly rise in the neck of the flask and come in contact with the cotton wool stopper. In the event of this happening and the wool stopper becoming damp with wort, a suitable field is thereby provided for infection from moulds, etc., which might settle upon it, develop, and contaminate the whole of the contents of the flask.

Plenty of space for the wort to ferment without any chance of contaminating the cotton wool stopper becomes obviously a matter of paramount importance.

The 50 c.c. flask we propose inoculating having been prepared according to the directions given is set upon the bench whilst we remove from the dust-

proof chamber the slip glass with its cover (fixed by vaseline) upon which the droplet is suspended.

The slip glass is then supported by being laid across the rim of a suitable glass or beaker.

A pair of forceps is then sterilised by flame and placed under a cover until cool, whereupon a small piece of sterilised paper is seized by them.

Next the cover glass whereupon the droplet is suspended is raised from the slip glass sufficiently by a sterilised spatula to allow the sterilised paper held by the forceps to come in contact with the colony of cells growing in the droplet.

The forceps are then quickly withdrawn and placed with their points under the tin sterile cover on the bench in such a manner as not to drop the piece of paper conveying the cells.

The wool stopper is then immediately removed from the 50 c.c. flask by another pair of forceps (hold the flask on its side while doing this, dust is less likely to fall down its neck), the piece of paper infected with the yeast cells ready and waiting

under the sterile cover is dropped in, the wool stopper is replaced ablaze, and the operation is complete.

The flask is then set to rest in the dust-proof chamber or thermostat should the requirements of temperature favourable to the development of the type under consideration be a matter of importance.

Observations taken in a few hours will disclose whether or no fermentation has commenced.

Some exercise will be necessary before the operations detailed may be performed with certainty of success. It is as well, therefore, to practise with empty flasks or flasks containing nothing of importance. It is not an easy matter at first to hold the slip glass, lift its cover glass, introduce the forceps holding the paper, lay them aside without dropping the paper, withdraw the stopper from the 50 c.c. flask, drop the paper in, replace the wool ablaze; these details are not accomplished without considerable chances of infection. But practice will make perfect, and in a surprisingly short time the operations will be performed with facility.

Inoculation from a Plate Culture.

First make a microscopical examination of a few cells taken from a colony according to the directions already given. (See Inoculation from a Droplet.)

The possibility of infecting a plate culture by bacteria (owing to its larger surface) being greater than in the case of a droplet, minute care must be exercised at the moment of lifting its cover, whilst transferring cells to flasks of sterilised wort.

All that has been said in the descriptions given under the heading of the droplet system respecting the placing of forceps under a sterile cover until cool applies also to a platinum wire, glass rod, or any other instrument employed for the purposes of conveying cells from colony to culture flask.

Plate cultures in comparison with droplet cultures have one advantage in their favour, for the reason that a greater number of cells may be transferred from them at one operation, and conse-

quently a somewhat larger flask than 50 c.c. capacity may be employed for their development.

For instance, assuming that a microscopical examination of a colony reveals nothing but pure yeast, there is no reason why in the place of a platinum wire, or forceps holding a speck of sterile paper, a glass rod with flattened ends should not be employed.

Such an instrument would convey yeast sufficient to inoculate at least 50 c.c. of wort or even more, the usual precaution being taken regarding the size of the flask employed, which in every case should be of about double the capacity of the volume of wort to be inoculated.

Speaking generally 12 to 18 hours should suffice for the complete fermentation of the flasks of wort inoculated as described, whereupon their contents may be transferred to flasks containing larger volumes of wort.

CHAPTER VII.

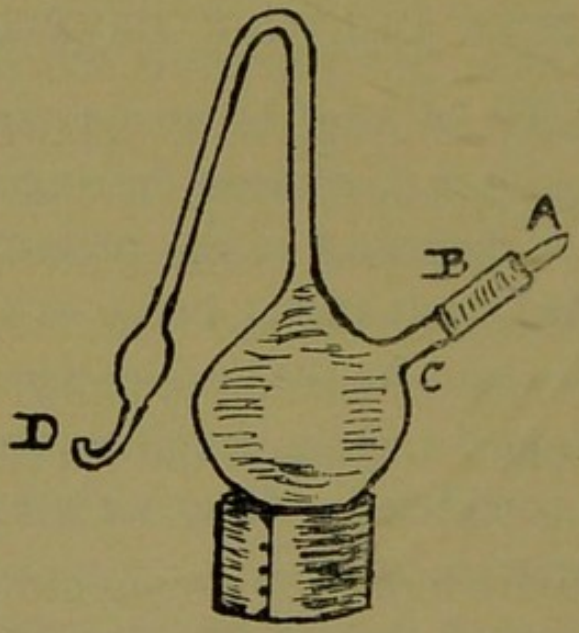
THE PURE CULTURE DEVELOPED.

BOTTLES of wort of 50 c.c. capacity containing 25 c.c. of wort having been inoculated as described in the preceding chapter, and having been left for 24 hours at the temperature most favourable to the development of the type of yeast contained therein, fermentation should by this time be completed. If so we are prepared to take the next step.

It is understood that in no case are cells to be transferred from one vessel to another without previous microscopical examination.

This being satisfactory, we may prepare to inoculate Pasteur flasks of half a litre capacity containing 250 c.c. of sterilised wort.

The Pasteur flask is placed upon a cylindrical cardboard support, and a Bunsen flame is allowed to play around the spout *C*, the glass stopper *A*, and rubber connection *B*, care being taken not to burn the rubber unnecessarily.



The 50 c.c. flask of fermented wort containing the pure yeast is then taken, shaken a little in order to distribute the cells equally over the whole volume, whereupon the wool is set alight and removed ablaze, thus preventing chance infection

from anything that may have settled upon the wool.

A sterilised pipette is then inserted into the 50 c.c. flask, and as much of the solution taken up as may be possible without suction (by the mouth) when the rubber *B* is removed from the neck *C* of the Pasteur flask, the pipette is inserted, and the liquid allowed to run in, whereupon the rubber *B* is immediately replaced.

Note that in the performance of this operation the stopper *A* is not removed from the rubber *B*.

The foregoing operations should be practised with flasks containing water until facility has been acquired.

After 24 hours fermentation should be complete, and the next step is easier than anything we have yet had to accomplish.

A Pasteur flask of 2 litres capacity containing 1 litre of unfermented wort is now taken supported by a cardboard cylinder and placed upon the bench (see Figure 1).

The half-litre flask containing the fermented wort and yeast cells is placed opposite to it (Figure 2).

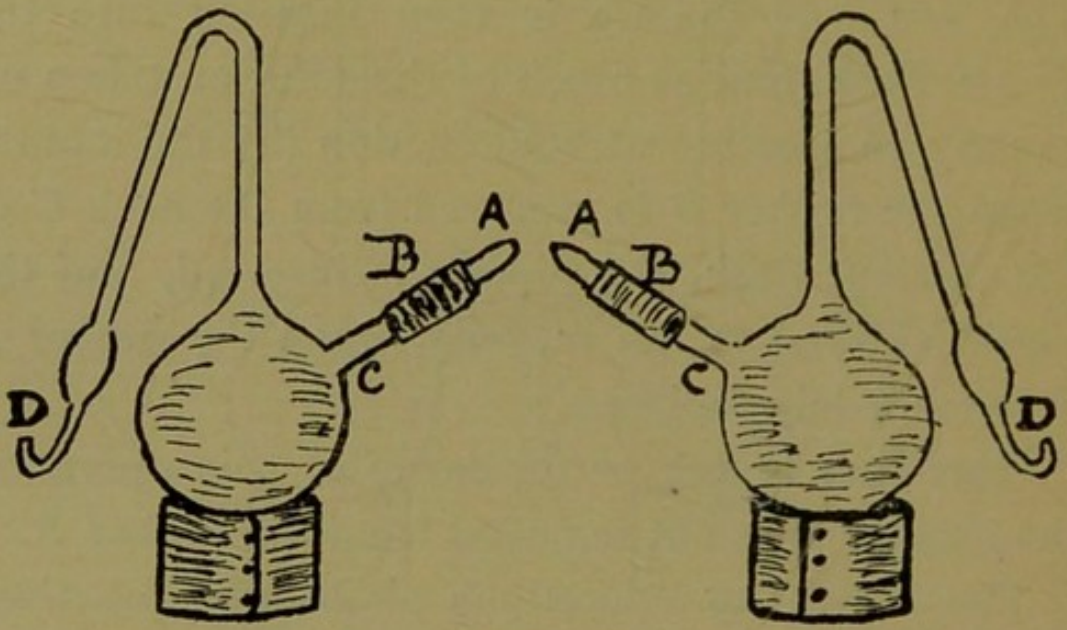


Fig. 1 Fig 2

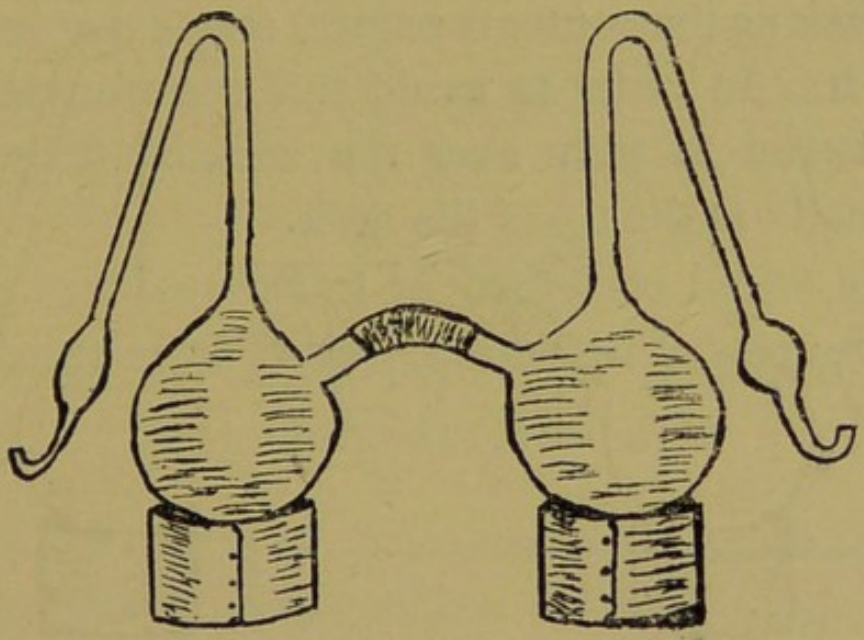
The Bunsen flame is then allowed to play over *A*, *B* and *C* in both cases (Figure 1 and Figure 2).

The glass stopper *A* (Figure 1) is then removed.

The glass stopper *A*, together with the rubber neck *B*, is then removed from Figure 2.

The spout *C* (Figure 2) is then introduced into the rubber connection *B* (Figure 1).

An illustration will show the result of the foregoing attachment.



Now it is a simple matter to pour all or any of the contents of Figure 2 into Figure 1, but in this respect a most important direction becomes necessary.

It will be obvious that as the liquid empties from Figure 2 into Figure 1 air must flow in at the neck *D* (Figure 2), or a vacuum would be created, and a cotton wool plug if placed at *D* would not allow the air to pass quickly enough to prevent gurgling

in the flask. The plug is therefore removed. Now it is equally obvious that the air (passing into the flask as the contents empty) may carry infection with it. In order to avoid this the Bunsen flame is allowed to play over the neck *D* (Figure 2), especially at the tip of the neck.

The liquid may then be transferred to Figure 1 without fear of becoming itself (Figure 2) infected.

CHAPTER VIII.

PURE YEAST PROPAGATED IN COMMERCIAL QUANTITIES.

WE are now in a position to consider the ways and means whereby the pure cultures contained in the Pasteur flasks may be propagated so that a sufficiency of yeast may be produced to ferment volumes of wort approaching commercial quantities.

A succession of vessels must be provided on a graduated scale; for example, the yeast from a two litre Pasteur flask may be sown in two or three gallons of wort, the yeast from the latter volume may be sown in 12 to 18 gallons, and so on.

But, as at this stage of the proceedings our experiments will have become somewhat cumbersome and bulky, a few further explanations and a short digression will be advantageous.

Hansen, in order to obtain a sufficiency of pure yeast for commercial purposes, devised a machine capable of supplying sterilised wort from a reservoir to a fermenting vessel constructed (so far as essential conditions are concerned) on the principle of a Pasteur flask.

Suitable means were provided for introducing sterilised air and withdrawing carbon dioxide as fermentation progressed. Cocks were also furnished for the introduction of steam for the purpose of sterilisation after cleaning, the withdrawal of yeast, and so forth.

In short, it was constructed in such a manner as to assure scientific accuracy in results and to be at all times under control.

It will be seen that by adopting Hansen's method any amount of pure yeast may be propa-

gated, the quantity being regulated by the capacity of the machine designed for the purpose.

But, as the cost of such a machine would be prohibitive to the majority of students and many breweries, we will consider the following alternative.

A can of 4 or 5 gallon capacity, such as painters use for oil or varnish, is a suitable vessel to employ during the first stage of developing the pure culture contained in the Pasteur flask.

The usual precautions respecting sterilisation of both can and wort being carefully observed before the introduction of the yeast.

If possible a can should be procured with an additional neck corresponding in size to the spout of a Pasteur flask, so that the transference of the contents of the Pasteur flask to itself may be accomplished by connecting them (the can and the flask) by a piece of rubber tubing in the same manner as described for the transference of the contents of one Pasteur flask to another.

Always bear in mind that it is inadvisable to fill a can to more than two-thirds of its capacity, and never omit the plugging of its neck with cotton wool ablaze at the earliest moment possible after the introduction of the yeast.

We have next to consider the propagation of the contents of the can after fermentation has been completed; and, as in all probability we shall no longer be able to work under aseptic conditions, it may be as well to think how suitable means may be devised for obtaining the best results, always bearing in mind that our devices must be inexpensive and practical.

There are few breweries that do not contain small copper vessels of some sort, ranging in capacities from 9 gallons to 4 or 5 barrels, employed for the storage of yeast, the dissolving of sugar, the boiling of primings, and so forth.

Such vessels will be found suitable for our present purpose. Every care must be taken that they are properly cleaned and burnished and

sterilised by the action of steam or by being filled with boiling water.

If convenient it is better to take the wort we intend to ferment straight from the hop back main as hot as possible, thus avoiding infection from coolers, refrigerators, and the pipes and connections incidental to them. Cooling may be achieved either by jacket or attemperator. Objections respecting the lack of aeration of wort owing to the neglect of coolers need not be seriously considered as long as we are working with small volumes and our purpose is confined to increasing yeast in preference to perfection in the fermented wort.

The wort having been cooled to the degree most favourable for fermentation (60F., for example), the yeast (contained in the can) we desire to increase should be emptied into it and immediately stirred in by a thick glass rod or something else suitable.

It is important to bear in mind that by the adoption of vessels no longer under control regard-

ing infection—that is to say, open instead of closed vessels—we are running the risk of our cultures (over which we have taken such elaborate care) becoming infected either with yeasts of undesirable types or any other micro-organisms. We must depend, therefore (or rather we shall have to later when working on the full scale of the brewery), on the survival of the fittest. There is no reason, however, why simple precautions should not be taken whilst we are still at work in our progressive stages, that is to say, whilst fermenting small volumes of wort.

Therefore it is well to provide some suitable cover to the fermenting vessel, which, whilst allowing the escape of CO_2 and the admission of air, does not allow serious infection from outside sources.

A clean linen cloth answers the purpose well; it should be either thrown lightly across the opening, or in the case of a round vessel secured and stretched more or less tightly by a hoop, just as,

for example, in the case of yeast being prepared for transit a piece of hop cloth is supplied instead of a "head" to the cask.

A further precaution may be taken by rinsing the linen cloth in water containing either salicylic acid or bisulphite of lime or potash. When removing the cover, care must be exercised to avoid its being shaken over the fermenting liquor, for should any micro-organisms have settled upon it they would become intermixed with the pure yeast.

The foregoing operations being repeated each time with a larger volume of wort should result eventually in the possession of sufficient pure yeast for adequate working on a commercial scale.

Whenever practicable, it is better to use vessels of capacities slightly above the volume of wort employed rather than to ferment, for example, 10 barrels of wort in a large fermenting vessel.

As far as possible the same precautions should be observed when working to the full capacity of

the brewery as have been taken during the intermediary stages.

Should copper vessels not be available for the development of cultures, the next alternative would be to employ wooden vessels, vats or backs, care being taken to thoroughly pitch them with boiling pitch before putting them to use.

Wooden fermenting vessels are undoubtedly the cause of serious infection in the brewery. For this reason it is the general custom on the Continent to pitch them. Unfortunately this is not the case at home, but as long as we are compelled to employ them without being pitched we must do all in our power to minimise their undesirable influence, either by filling them with boiling water, with or without antiseptics, according to their condition, or by the application of steam, or by cauterising their surface with a flame, or by any other method appropriate to their sterilisation.

So far we have limited our discussion to means suitable to the development of yeast, it may be well

before closing this chapter to give a few moments' consideration to the source from whence we propose to obtain the type most suitable for propagation.

Assuming that we are at work in a brewery or distillery, it is unlikely that we should be unable to find some yeast at hand from whence we may take a supply of cells in a more or less healthy condition. Even should it be badly infected with bacteria the type is probably better suited to the locality than any to be obtained from outside sources, and the bacteria themselves matter nothing to us now that we know how to eliminate them.

Should, however, the yeast be really weakened and exhausted owing to successive sowings in unsuitable wort (wort composed of carbohydrates resulting from excessive percentages of raw grain, for example), it is better to take it with all of its impurities and let all grow together in a flask or beaker of wort prepared according to the method set forth in a previous chapter, in order to secure

its return to a healthy and vigorous condition before attempting to isolate a single cell from it.

Single cells if emaciated are liable to dry up both on cover glasses (should we adopt the droplet system), or agar surfaces should we be working with flasks or tubes.

Time, therefore, will be gained by first giving attention to the feeding of the cells upon such food as may strengthen them.

CHAPTER IX.

DIFFERENTIATION BETWEEN SPECIES OF YEAST.

THE differentiation between species of yeasts becomes increasingly important as further knowledge is gathered by investigators respecting the technical effects resulting from the use of definite types.

Thus where many characteristics are found in common between two species it becomes necessary to establish some test whereby each may be determined.

The microscope is of little or no assistance to us in this respect—in fact, excepting for certain obser-

vations it is useless, for the reason that many types of yeast are able under certain conditions to take to themselves the contour of others, and one may, therefore, be easily drawn to false conclusions by depending upon the test of microscopic observation alone.

Chief among the reliable tests whereby one type may be distinguished from another is what is technically known by the name of "sporulation" or spore formation.

Spore formation furnishes one of those curious instances of the persistence of nature asserting itself under abnormal conditions, and besides being remarkable in itself provides an instance of "overlapping" between the animal and vegetable kingdoms.

Sporulation (so far as yeasts are concerned) is neither more nor less than the giving birth (by micro-organisms belonging to the vegetable kingdom) to their kind in their complete form just as an animal gives birth to its young.

Temperature and time have their marked influences on spore formation, and in order to determine with certainty the actual type of yeast under observation both of these have to be remarked with accuracy.

The method by which spore formation is accomplished is by sowing yeast cells (from pure cultures) on gypsum blocks.

The details incidental to the experiment are as follows :—

Plaster of Paris is mixed with water to form a stiff paste, and whilst in the plastic state is formed into cones of about 1 inch diameter at the base. Each cone is then divided about the centre (parallel to the base) to form a circular plane. They are then allowed to dry, and may be sterilised by baking. To ensure perfect sterilisation, it is as well before putting them to use to pour a little absolute alcohol over them and set it ablaze, and whilst still hot place

them on a sterilised tile or other suitable object and cover them with a glass bell embedded in vaseline.

When cold their surface is spread with a thin layer of yeast cells from a pure culture by the aid of a sterilised platinum spatula, the bell cover is replaced over them, and they are ready to be conveyed to the thermostat.

As a rule it is well to conduct several experiments at the same time, that is to say, if a thermostat is available possessing chambers at various temperatures.

In the Institüt für Garüngsgewerbe in Berlin there is a thermostat containing some 25 or more chambers, one for every four degrees of the Centigrade scale. With such facilities at command work is reduced to a minimum, and it only remains to introduce experimental objects into chambers at temperatures probably favourable to their respective requirements and note the results at as many intervals as may be considered necessary.

The actual phenomenon of sporulation may be

described as a lengthening-out of a cell, until its familiar circular shape is entirely dispelled, eventually giving place to a more or less elliptical form somewhat resembling a pea or bean pod.

Once the elongation phase in the metamorphosis is observed, the next thing to look for is a cell or cells formed or forming in the ellipse, and here again the resemblance to a bean pod containing beans is more remarkable.

At this stage it is merely a question of time for the parent cell or pod to burst and give forth the new cells it has formed within itself.

Another essential condition to spore formation in addition to temperature and time is restriction of nutriment, as we have seen in the case of imposing yeast cells upon gypsum blocks.

Samples of yeast taken from pure cultures grown on agar surfaces are often to be observed exhibiting spores, the reason being that owing to the compression of cells in the colonies, with consequent

restriction of nutriment, a result similar to that obtained from the gypsum block has been achieved without either assistance or intention.

The following results in respect of temperature and time favourable and unfavourable to spore formation have been determined by Professor Hansen, and are given here in order to guide the student in his researches.

SACCHAROMYCES CEREVISIÆ I.

Temp. F.		Time.
100·0	no development.	
97·0	appearance of distinct rudiments after	29 hours.
95·0	,, ,, ,, ,, ,,	25 ,,
92·0	,, ,, ,, ,, ,,	23 ,,
86·0	,, ,, ,, ,, ,,	20 ,,
77·0	,, ,, ,, ,, ,,	23 ,,
73·4	,, ,, ,, ,, ,,	27 ,,
63·0	,, ,, ,, ,, ,,	50 ,,
61·0	,, ,, ,, ,, ,,	65 ,,
52·0	,, ,, ,, ,, ,,	10 days.
48·2	no development.	

Saccharomyces Pastorianus I.

Temp. F.		Time.
89·5	no development.	
85·0	appearance of distinct rudiments after	30 hours.
84·2	,, ,, ,, ,, ,,	27 ,,
83·0	,, ,, ,, ,, ,,	24 ,,
74·0	,, ,, ,, ,, ,,	26 ,,
64·4	,, ,, ,, ,, ,,	35 ,,
59·0	,, ,, ,, ,, ,,	50 ,,
50·0	,, ,, ,, ,, ,,	89 ,,
47·0	,, ,, ,, ,, ,,	5 days.
44·6	,, ,, ,, ,, ,,	7 ,,
38·0	,, ,, ,, ,, ,,	14 ,,
33·0	no development.	

Saccharomyces Pastorianus II.

Temp. F.		Time.
84·2	no development.	
81·0	appearance of distinct rudiments after	34 hours.
77·0	,, ,, ,, ,, ,,	25 ,,
73·4	,, ,, ,, ,, ,,	27 ,,
62·6	,, ,, ,, ,, ,,	36 ,,
59·0	,, ,, ,, ,, ,,	48 ,,
52·5	,, ,, ,, ,, ,,	77 ,,
44·6	,, ,, ,, ,, ,,	7 days.
38·0	,, ,, ,, ,, ,,	17 ,,
33·0	no development.	

Saccharomyces Pastorianus III.

Temp. F.		Time.
84·2	no development.	
81·0	appearance of distinct rudiments after	35 hours.
79·0	,, ,, ,, ,, ,,	30 ,,
77·0	,, ,, ,, ,, ,,	28 ,,
71·6	,, ,, ,, ,, ,,	29 ,,
62·6	,, ,, ,, ,, ,,	44 ,,
60·8	,, ,, ,, ,, ,,	53 ,,
51·0	,, ,, ,, ,, ,,	7 days.
47·0	,, ,, ,, ,, ,,	9 ,,
39·2	no development.	

Saccharomyces Ellipsoideus I.

Temp. F.		Time.
90·0	no development.	
87·0	appearance of distinct rudiments after	31 hours.
85·0	,, ,, ,, ,, ,,	23 ,,
77·0	,, ,, ,, ,, ,,	21 ,,
64·4	,, ,, ,, ,, ,,	33 ,,
59·0	,, ,, ,, ,, ,,	45 ,,
51·0	,, ,, ,, ,, ,,	4½ days.
45·0	,, ,, ,, ,, ,,	11 ,,
39·2	no development.	

SACCHAROMYCES ELLIPSOIDEUS II.

Temp. F.						Time.	
95·0	no development.						
92·0	appearance of distinct rudiments after					38	hours.
91·4	,,	,,	,,	,,	,,	27	,,
88·2	,,	,,	,,	,,	,,	23	,,
84·2	,,	,,	,,	,,	,,	22	,,
77·0	,,	,,	,,	,,	,,	27	,,
64·4	,,	,,	,,	,,	,,	42	,,
51·8	,,	,,	,,	,,	,,	5½	days.
46·4	,,	,,	,,	,,	,,	9	,,
39·2	no development.						

Another valuable test whereby types may be differentiated is that technically named by Hansen "voile," and freely translated by Gordon Salamon by "Mothering."

This term "Mothering" is used to describe a film formation, which is capable of being produced under suitable conditions by true saccharomyces on the surface of wort at the close of fermentation, just as the common mould Penicilium glaucum forms a more or less thick felt on the surface of exposed jam.

The essential condition to "Mothering" is free access of filtered air to a flask containing pure yeast sown in sterilised wort.

This access of air is accomplished by covering the neck of a flask with sterilised filter paper. At the close of fermentation specks or films will exhibit themselves on the surface of the liquid, which in time will spread over the entire surface. Time and temperature have their marked effects on "Mothering," just as they have on spore formation, and the following tables provided by Hansen will be found of assistance:—

LIMITING TEMPERATURES OF "MOTHERING" FORMATIONS.

S. Cerevisiæ I.

Deg. Fahr.					Time.
At 100·4	no formation.				
,, 92·0	flecks feebly developed	after			9·18 days.
,, 81·0	,,	,,	,,	,,	7·11 ,,
,, 70·0	,,	,,	,,	,,	7·10 ,,
,, 57·0	,,	,,	,,	,,	15·30 ,,
,, 43·0	,,	,,	,,	,,	2·3 months.
,, 41·0	no formation.				

S. Pastorianus I.

Deg. Fahr.		Time.
At	93·2 no formation.	
,,	81·0 flecks feebly developed after	7·10 days.
,,	70·0 ,, ,, ,, ,,	8·15 ,,
,,	57·0 ,, ,, ,, ,,	15·30 ,,
,,	43·0 ,, ,, ,, ,,	1·2 months.
,,	39·0 ,, ,, ,, ,,	5·6 ,,
,,	36·0 no development.	

S. Pastorianus II.

Deg. Fahr.		Time.
At	93·2 no formation.	
,,	81·0 flecks feebly developed after	7·10 days.
,,	70·0 ,, ,, ,, ,,	8·15 ,,
,,	57·0 ,, ,, ,, ,,	10·25 ,,
,,	43·0 ,, ,, ,, ,,	1·2 months.
,,	39·0 ,, ,, ,, ,,	5·6 ,,
,,	36·0 no formation.	

S. Pastorianus III.

Deg. Fahr.		Time.
At	93·2 no formation.	
,,	81·0 flecks feebly developed after	7·10 days.
,,	70·0 ,, ,, ,, ,,	9·12 ,,
,,	57·0 ,, ,, ,, ,,	10·20 ,,
,,	43·0 ,, ,, ,, ,,	1·2 months.
,,	39·0 ,, ,, ,, ,,	5·6 ,,
,,	36·0 no development.	

S. Ellipsoideus I.

Deg. Fahr.					Time.
At 100·4	no formation.				
,, 92·0	flecks	feebly	developed	after	8·12 days.
,, 81·0	,,	,,	,,	,,	9·16 ,,
,, 70·0	,,	,,	,,	,,	10.17 ,,
,, 57·0	,,	,,	,,	,,	15·30 ,,
,, 43·0	,,	,,	,,	,,	2·3 months.
,, 41·0	no formation.				

S. Ellipsoideus II.

Deg. Fahr.					Time.
At 104·0	no formation.				
,, 98·0	flecks	feebly	developed	after	8·12 days.
,, 92·0	,,	,,	,,	,,	3·4 ,,
,, 81·0	,,	,,	,,	,,	4·5 ,,
,, 70·0	,,	,,	,,	,,	4·6 ,,
,, 57·0	,,	,,	,,	,,	8·10 ,,
,, 43·0	,,	,,	,,	,,	1·2 months.
,, 38·0	,,	,,	,,	,,	5·6 ,,
,, 36·0	no formation.				

The degree of temperature at which yeast cells resist destruction, taken in conjunction with their particular habits of spore and film formations, is also a valuable test towards the determination of types.

Thus, for example, *Saccharomyces Thermantitonum* can form spores under conditions corresponding in temperature and time to other yeasts, but its temperature of resistance to destruction at 180F. is greatly in excess of any saccharomyces so far discovered.

The following details, whereby the temperature of resistance to destruction of types may be determined, are given from the author's personal notebook. Their value consists in that they provide not merely additional data to that already collected by others, but illustrate further that there is probably a large field of research awaiting investigation respecting fermentation at high temperatures by yeasts possessing abnormal powers of resistance.

The experiments are performed in the following manner:

Ten pieces of glass tubing, each about 4 in. long, are taken, whereupon they are sterilised by

flame, and both ends are then drawn out in the manner of a pipette.

Sufficient yeast is then scraped off the surface of a pure culture and stirred into sterilised water until it becomes milky in colour.

A few drops of the milky-coloured solution are then drawn up into each pipette, whereupon both ends are sealed in the Bunsen flame.

The whole of the ten tubes are then submerged in a water bath containing cold water, whereupon a Bunsen burner is placed underneath to raise its temperature.

By the time that the temperature has risen to 122F. the time has to be noted and one tube of yeast has to be withdrawn.

Another tube is to be withdrawn at every rise of 9 degrees in temperature, the time being noted upon every occasion, until all have been removed.

Ten small flasks of wort are then taken and inoculated—one from each tube. The ends of the tubes are broken and the contents allowed to flow

into the flasks. The inoculated flasks are then placed in the thermostat (in the case of *Saccharomyces Thermantitonum* at a temperature of 105F.), the time being noted at the first sign of fermentation.

An example will illustrate the foregoing clearly.

No.	Time of withdrawal of tube from the water bath.	Temp. F.	No. of hours that expired before fermentation commenced.
1.	12.25	122	40
2.	12.35	131	40
3.	12.45	140	48
4.	1. 0	149	50
5.	1. 8	158	no fermentation.
6.	1.30	167	,,
7.	1.35	176	,,
8.	1.42	185	,,
9.	1.50	194	,,
10.	2. 0	203	,,

From the foregoing table it will be seen that in the case of tube No. 4, thirty-five minutes elapsed whilst the temperature was being raised from

122F. to 149F. Yet the yeast was not destroyed, but became active and vigorous within 50 hours after having been introduced into the small flask of wort.

Reference to tube No. 5 will show that 43 minutes elapsed whilst the temperature was being raised from 122F. to 158F., but in this case the temperature taken in conjunction with the time occupied will be seen to have been sufficient to have caused the destruction of the yeast. No fermentation had taken place in the bottle of wort inoculated by it.

Another useful experiment in respect of resistance to temperature may be performed in the following manner:

Take three tubes as before, draw out their ends and introduce *water* and yeast, seal them. We will call these A1, A2 and A3.

Take three more tubes and introduce *wort* and yeast, seal them. We will call these B1, B2 and B3.

Now take a water bath and raise its temperature to 140F. Insert tubes A1 and B1 and submerge them for ten minutes, maintaining the temperature of 140F., and then withdraw.

Now raise the water bath to 160F. Insert tubes A2 and B2 and submerge them for ten minutes, maintaining the temperature to 160F., and then withdraw.

Now raise the water bath to 180F. Insert tubes A_3 and B_3 and submerge them for ten minutes, maintaining the temperature at 180F., and then withdraw.

To make all clear—

A1 and B1 have been submitted to a temperature of 140F., A2 and B2 to a temperature of 160F., and A_3 and B_3 to a temperature of 180F.—in every case for a period of ten minutes.

Six flasks of wort are now taken and inoculated by the contents of the several tubes. When fermentation commences, the time is to be noted.

As an example, the above experiments were con-

ducted with *Saccharomyces Thermantitonum,* and in every case the bottles of wort maintained at a temperature of 110F. commenced to ferment within 48 hours after having been inoculated.

It will be observed from perusal of the details of the foregoing experiments that the yeast employed resisted destruction whether mixed with wort or water in the tubes.

This power of resistance, however, cannot be expected with all yeasts.

Some species would be able to resist destruction by wort, but would be destroyed by water at the same temperature.

CHAPTER X.

THE SUBJECT CONTINUED.

WE have to thank Professor Lindner, of Berlin, for a remarkable series of observations he has made in respect of the form or contour taken by the mass of cells composing pure cultures whilst growing upon agar or gelatine surfaces.

He has divided his experiments into two parts under the headings of giant cultures and stripe cultures.

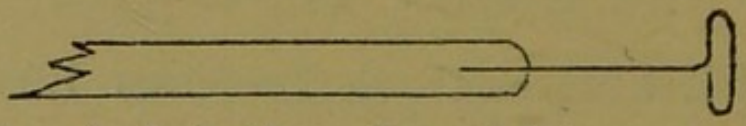
The details of the giant culture experiment are as follows:

A flask of about 250 c.c. capacity is filled to about half of its volume with wort agar, it is then

sterilised and plugged with cotton wool according to the usual rules, and allowed to cool and solidify.

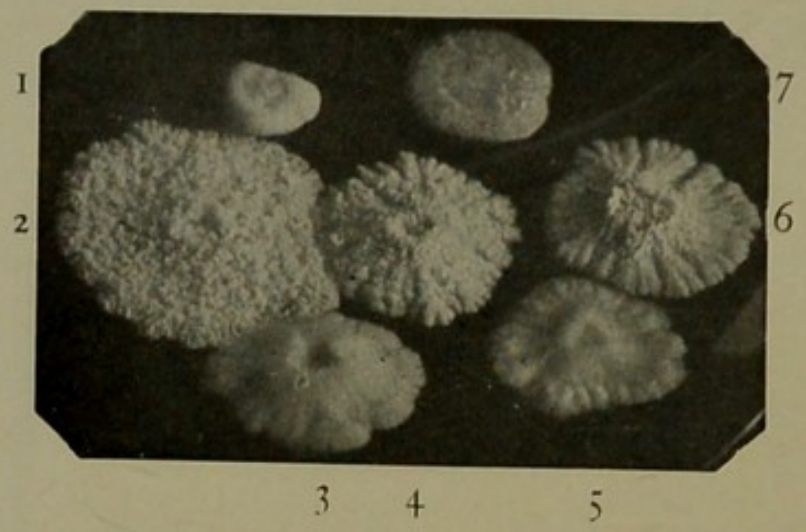
A platinum wire fixed into a glass rod is then taken and its end is formed into a circle about a quarter of an inch in diameter, and then turned at right angles to the wire.

Thus—



The wire circle is then dipped into a pure culture of yeast growing upon wort agar, care being taken to distribute cells of yeast as evenly as possible over the whole of its circumference.

The wire is then introduced into the flask above mentioned and an impression is made with it upon the centre of the surface of wort agar. Should the operation have been performed successfully a complete circle of yeast cells should be visible, just as a printed impression is made by imposing an inked printer's block upon the surface of a sheet of paper.



Giant Cultures of Seven Species of Yeast

PROF. LINDNER.

1. Yeast from Cane Sugar Molasses.
2. Yeast from a Russian Beer.
3. Saccharomyces Cratericus.
4. Yeast from Russian "Kwass."
5. A Pastorianus Shaped Yeast.
6. Saccharomyces Cartilaginosus.
7. Yeast from Concentrated Sicilian Wine-Must.

To face page 115

The usual precautions are to be taken respecting chance infection during the operation and whilst plugging the flask's neck with cotton wool ablaze.

After a short period, the growth of yeast cells will fill up the space within the circle and spread beyond the initial circumference until a more or less circular object of a diameter of $\frac{3}{4}$ to 1 inch is formed. As the diameter increases so do the yeast cells pile themselves up into thicker layers, and the result is the giant culture. The form this culture exhibits when well developed is a reliable test of the type of yeast under observation.

Reference to the illustration of giant cultures, kindly lent by Professor Lindner, will show the forms taken by various types in a marked degree.

For stripe cultures a wort gelatine or wort agar film is taken and inoculated by painting upon it three stripes. The first stripe should be painted with water containing a sufficiency of cells to render it milky, the second stripe should be painted from a 10 per cent. solution of the mixture used for

the first stripe, and the third should be painted from a 10 per cent. solution of the second.

Minute care must be taken that the yeast cells employed are from pure cultures.

The film is then covered and placed in the thermostat at the temperature most favourable to the type under observation, and the contour of the outside edges of the stripes is noted as they become distinct.

The difference between the contours of the edges of the stripe cultures according to varieties of saccharomyces is most striking and interesting.

The student should, while making his experiments, take accurate notes of the time occupied during the development of the giants or stripes—the temperature at which they were propagated—and, further, he should draw a picture, to the best of his ability, showing the outside edges and general aspect of his cultures to keep for reference.

Another valuable observation may be made in respect of the horizontal aspect of yeast after completing fermentation.

For example, a rectangular bottle is filled to half its capacity with wort and inoculated from a pure culture. It is then placed in the thermostat until fermentation is finished and the cells allowed to deposit. The horizontal aspect of the yeast is then noted—whether it is perfectly flat, whether there are mountains, whether there are undulations, whether there are craters. All of these observations, strange as it may seem, are of the greatest assistance towards the determination of types.

Reference has been made to the fact that some yeasts are able to liquefy wort gelatine, but are yet unable to do so with wort agar.

In the event of many characteristics having been observed in common between two types of yeast, recourse may be had to this method of differentiation. An example will make this clear.

A species of yeast which we will call *x*, if sown as a giant culture on 500 c.c. of wort gelatine contained in a litre flask, is capable of liquefying the entire volume, but another yeast which we will

call *z*, although having many characteristics in common with the type we have designated *x*, is unable to do this.

Here at once is to be found an undoubted method of differentiation between the two.

The ability to ferment different sugars is again an important test by which types may be determined—for example, some yeasts cannot ferment maltose; again, others are unable to ferment dextrin; another type may be unable to ferment glucose, and so forth.

It will be seen, therefore, that by this method alone—viz., the ability or inability of various types of yeasts to ferment certain sugars—may be formed a basis of analysis as valuable as any of the other means we have enumerated.

Attenuation limit is also another matter of the greatest importance. For example, a certain yeast isolated from cane juices is capable of reducing the gravity of a wort from 1·050 to 1·015, but it is incapable of reducing it further.

But upon sterilising the above fermented wort (this may be done by distilling half of the volume and then returning the distillate, when cool, to the remainder from which it was distilled) and then adding another molasses yeast, a further reduction of gravity—viz., from 1·015 to 1·008—may be accomplished.

Here again is a most valuable indication of the differentiation between the two types.

In appearance, in their temperature favourable to development, and for fermentation, in their ability to precipitate, and in other characteristics they have much in common, yet they are not the same yeasts, for the reason that one can reduce the gravity of the same wort to a lower degree than the other.

Enough has been said to indicate to the student the means whereby he may establish a reliable basis upon which he may construct methods of his own for the differentiation of types.

CHAPTER XI.

THE TRUE ALIMENTATION OF YEAST.

THE necessity of supplying the true alimentation of yeast—that is to say, soluble nutritive matter most suitable to the type employed—does not seem to have received the attention due to its importance. It is one thing to isolate a yeast cell and propagate it successfully in the laboratory; it is quite another to employ it commercially in such a manner as to obtain the best results from its use.

To take an instance: we will suppose that a yeast has been successfully propagated in the laboratory on a wort composed entirely of malt extract.

It might easily be assumed that the same alimentation in practice would be not merely necessary, but that the best results would be obtained by carefully observing the same rule upon every occasion.

This does not follow, however, as we shall see if we proceed to consider the subject further.

There are other matters beside actual yeast propagation to be taken into consideration, such as, for example, good fining properties, agreeable flavours, fullness or the reverse of the finished beer. All these factors, and many others, will occur to the practical brewer as important considerations calling for his attention.

Speaking broadly, the percentage of nitrogen in the form of soluble albumen, or as nitric acid (forming nitrates) present in a wort, is a controlling factor in the healthy or unhealthy development of yeast.

We will consider this statement in detail and

endeavour to adduce evidence to prove whether or not this is so.

All who have had the advantage of practical experience must have remarked that under varying conditions yeast gathered from brewings may be classified under three headings.

Unhealthy Signs Due to Feeding upon Worts too Rich in Nitrogen.

A peculiar reddish colour varying in shades between ochre and orange. Such colour generally being associated with sluggishness in fermentation, difficulty in separation from the finished beer and an extremely pasty consistence.

Healthy Signs.

Yeast of a light creamy colour which, rushing through the swan necks of the unions, soon fills the troughs to overflowing. When "wisped" in order to lessen the bulk it resembles beating a bag of feathers, so light is it and so persistently flocculent.

Unhealthy Signs Due to Feeding upon Worts too Poor in Nitrogen.

A blistery meagre appearance, exhibiting a greenish-yellow colour generally associated with yeast collected from a boiling fermentation.

Let us endeavour for a moment to try to see whether we can reasonably attribute the foregoing results under the three headings to the presence of nitrogen in greater or less degree.

We will take them in order.

Every observant practical brewer will have remarked that the qualities of extreme redness of yeast and sluggishness in fermentation are associated with worts derived from heavy English barley malts, probably carrying a high percentage of albuminous matter.

To continue brewing a long series of worts from such malts would in time so aggravate the trouble of sluggishness in fermentation that at last extreme

turbidity would ensue, and beers from every succeeding wort would taste increasingly yeasty and would eventually become actually yeast-bitten.

Brewing waters containing excessive nitric acid (as nitrates) used in conjunction with grain carrying a small percentage of albumen produce a very similar effect, and in cases where iron plant is used—especially iron hop backs or coolers—the redness not only of the yeast but also of the beer fermented by it becomes conspicuously noticeable.

Sufficient evidence is, therefore, at hand to prove that nitrogen, no matter how it may be combined, is objectionable if present in excess of requirements.

The best way of overcoming the trouble arising from water containing nitrates in undesirable quantities is to reject it and brew from another source, but in the case of malts carrying high percentages of albuminous matter it becomes a question of serious loss should it not be used, and the best way out of the difficulty—in fact, the only

remedy—is to blend it with grain such as rice, or maize, or invert sugar, or any other material carrying little or no albuminous matter, in order that as a result of such judicious blending the average percentage of nitrogen may be reduced.

The creamy colour of yeast, associated as a rule with great vigour and the greatest facility in separating from the fermented wort, may generally be taken as an index of satisfactory alimentation.

The above conditions usually result from the fermentation of worts composed of 10, 15 or even 20 per cent. of flaked rice or maize employed in conjunction with malts derived from well-ripened barleys grown on light soils, especially foreign varieties, such as Chilian, Smyrna or others grown in sunny climates.

Malts derived from such sources contain, as a rule, considerably less albuminous matter than those manufactured from heavy English varieties. And here we have an illustration of successful

results, undoubtedly due to the restriction of nitrogenous bodies.

It must not be concluded, however, from the foregoing observations that malt alone is unable to provide satisfactory alimentation over a long succession of brewings—experience proves the contrary to be true—the point, however, to be kept uppermost in mind is that malts should be chosen that do not carry high percentages of nitrogen, if malt alone is to be employed.

But, lest we may be inclined to restrict albuminous matters unduly, let us look at the results due to such practice, and take as an example worts made up of malt and grain or raw sugar, in which the percentages of the latter materials have become excessive. We find that poor, thin, blistery heads rise on the surface of the fermenting tuns—sometimes the fermentations "boil"—the yeast collected is poor both in quantity and quality and oftentimes exhibits the greenish-yellow colour we have mentioned in our third classification.

Beers produced from such worts may be poor and thin, and may or may not possess an objectionable flavour. What is the inference to be deduced? Is it not that the worts, having been composed of materials carrying insufficient nitrogen, have been unable to provide the true alimentation of yeast?

Enough has been said to support the contention that nitrogen, no matter how combined, is a controlling factor in the healthy or unhealthy development of yeast cells which, in their turn, affect the finished beer for good or ill materially.

In the case of lager beer breweries the percentage of albuminous matter in a wort is not a matter of the same importance as in the case of a high fermentation brewery, for it must be remembered, apart from the question of the varieties of yeast employed, that from the moment of commencing the fermentation of lager beer the temperature is lower by many degrees than in the case of high fermentation beer, such low temperature

being favourable to the precipitation of albuminous matter, in varying quantities, according to the length of time occupied in fermentation, the percentage of alcohol formed, the mineral salts present in the brewery water, and so forth.

In a paper read before the Institute of Brewing on June 19th, 1905, on the subject of *Saccharomyces Thermantitonum,* reference to the alimentation of yeast will be found, and as it presents the idea now under discussion in a concise form it may be as well to quote the following paragraph :

"I suggest that such troubles as turbidity in summer time may oftentimes be due to the fact that many wild ferments are present in our brewery yeasts in the hot season, which, although if isolated and their proper alimentation provided, might yield good results, yet their presence in a wort probably unsuitable to their normal habits causes those difficulties of clarification which render the Operative Brewers' position so perplexing."

The importance of finding the true alimentation

of yeast is well illustrated by the discovery of *Saccharomyces Thermantitonum*.

Here is an instance of the isolation of a new type possessing unknown properties, which had to be fully investigated before any practical advantage could be gained by its use.

The initial laboratory experiments more than justified the supposition that a new era in fermentation had dawned, at any rate so far as its tropical application was concerned, but before it became possible to reap the benefits of the discovery every conceivable combination of soluble carbohydrates had to be tried in order that the best results might be obtained.

All the factors enumerated regarding flavours, secondary fermentation, and so forth, had not merely to be faced, but steps had to be taken to ensure the results aimed at by establishing something in the nature of a basis for future guidance.

The modifications in the composition of worts that from time to time became necessary may be

useful to illustrate the importance of minute and accurate observation.

In the first instance—that is to say, within a short time of the discovery of *Saccharomyces Thermantitonum*—it had been fed on wort composed of 80 per cent. malt extract and 20 per cent. maize, and this with most excellent results. The flavour was in no way different to ordinary fermented wort, and it fined with extraordinary facility.

The first time, however, that it was introduced into a brewery on a commercial scale the wort of the day happened to be composed of about 90 per cent. malt and 10 per cent. invert sugar.

The flavour of the resultant beer was objectionable, and nothing but filtering would clear it. Isinglass finings had no more effect on it than had they never been introduced.

It would seem that in order to keep certain types of yeast healthy it is not merely necessary to feed them, but also to provide them with something

calling into the fullest action their inverting power. Apparently, food too easily assimilable in some cases causes types to deteriorate, fermentation may become sluggish, beer may remain turbid, with the utmost persistence, flavours may be anything but agreeable.

It would appear that such yeasts as the above require to ferment worts containing 5 or 10 per cent. of raw cane sugar in order that they may multiply successfully.

From experiments undertaken in certain German lager beer breweries, where low temperatures ruled and only malt was used in the composition of the wort, a most pronounced and persistent yeast bite resulted when fermented with *Saccharomyces Thermantitonum,* which seemed to point to the necessity of employing worts containing some proportion of raw grain or sugar.

This conclusion proved to be correct, taking the temperature of fermentation (about 38 to 40F.) into consideration.

But to illustrate the rashness of jumping to the conclusion that favourable conditions under one set of circumstances must be favourable under all, it may be cited that the exact contrary was found to be true whilst fermenting with *Saccharomyces Thermantitonum* under a tropical sun.

Here it was proved that worts composed entirely of malt were absolutely essential at intervals—that is to say, that yeast weakness was exhibited to a startling degree after fermenting a succession of brewings containing much rice, maize or sugar. But the yeast when regrown in malt wort regained its vitality, leaving the finished beer without the smallest trace of yeast bite.

Again, successive brewings of all malt wort resulted in excessive turbidity, and more than a little knowledge and observation were necessary to determine the exact stage when it became advisable to increase or decrease the percentage of malt.

Much more might be written, but sufficient has

been said to show that there is no royal road to success.

It may be urged, of what use, then, is it to discuss the question of alimentation? How is one to know what to do upon every occasion? There seems to be no rule by which one may be guided.

To such an argument the reply is that there is a rule, and that a reliable one—it is the rule of observation.

A very accurate record should be kept of the condition of the yeast collected from every brewing, and when introducing it into another, the number of the brewing from which it was taken should be recorded.

Such details are essential. They provide a sequence of the happenings during the life's history of the yeast for successive stages, and an observant student will soon be able by many little signs to gauge from time to time the conditions necessary to be observed in order to control continuous success.

Modifications both in material and mashing temperatures may become important. Another way would be to so arrange brewings that yeast weakened by excess of raw grain might be resown in a wort composed of as much malt as may provide its true alimentation.

Hitherto it has not been usual to look at matters in this light, but it will become increasingly obvious to those who will spend time in elucidating the causes which produce effects, to those who endeavour to find a reason for everything.

CHAPTER XII.

OBJECTIONS URGED AGAINST THE USE OF PURE CULTURES.

THE objections urged against the commercial use of pure yeast will be seen for the greater part to be founded on misapprehension, if we examine the facts more closely in connection with the practice.

It is as well to remember that yeasts may still be regarded as pure, though of composite types, as opposed to single-cell cultures.

It may be true that instances could be cited to support the contention that by the use of single-cell cultures flavours have been altered, secondary

fermentations have been exceedingly difficult and slow, or lacking entirely, flat beers have resulted, and so forth.

But because these objections may be true in individual cases it does not follow that experiments carried out scientifically may not produce other and widely different results.

In opposition to an idea more or less generally held, secondary fermentation is not impossible by yeast propagated from a single type, as witness Hansen's Carlsberg No. 1, which is able to effect this, always provided that the worts are suitably prepared, that sufficient time is allowed, and that favourable temperatures are controlled.

The same fact holds good with other lager beer yeasts, and the difficulty has not been so much with these as with those belonging to the high fermentation types.

The flavours of lager beers are, generally speaking, due to pure alcoholic fermentation by true saccharomyces, but this is not the case with many

of our English beers, whose flavours are due not merely to yeast alone but also to a variety of torulæ and bacteria.

The first important point to aim at when proposing to employ pure cultures is to decide upon the result desired and then provide the necessary means to attain it.

In spite of what has been said respecting flavours of English beers being due in some part to torulæ and bacteria, modern requirements seem to tend in the direction of the production of light chilled, filtered and aerated beer, more or less neutral in flavour, approximating to the style of lager beers—at any rate, so far as the absence of those aromatic principles generally associated with secondary fermentation is concerned.

Therefore we will first take this as an example of something to aim at, and see whether or no single-cell cultures may help us.

Considering the aspect of the case broadly, cultures propagated from the Burton or London

types of *Saccharomyces Cerevisiæ* produce this more or less neutral flavour, provided that worts are prepared in such a manner as to ferment easily to their attenuation limit. The employment of such a type of yeast will result in a beer, flat without doubt, but with a certain advantage, as will be seen if we consider the matter further.

Now one of the principal objects in producing this type of chilled, filtered and aerated beer is that it may remain bright in bottle; this involves a condition inseparable from it, viz., that fermentation should not take place therein; for, should such fermentation ensue, a deposit of yeast cells becomes inevitable.

Clearly, therefore, the absence of yeasts causing secondary fermentation is a point greatly to the benefit of our requirements in such a case as that we have considered.

But, supposing we require beers possessing aromatic flavours, the foregoing conditions do not apply at all.

The public demanding aromatic beers, such as, for example, bottled Burton ale, are accustomed to a certain cloudiness or haziness due to the secondary fermentation which has taken place in the bottle—the question of deposit of cells resulting from the secondary fermentation becomes in such instances a matter of secondary importance to flavour.

It has often been said in a jocular vein that a bottle of well-matured and highly aromatic Burton ale contains enough bacteria to digest a horseshoe, as well as the ostrich whose fabulous food it is.

But it must not be supposed that aromatic flavours are dependent on bacteria alone. Recently the discovery and isolation from a bottle of Burton ale by Claussen of a species of torula, which he has named Brettanomyces, has been proved to have a very great influence on the flavour usually associated with ales matured in bottle.

When visiting the Carlsberg Laboratory, Copenhagen, the author was given a bottle of

stout fermented by Brettanomyces, and the flavour undoubtedly resembled an old bottled stout brewed by Guinness.

This flavour could not have been produced had the wort been fermented by a pure culture of a true yeast.

Here we have proof, then, that Brettanomyces produce the aromatic flavours associated with bottled beer, and as these torulæ are almost invariably to be found in British ales matured in bottle it will be seen that they are as important in their relation to flavour, as yeasts are necessary to primary fermentation.

Again, pure cultures of true yeasts would be of no avail in the production of West of England vatted beers, for they would not and could not produce the acidity considered desirable, which in some cases has been found to be in excess of that usually contained in ordinary table vinegar. Neither in such cases would Brettanomyces provide the means of supplying all requirements, for,

although whilst producing alcohol these torulæ also produce considerable acidity, they do not provide those flavours which play so important a part in vatted beers.

Ethers to which the flavours of Bristol beers are directly attributable are due to the presence of acid-producing bacteria such as butyric, acetic, etc.

The acid having been formed, the alcohol radicle is replaced by the hydrogen of the acid according to the following equation :

$$\underset{\text{acetic acid}}{CH_3\,COOH} + \underset{\text{alcohol}}{C_2\,H_5\,(OH)} = \underset{\text{ethyl. acetate}}{CH_3\,COO\,(C_2\,H_5)} + \underset{\text{water}}{H_2O}$$

From the cases enumerated it will be seen that a very wide range of conditions has to be met in order to comply with individual requirements.

It would be an error to suppose that West of England vatted beers are produced in so hopelessly an unscientific manner that nothing matters in respect of what one does, or how they are treated.

One of the first essential conditions in regard to them is that they should fine with facility, for it is

the custom to blend them with newer and milder beers.

Now, should they be blended whilst cells, either of yeast or bacteria, still remain in suspension, the newer beer would provide suitable alimentation for the further development of micro-organisms, and the result of mixing the old with the new would be (in a few hours' time) a beer of about the same clarity as cold hopped unfermented wort.

We can see, therefore, that scientific investigation in this case may be of the greatest assistance, by first proving what to avoid in the nature of cells that remain too long in suspension among the heterogeneous mass of organisms which go to make up a result of so complex a nature.

The idea of resorting to the expedient of a change of yeast from an adjacent brewery when trouble arises is not to be recommended for many reasons; the first and foremost being that a brewery free from bacteria may easily be infected by such means. Again, a brewer supplying a

change of yeast could scarcely be expected to furnish the history of its development during even the last five consecutive brewings, for example, yet such information would be not merely helpful, but would be a necessity in some instances, in order that a decision could be arrived at respecting its suitability or otherwise to the wort which it is intended to ferment.

Instances could doubtless be furnished to prove that by changing yeast with another brewery difficulties had been ended, yet the most cursory examination of such a proceeding must disclose the enormous risks inseparable from it.

It is conceivable that yeast from another brewery has already been exhausted by fermenting a prolonged series of worts of a particular type. To ensure its return to a vigorous state it might require conditions differing essentially from those obtaining in the brewery proposing to employ it.

As this subject has been considered in the chapter devoted to alimentation we need not discuss it further from this point of view.

In all probability the majority of breweries are fermenting with yeasts of more than one type, and in order to perpetuate the general characteristics of the beers resulting from such a mixture, the individual types should be studied in accordance with the rules with which we have by now become acquainted.

It should, therefore, be our aim to prepare a mixed culture containing the various types in approximately the same proportions as were found under normal conditions in the brewery.

The following device, although imperfect, may be adopted for the purpose of retaining the original percentages of species, as it involves but small liability to error.

It is understood that the usual care must be taken to avoid infection during the operations.

A glass culture box containing a wort agar film is painted with five stripes with water containing yeast cells from the brewery. Excessive dilution is, of course, understood.

We will suppose that three colonies develop in each stripe—15 colonies in all.

Now take a platinum wire brush and dip it successively into each colony and then inoculate a small bottle of wort with it.

The bottle should then contain composite yeasts, approximately in the same proportions as that existing in the yeast infected by bacteria from which we have propagated our pure supply.

With care a series of Pasteur flasks may be inoculated from one another by a mixed culture prepared as described, thus providing the means of perpetuating the types without having to prepare a plate culture.

For the purpose of propagating a quantity of yeast, sufficient for commercial purposes, a Pasteur flask containing the mixed culture should be taken and the contents developed according to the rules already given.

During the operations of propagating a quantity sufficient for the entire brewing plant, observations

should be taken and recorded respecting flavour of the fermented wort, its fining properties, and so forth.

These observations should be taken in every successive stage, beginning at the first residue from the culture flask. Possible waste of large quantities of wort by these means may be avoided.

CHAPTER XIII.

A COMPARISON OF RESULTS ACHIEVED UNDER A VARIETY OF CIRCUMSTANCES.

AS in all probability a considerable development in the Tropical and Colonial brewing industries may be expected during the next few years, the following few general remarks addressed to those about to embark upon such an enterprise may be found useful.

In the first place, we will endeavour to see how we may apply the knowledge we have gained by having considered point by point the details which collectively constitute the study of bacteriology.

With this end in view we will compare a series of brewings of various characteristics—note their original and final gravities—and discuss the probable causes that have produced the effects in the individual cases.

The following list (compiled from the author's notebook) of British and foreign types of malt liquors, provides an illustration of the widely divergent qualities which may from time to time and in different localities have to be met in order that the markets may be controlled.

All of the determinations have been made from bottles of malt liquors of well-known brands.

	Degrees fermented.	Degrees unfermented.	Total gravity.
German Lager Beer	1·042	1·006	1·048
Pilsener Beer	1·045	1·008	1·053
Dark Münchener Beer	1·037	1·020	1·057
Burton Ale	1·049	1·012	1·061
London Luncheon Ale Matured in Bottle	1·046	1·001	1·047
London Stout	1·050	1·015	1·065

It will be seen by the perusal of the foregoing table that the differences between the German lager and Pilsener beers are but slight, but either of the two compared with the dark Münchener beer exhibits a marked divergency in the degrees fermented as well as in the degrees unfermented.

This is only to be expected. Anyone accustomed to drinking dark Münchener beers must have observed their extreme fulness in comparison to the lighter varieties.

Now should it be an essential condition in some far country to imitate the dark full beer in preference to the lighter sorts, it would obviously be a matter of the first importance to select a type of yeast with restricted attenuative powers in order that the result aimed at may be achieved.

On the other hand, should the lighter varieties of beers be required, a yeast with a greater attenuative power should be chosen.

Secondary fermentation in lager beers is not a matter of the same importance as in the case of

British beers, the reason being that lager beers owing to their long storage in vats at an exceedingly low temperature, pass into solution sufficient carbon dioxide gas for their adequate aeration at the time of bottling.

The bottling having been accomplished, the bottles containing the beer may be at once raised in temperature to 140F. in order that the yeast cells may be destroyed (Pasteurized). We have noted the fact that sufficient gas has been generated by storage in the vat, it becomes obvious, therefore, that no further fermentation (with the inevitable deposit of yeast cells) is necessary in the bottle.

The Burton ale mentioned in our list is typical of the best qualities of British manufacture, though the degrees unfermented, viz., 1·012, are higher than would be found in many instances.

Generally speaking, a high final gravity in the case of British bottled beer is an indication of comparatively recent bottling.

After prolonged storage, that is to say after the lapse of some months from the date of bottling, a

further reduction in gravity may be expected, always provided that the beer has not been Pasteurized.

Upon examination of the deposit contained in the bottle of beer under consideration, it was found to consist of a variety of micro-organisms the majority of which proved to be Brettanomyces, which we have mentioned under the heading of torulæ and will be remembered as non-sporulating alcoholic fungi.

The London luncheon ale is typical of the class of beer that has been bottled for a period of three or four months, the secondary fermentation having taken place in the bottle.

This is evident from the excessively low final gravity. Practically speaking, the whole of the saccharine matter has become fermented.

In all probability the ale had been bottled at a gravity of 1·005, the discrepancy of 4 degrees at the time of estimating its final gravity being accounted for by the secondary fermentation.

The London stout does not call for much comment; it had probably been bottled at a gravity of 1·020, and the fermentation in the bottle accounting for a further 5 degrees seemed to be actually due to a continuation of the primary fermentation.

Those aromatic flavours usually associated with bottled stout were entirely absent, and the deposit from the bottle developed on a culture plate revealed few micro-organisms other than *Saccharomyces Cerevisiæ*.

Had the stout actually undergone a secondary fermentation the culture plate would undoubtedly have revealed many colonies of torulæ which, whether introduced by accident or design, would have provided the means of producing those aromatic flavours generally associated with bottled stout that has reached the age of maturity.

The best recommendation that could be given to a brewer in a distant country, should he desire to imitate ale of the Burton type for example, would be to take a bottle of any well-known brand (not

Pasteurized) and prepare a culture plate from the deposit.

The various types of micro-organisms that may develop may be studied in accordance with the rules we have learned, and such pure cultures may be made from the varieties as may be appropriate to the particular end desired.

Van Laer, of Brussels, has put the case of the British brewer in so concise a manner that it may be as well to look at things from his point of view for a moment. "A good commercial yeast," he said, "is that containing a variety of types all of which have been proved to be suitable individually. In fact it is a mixture free from bacteria or such fungi as may have been proved to produce injurious results."

This view of matters is undoubtedly the correct one, but just as a great painter explained to his students that the painting of a picture was merely the placing of the proper colours in their right positions on the canvas, so may we paraphrase his

lesson and say, that all that is necessary for us to do is to select such types of yeast as may be suitable to our purpose and introduce them into worts of a proper constitution.

A special word of warning is necessary to brewers proposing to commence operations under a tropical sun.

It must be borne in mind that in hot climates the whole atmosphere, as well as every organic or inorganic object, abounds with the lowest forms of life.

The brewer's wort provides one of the most favourable fields for the development of germs, and cooler infection is therefore out of all proportion (in the tropics) to anything that we are acquainted with at home.

The most minute care must be taken that every pipe used for wort—every plate whether belonging to mash tun or hop back—all bottling plant and filter pulp—in fact everything with which beer and wort may come in contact, must be cleaned in such

a manner as to minimise infection as far as possible.

The pace at which Life lives in equatorial zones cannot be compared to the comparative sluggishness of development in temperate climates, and such highly albuminous organic matter as yeast is more prone to decomposition than substances such as meat or fish.

To give an instance, yeast collected and stored as it might be in England until the day following collection would become a seething mass of bacteria in the space of three or four hours.

In appearance it would bear no resemblance to yeast, that is to say regarding it superficially by the naked eye—the only thing to which it could be compared in the mass would be a thick soup, and this, be it remarked, after having been collected but three or four hours previously from a healthy and vigorous fermentation.

To combat the enormous chances of infection such as have been described, there are but two

ways to meet it. First, by re-sowing yeast as soon as possible after collection, and secondly, by perpetually propagating new cultures under aseptic conditions.

To preserve such a high temperature yeast as *Saccharomyces Thermantitonum* by placing it in an ice chamber is out of the question, for cooling retards its life's progress in the same manner as when exposing it to temperatures in excess of those favourable to the performance of its functions.

Now, unless fermentation commences at the moment of collecting wort, decomposition by the agency of bacteria at once sets in.

It will be seen, therefore, that by the adoption of any plan, which although favourable to the preservation of yeast may at the same time retard its development, must be studiously avoided.

CHAPTER XIV.

YEAST CONSIDERED IN ITS RELATION TO THE DISTILLING INDUSTRY.

DISTILLERIES, especially those situated in tropical climates, provide the bacteriologist with the widest possible field for the exercise of his energy; for, in their case, the provision of a suitable type of yeast or certain variations in the accustomed mode of propagating it may turn the scale between possible profit and certain failure.

We have noted at the outset of our practical studies in yeast culture that the distiller's principal object should be to reduce the specific gravities of his washes to the lowest degree possible in order

that he may obtain the highest percentage of alcohol from his raw materials.

But one of the principal obstacles standing between him and the result he is desirous of achieving is that his washes do not as a rule supply the proteins necessary for the adequate alimentation and consequent reproduction of yeast.

Restriction in reproduction of cells results as a matter of necessity in the diminution of the supply of zymase, which is, as we have already learned, the active principle causing fermentation.

Now we have also learned that yeast is a plant, and that it is, therefore, able to build up protein bodies from mineral salts. We should ask ourselves, therefore, whether or not we may rectify the deficiency of protein matter contained in distillers' washes by the supply of chemical salts possessing those elements necessary to the elaboration of yeast cells.

This subject is worthy of very serious consideration and does not seem at all to be met by the

common device of adding various percentages of sulphate of ammonia to distilling washes. There seems to be no evidence whatever to suggest that artificial yeast food composed solely of ammonium salts, although probably suitable to one type of yeast under definite conditions, must, therefore, be suitable to all types upon every occasion.

Experiments should not be confined to establishing the true alimentation of yeast by mineral salts alone.

For example, it is conceivable that a wash composed of molasses without other carbohydrates in solution might be incapable of fermentation by a particular type of yeast.

Yet the same yeast introduced into a wash composed of molasses to which a certain percentage of malt extract had been added might ferment with facility.

Some distilling experiments conducted with *Saccharomyces Thermantitonum* clearly illustrate the importance of providing the true alimentation of yeast.

Upon one occasion a wash, composed entirely of raw cane sugar of a specific gravity of 1·080, declined to ferment below 1·020.

Another trial was made of the same gravity, but the wash on this occasion was composed of 90 per cent. of raw cane sugar and 10 per cent. of malt extract. This fermented with the greatest facility to 0·995.

At another time a trial was made in a distillery to test its ability to ferment washes composed of 80 per cent. of maize. This wash *Saccharomyces Thermantitonum* declined to ferment at all—for all practical purposes, although infected with yeast in a healthy and vigorous condition, the solution might have remained sterile.

Why? Simply because maize extract did not provide the true alimentation of this particular yeast.

It has been reiterated from time to time that *Saccharomyces Thermantitonum* possesses remarkable inverting power and that this fact has been

taken advantage of in several directions. But this power has been exaggerated in some quarters so unduly that its discoverer may be excused should he fail to recognise his own yeast.

The truth is actually this: *Saccharomyces Thermantitonum,* if propagated upon food which provides its true alimentation, has undoubtedly remarkable power in inverting and fermenting raw cane sugar in solution.

But, let it be remarked with accuracy, this can only follow should the yeast be at the very zenith of its power—in other words, that it has been propagated under favourable conditions.

Immediately after fermenting or endeavouring to ferment unsuitable washes (those washes which do not supply its true alimentation)—raw cane sugar solution, for example—its inverting power, and, consequently, its fermenting power, is diminished and cannot be replenished until the cells have been again brought to a vigorous condition by the supply of suitable food.

This is also probably true of all species, and accounts for the greater part for the fact that the distiller loses all or the greater part of his yeast in the still.

His washes, lacking those carbohydrates necessary for the adequate reproduction of cells, result also in a crop of yeast which separates with difficulty, or not at all, from the fermented solution.

Some washes ferment with greater facility if rendered slightly acid, and for this purpose sulphuric acid is usually employed. Experiments, however, should certainly be made with any other acids capable of production locally.

There can be no question that a brewery operating in conjunction with a distillery would be of the greatest possible assistance, for by simultaneous working fresh yeast would be available from day to day.

Purity of culture is not a matter of the same importance to distilleries as to breweries, the reason being that washes as a rule are already fully

charged with a great variety of micro-organisms, and an attempt to sterilise and then ferment them with a pure culture seems to be out of the question from the point of view of economy.

Again, composite sugars require composite yeasts to split them up, and, therefore, a good, healthy brewery yeast used in conjunction with those micro-organisms already present in many washes would, in all probability, reduce their gravity to a lower degree than if allowed to ferment spontaneously.

The studies of the bacteriologist should not be confined to yeasts, but to all causes which produce the effects considered desirable or undesirable in the several cases.

It should not be forgotten that there are other volatile matters in addition to alcohol which play an important part in the character and flavour of the spirit passed over in the still.

The flavours of West Indian rums vary considerably according to their origin. Thus we find that

the products of Jamaica are preferred to those from Demerara, and consequently command a higher price.

The vatted beers of Bristol supply a complete analogy to the rum distilled in Jamaica; in both cases certain aromatic flavours are esteemed; further, they are directly attributable to the antithesis of pure alcoholic fermentation.

Experiments undertaken towards the elucidation of variations in flavour have elicited the fact that a considerable amount of butyric ether may generally be found in Jamaica rum, whilst the quantity in that from Demerara is almost negligible.

Now the coincidence is worthy of remark that the butyric bacterium may be commonly found on the green top of a pineapple and may be isolated therefrom, and that the pineapple flourishes to a greater extent in Jamaica than it does in Barbados or Demerara.

Admitting that germ life may be easily distributed by an air current, it is not unreasonable to

suppose that these bacteria find their way into, and have provided for them, a suitable field for development, in Jamaican molasses washes, fermented as they are without any protection from aerial contamination.

In any case, this subject is worthy of research, and experiments should be directed towards the determination of the correct percentages of butyric bacteria advisable, as well as the psychological moment at which they should be introduced into the fermenting vessels.

In cases of difficulty in reducing the specific gravity of molasses washes to zero an examination should be made of matter accounting for any degrees remaining unfermented.

Thus we should determine whether fermentable sugars (if any) might have been reduced by a suitable yeast or by some variation in its mode of propagation.

Again, perhaps the degrees of gravity already reduced have been sufficient to cause the pro-

duction of enough alcohol to suspend fermentation. It must not be forgotten that some yeasts can work in solutions containing higher percentages of alcohol than others.

Another point: clarity has a greater influence than most people imagine on the possible reduction of gravity by fermentation. Thus it is not uncommon for distillers to allow any foreign substance to flow into the fermenting vessels, flattering themselves that all extraneous matters will be separated in the still and that nothing of the kind indicated is of any importance.

Such a point of view is the greatest possible error; turbid washes have been proved to be inordinately stubborn, whilst clear washes of the same gravity and from the same sources have fermented with the greatest facility.

It is conceivable that soluble matters alien to the development of yeast cells are capable of passing by osmosis into the cells, whereby they may become clogged in such a manner as to diminish

their ability in exerting their normal functions to the full.

. . . . . .

At the close of a lesson it is not an uncommon practice for a tutor to take his class into his confidence and for a moment allow a certain degree of personality to penetrate the scholastic atmosphere.

In accordance with this custom, the author addresses the following few words of a personal nature to his students:

Do not be dismayed should your work at first sight seem unprofitable.

Careful overhauling of facts, which have led up to apparent failure, will oftentimes lead to the discovery of means whereby inaccessible barriers may be scaled. Failure, therefore, in the sincere labourer's vocabulary should be translated "another step in the educational process."

Do not jump to conclusions hastily, and do not assume a superior air towards the man who, lack-

ing the benefits of a scientific training, may yet possess valuable knowledge acquired by observation.

The amateur chemist lacking experience in the application of facts acquired by laboratory research is oftentimes as great a danger to the capitalist as the type of man who, possessing no scientific knowledge, attempts to cloak his ignorance by assuming an air of contempt towards the man of higher education.

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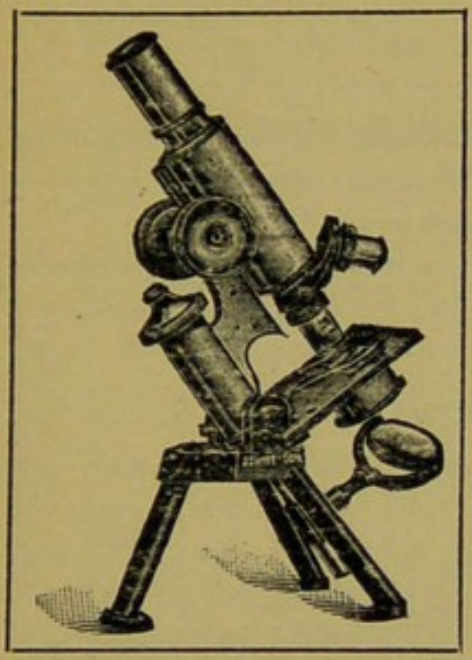

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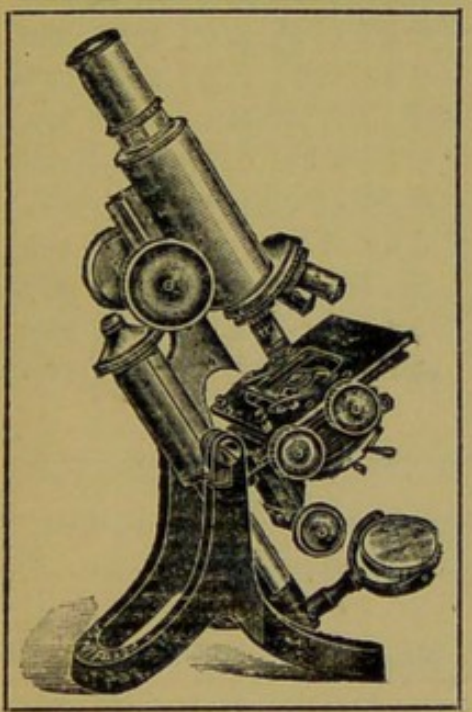

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Degree of Spirit Indication.	·0	·1	·2	·3	·4	·5	·6	·7	·8	·9
0		·3	·7	1·0	1·4	1·7	2·1	2·4	2·8	3·1
1	3·5	3·8	4·2	4·6	5·0	5·4	5·8	6·2	6·6	7·0
2	7·4	7·8	8·2	8·7	9·1	9·5	9·9	10·3	10·7	11·1
3	11·5	11·9	12·4	12·8	13·2	13·6	14·0	14·4	14·8	15·3
4	15·8	16·2	16·6	17·0	17·4	17·9	18·4	18·8	19·3	19·8
5	20·3	20·7	21·2	21·6	22·1	22·5	23·0	23·4	23·9	24·3
6	24·8	25·2	25·6	26·1	26·6	27·0	27·5	28·0	28·5	29·0
7	29·5	30·0	30·4	30·9	31·3	31·8	32·3	32·8	33·3	33·8
8	34·3	34·9	35·5	36·0	36·6	37·1	37·7	38·3	38·8	39·4
9	40·0	40·5	41·0	41·5	42·0	42·5	43·0	43·5	44·0	44·4
10	44·9	45·4	46·0	46·5	47·1	47·6	48·2	48·7	49·3	49·8
11	50·3	50·9	51·4	51·9	52·5	53·0	53·5	54·0	54·5	55·0
12	55·6	56·2	56·7	57·3	57·8	58·3	58·9	59·4	59·9	60·5
13	61·0	61·6	62·1	62·7	63·2	63·8	64·3	64·9	65·4	66·0
14	66·5	67·0	67·6	68·1	68·7	69·2	69·8	70·4	70·9	71·4
15	72·0									

Original Gravity Tables.

Spirit Indication, with corresponding Deg. of Gravity lost in Malt Worts by the "Distillation Process."

Degree of Spirit Indication.	·0	·1	·2	·3	·4	·5	·6	·7	·8	·9
0	0·0	·3	·6	·9	1·2	1·5	1·8	2·1	2·4	2·7
1	3·0	3·3	3·7	4·1	4·4	4·8	5·1	5·5	5·9	6·2
2	6·6	7·0	7·4	7·8	8·2	8·6	9·0	9·4	9·8	10·2
3	10·7	11·1	11·5	12·0	12·4	12·9	13·3	13·8	14·2	14·7
4	15·1	15·5	16·0	16·4	16·8	17·3	17·7	18·2	18·6	19·1
5	19·5	19·9	20·4	20·9	21·3	21·8	22·2	22·7	23·1	23·6
6	24·1	24·6	25·0	25·5	26·0	26·4	26·9	27·4	27·8	28·3
7	28·8	29·2	29·7	30·2	30·7	31·2	31·7	32·2	32·7	33·2
8	33·7	34·3	34·8	35·4	35·9	36·5	37·0	37·5	38·0	38·6
9	39·1	39·7	40·2	40·7	41·2	41·7	42·2	42·7	43·2	43·7
10	44·2	44·7	45·1	45·6	46·0	46·5	47·0	47·5	48·0	48·5
11	49·0	49·6	50·1	50·6	51·2	51·7	52·2	52·7	53·3	53·8
12	54·3	54·9	55·4	55·9	56·4	56·9	57·4	57·9	58·4	59·9
13	59·4	60·0	60·5	61·1	61·6	62·2	62·7	63·3	63·8	64·3
14	64·8	65·4	65·9	66·5	67·1	67·6	68·2	68·7	69·3	69·9
15	70·5	71·1	71·7	72·3	72·9	73·5	74·1	74·7	75·3	75·9
16	76·5									

Lists of Elements.

Name	Symbol	Atomic Weight	
Hydrogen (Normal Element)	H.	1	
Aluminium	Al.	27.5	
Antimony	Sb.	122	
Arsenic	As.	75	
Barium	Ba.	137	
Boron	B.	11	
Bromine	Br.	80	
Calcium	Ca.	40	
Chlorine	Cl.	35.5	
Chromium	Cr.	52.5	
Cobalt	Co.	59	
Carbon	C.	12	
Copper	Cu.	63	
Fluorine	F.	19	
Gold	Au.	196.7	
Hydrogen	H.	1	
Iodine	I.	127	
Iron	Fe.	56	
Lead	Pb.	207	
Lithium	Li.	7	
Magnesium	Mg.	24	
Manganese	Mn.	55	
Molybdenum	Mo.	96	
Mercury	Hg.	200	
Nitrogen	N.	14	
Nickel	Ni.	58.8	
Oxygen	O.	16	
Phosphorus	P.	31	
Platinum	Pt.	197.18	
Potassium	K.	39	
Sulphur	S.	32	
Silver	Ag.	108	
Silicon	Si.	28	
Sodium	Na.	23	
Strontium	Sr.	87.5	
Tin	Sn.	118	
Zinc	Zn.	65	

Comparison of Thermometer Degrees

Fahrenheit	Centigrade	Fahrenheit	Centigrade	Fahrenheit	Centigrade	Fahrenheit	Centigrade
32	0°00	77	25°00	122	50°00	167	75°00
33	0°56	78	25°56	123	50°56	168	75°56
34	1°11	79	26°11	124	51°11	169	76°11
35	1°67	80	26°67	125	51°67	170	76°67
36	2°23	81	27°23	126	52°23	171	77°23
37	2°78	82	27°78	127	52°78	172	77°78
38	3°34	83	28°34	128	53°34	73	78°34
39	3°90	84	28°90	129	53°90	174	78°90
40	4°45	85	29°45	130	54°45	175	79°45
41	5°00	86	30°00	131	55°00	176	80°00
42	5°56	87	30°56	132	55°56	177	80°56
43	6°11	88	31°11	133	56°11	178	81°11
44	6°67	89	31°67	134	56°67	179	81°67
45	7°23	90	32°23	135	57°23	180	82°23
46	7°78	91	32°78	136	57°78	181	82°78
47	8°34	92	33°34	137	58°34	182	83°34
48	8°89	93	33°90	138	58°90	183	83°90
49	9°45	94	34°45	139	59°45	184	84 45
50	10°00	95	35°00	140	60°00	185	85°00
51	10°56	96	35°56	141	60°56	186	85°56
52	11°11	97	36°11	142	61°11	187	86°11
53	11°67	98	36°67	143	61°67	188	86°67
54	12°23	99	37°23	144	62°23	189	87°23
55	12°78	100	37°78	145	62°78	190	87°78
56	13°34	101	38°34	146	63°34	191	88°34
57	13°90	102	38°90	147	63°90	192	88°90
58	14°45	103	39°45	148	64°45	193	89°45
59	15°00	104	40°00	149	65°00	194	90°00
60	15°56	105	40°56	150	65°56	195	90°56
61	16°11	106	41°11	151	66°11	196	91°11
62	16°67	107	41°67	152	66°67	197	91°67
63	17°23	108	42°23	153	67°23	198	92°23
64	17°78	109	42°78	154	67°78	199	92°78
65	18°34	110	43°34	155	68°34	200	93°34
66	18°89	111	43°90	156	68°90	201	93°90
67	19°45	112	44°45	157	69°45	202	94°45
68	20°00	113	45°00	158	70°00	203	95°00
69	20°56	114	45°56	159	70°56	204	95°56
70	21°11	115	46°11	160	71°11	205	96°11
71	21°67	116	46°67	161	71°67	206	96°67
72	22°23	117	47°23	162	72°23	207	97°23
73	22°78	118	47°78	163	72°78	208	97°78
74	23°34	119	48°34	164	73°34	209	98°34
75	23°90	120	48°90	165	73°90	210	98°90
76	24°45	121	49°45	166	74°45	211	99°45
						212	100°00

Conversion of Thermometer Degrees

°C to °F, multiply by 9, divide by 5, then add 32.
°F to °C first subtract 32, then multiply by 5, and divide by 9.

Relation of Saccharometer Pounds per Barrel to Specific Gravity.

Lbs. per Brl.	Specific Gravity	Lbs. per Brl.	Specific Gravity	Lbs. per Brl.	Specific Gravity	Lbs. per Brl.	Specific Gravity
·2	1000·55	·2	1025·55	·2	1050·55	·2	1075·55
·4	1001·11	·4	1026·11	·4	1051·11	·4	1076·11
·6	1001·66	·6	1026·66	·6	1051·66	·6	1076·66
·8	1002·22	·8	1027·22	·8	1052·22	·8	1077·22
1	1002·77	**10**	1027·77	**19**	1052·77	**28**	1077·77
·2	1003·33	·2	1028·33	·2	1053·33	·2	1078·33
·4	1003·88	·4	1028·88	·4	1053·88	·4	1078·88
·6	1004·44	·6	1029·44	·6	1054·44	·6	1079·44
·8	1005·00	·8	1030·00	·8	1055·00	·8	1080·00
2	1005·55	**11**	1030·55	**20**	1055·55	**29**	1080·55
·2	1006·11	·2	1031·11	·2	1056·11	·2	1081·11
·4	1006·66	·4	1031·66	·4	1056·66	·4	1081·66
·6	1007·22	·6	1032·22	·6	1057·22	·6	1082·22
·8	1007·77	·8	1032·77	·8	1057·77	·8	1082·77
3	1008·33	**12**	1033·33	**21**	1058·33	**30**	1083·33
·2	1008·88	·2	1033·88	·2	1058·88	·2	1083·88
·4	1009·44	·4	1034·44	·4	1059·44	·4	1084·44
·6	1010·00	·6	1035·00	·6	1060·00	·6	1085·00
·8	1010·55	·8	1035·55	·8	1060·55	·8	1085·55
4	1011·11	**13**	1036·11	**22**	1061·11	**31**	1086·11
·2	1011·66	·2	1036·66	·2	1061·66	·2	1086·66
·4	1012·22	·4	1037·22	·4	1062·22	·4	1087·22
·6	1012·77	·6	1037·77	·6	1062·77	·6	1087·77
·8	1013·33	·8	1038·33	·8	1063·33	·8	1088·33
5	1013·88	**14**	1038·88	**23**	1063·88	**32**	1088·88
·2	1014·44	·2	1039·44	·2	1064·44	·2	1089·44
·4	1015·00	·4	1040·00	·4	1065·00	·4	1090·00
·6	1015·55	·6	1040·55	·6	1065·55	·6	1090·55
·8	1016·11	·8	1041·11	·8	1066·11	·8	1091·11
6	1016·66	**15**	1041·66	**24**	1066·66	**33**	1091·66
·2	1017·22	·2	1042·22	·2	1067·22	·2	1092·22
·4	1017·77	·4	1042·77	·4	1067·77	·4	1092·77
·6	1018·33	·6	1043·33	·6	1068·33	·6	1093·33
·8	1018·88	·8	1043·88	·8	1068·88	·8	1093·88
7	1019·44	**16**	1044·44	**25**	1069·44	**34**	1094·44
·2	1020·00	·2	1045·00	·2	1070·00	·2	1095·00
·4	1020·55	·4	1045·55	·4	1070·55	·4	1095·55
·6	1021·11	·6	1046·11	·6	1071·11	·6	1096·11
·8	1021·66	·8	1046·66	·8	1071·66	·8	1096·66
8	1022·22	**17**	1047·22	**26**	1072·22	**35**	1097·22
·2	1022·77	·2	1047·77	·2	1072·77	·2	1097·77
·4	1023·33	·4	1048·33	·4	1073·33	·4	1098·33
·6	1023·88	·6	1048·88	·6	1073·88	·6	1098·88
·8	1024·44	·8	1049·44	·8	1074·44	·8	1099·44
9	1025·00	**18**	1050·00	**27**	1075·00	**36**	1100·00

Correction of Gravity of Wort for Temperature.

Gravity.	Temperature Fahrenheit.																																		Gravity.
	36°	38°	40°	42°	44°	46°	48°	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	74°	76°	78°	80°	82°	84°	86°	88°	90°	92°	94°	96°	98°	100°	
1010	—	1	1	1	1	1	1	1	1	—	—	—	To be deducted from Gravity as read on instrument.	—	—	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	1010
5	—	1	1	1	1	1	1	1	1	—	—	—		—	—	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	5
20	1	1	1	1	1	1	1	1	1	—	—	—		—	—	1	1	1	1	2	2	2	2	3	3	3	4	4	4	4	5	5	5	6	20
5	1	1	1	1	1	1	1	1	1	—	—	—		—	—	1	1	1	1	2	2	2	2	3	3	3	4	4	4	5	5	5	5	6	5
30	1	1	1	1	1	1	1	1	1	—	—	—		—	—	1	1	1	1	2	2	2	2	3	3	3	4	4	4	5	5	5	5	6	30
5	1	1	1	1	1	1	1	1	1	1	—	—		—	—	1	1	1	1	2	2	2	2	3	3	3	4	4	4	5	5	5	5	6	5
40	1	1	1	1	1	1	1	1	1	1	—	—		—	—	1	1	1	1	2	2	2	2	3	3	3	4	4	4	5	5	5	5	6	40
5	1	1	1	1	1	1	1	1	1	1	—	—		—	—	1	1	1	1	2	2	3	3	3	3	4	4	4	4	5	5	5	6	6	5
50	1	1	1	1	1	1	1	1	1	1	—	—		—	—	1	1	1	1	2	2	3	3	3	4	4	4	5	5	5	5	6	6	6	50
5	2	1	1	1	1	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	6	5
60	2	2	1	1	1	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	6	60
5	2	2	2	1	1	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	6	5
70	2	2	2	2	1	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	6	70
5	2	2	2	2	1	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	6	5
80	2	2	2	2	1	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	6	80
5	2	2	2	2	2	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	7	5
90	2	2	2	2	2	1	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	7	7	90
5	2	2	2	2	2	2	1	1	1	1	—	—		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	7	7	5
1100	2	2	2	2	2	2	2	1	1	1	1	1		—	—	1	1	1	2	2	2	3	3	3	4	4	4	5	5	6	6	6	7	7	1100

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SAMPLES *and* QUOTATIONS
ON APPLICATION

Table for calculating the amount of malt required to produce a given volume of wort.

A quarter (336 lbs.) of malt is capable of yielding 90 lbs. of extract, equivalent to 5 barrels (each 36 gallons) of a gravity of 18 lbs. or 1·050°.

The equivalent in round figures, calculated according to the metric system, is as follows: 152 kilos of malt are capable of yielding 818 litres of wort of a gravity of 1·050°.

On the above basis

100	kilos	of	malt	will	produce	538 litres
50	,,	,,	,,	,,		269 ,,
10	,,	,,	,,	,,		53·8 ,,
1	,,	,,	,,	,,		5·38 ,,
0·500	,,	,,	,,	,,		2·69 ,,

For ascertaining the Value of the Acetic Acid.

Excess per ct. of Acetic Acid in the Beer.	Corresponding Degrees of "Spirit Indication."									
	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09
·0		·02	·04	·06	·07	·08	·09	·11	·12	·13
·1	·14	·15	·17	·18	·19	·21	·22	·23	·24	·26
·2	·27	·28	·29	·31	·32	·33	·34	·35	·37	·38
·3	·39	·40	·42	·43	·44	·46	·47	·48	·49	·51
·4	·52	·53	·55	·56	·57	·59	·60	·61	·62	·64
·5	·65	·66	·67	·69	·70	·71	·72	·73	·75	·76
·6	·77	·78	·80	·81	·82	·84	·85	·86	·87	·89
·7	·90	·91	·93	·94	·95	·97	·98	·99	1·00	1·02
·8	1·03	1·04	1·05	1·07	1·08	1·09	1·10	1·11	1·13	1·14
·9	1·15	1·16	1·18	1·19	1·21	1·22	1·23	1·25	1·26	1·28
1·0	1·29	1·31	1·33	1·35	1·36	1·37	1·38	1·40	1·41	1·42