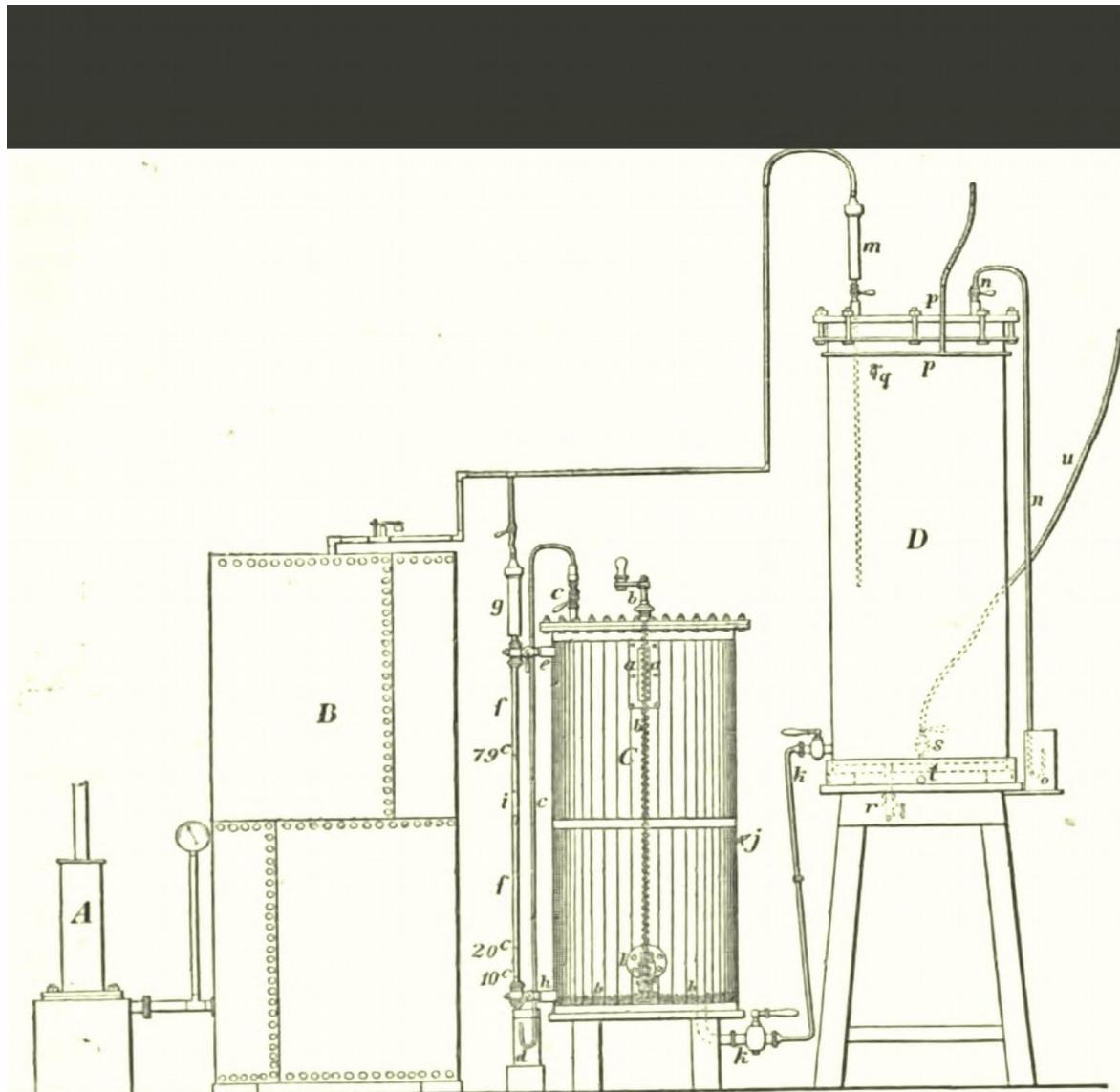


Practical studies in fermentation



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BEING

**CONTRIBUTIONS TO THE LIFE HISTORY
OF MICRO-ORGANISMS**

BY

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No doubt man selects varying individuals, sows their seeds, and again selects their varying offspring. But the initial variation on which man works, and without which he can do nothing, is caused by slight changes in the conditions of life. -Charles Darwin.

Nothing gives the scientific investigator greater pleasure than to make new discoveries; but his joy is redoubled when his observations prove to have a direct application in practical life. -Louis Pasteur

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PREFACE

EXPERIMENTAL studies on the micro-organisms readily lead to practical problems relating on the one hand to medicine and on the other to industry. The theoretical and practical problems in this field go hand in hand, and are frequently inseparable. This has also been the case with my investigations, as is seen in the first of them which appeared in 1878, and still more distinctly in the series published since 1881 under the common title "***Recherches sur la physiologie et la morphologie des fermentes alcooliques.***" Some of my researches are mainly of theoretical interest, whilst others have a more direct practical bearing, and according to whether the one or the other side predominates, they acquire importance for one or the other of the two classes of readers for whom they are written namely, scientific investigators who look for theoretical deductions, and practical men who wish to work in accordance with rational principles and thereby to obtain "a material gain. These considerations induced me to publish my investigations in two series since 1888, the theoretical studies appearing, as before, under the title given above, whilst those having a direct practical bearing were published in a new series.

The investigations brought together in this book treat in the main of the great questions of the circulation in nature of the alcoholic fungi, their relationship to the diseases of beer, the pure cultivation of yeast and the employment of systematically selected species and races. The main point is the reform which I succeeded in introducing into the brewing industry twelve years ago, and which has since found its way into the other branches of the colossal industry in which the cultivation of alcoholic ferments plays an important part, including distilleries, pressed yeast factories, and the wine, cider and fruit-wine industries. My work appeals, however, not only to those practically engaged in the fermentation industries, to technologists and chemists, but also to biologists, and I have, therefore, given it the additional title "Contributions to the Life History of Micro-Organisms."

This English edition is a translation of the new edition of my "***Untersuchungen aus der Praxis der Gärungsindustrie.***" Some additions have, however, been made here and there, and the book thus contains also an account of my most recent investigations. It consists of a series of treatises which have been published at different times. Some of these have been more or less remodeled, whilst others have been reproduced in the same form in which they were originally published; the latter can be recognized by the dates which are printed below the titles; and where it has been necessary to make any addition, this has been done in a foot-note.

At the time when I commenced my studies on the yeast fungi and their fermentations, the practice of starting new hypotheses was much in vogue; the journals contained abundant discussions concerning different possibilities, but a rigorous inquiry was avoided, and no account was taken of what was actually known and what was mere surmise. The problem in this field was, therefore, in the first place to apply strict method, and in the place of conjectures to substitute experimental investigations and accurate demonstrations. It was this

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view of the matter which led to both my practical and theoretical investigations.

The new ideas which I brought forward in my practical studies in fermentation were at first favorably 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 endeavors at reform, I had in reality to fight an unbroken battle for its progress; every step had to be gained by a struggle, and it is this which has to a large extent put a characteristic stamp upon the following researches. A great incitement to me in this case, as always, has been the desire to contribute to that literature, the object of which is to prove to the outside world that we in Denmark earnestly take our share of the work of progress, and that notwithstanding all political reverses, our little nation is still able to develop and carry out independent scientific research.

In conclusion I have much pleasure in expressing my best thanks to Dr. MILLER for the great interest and care which he has bestowed upon the translation of my work.

EMIL CHR. HANSEN.

Carlsburg Laboratorium, Copenhagen:
June 1895.

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CHAPTER I. Pure cultures of systematically selected yeasts in the fermentation industries.

1. Wherein the new advance consists.

IT will be known to many readers that in the year 1883 I succeeded in introducing pure cultures of systematically selected yeasts in the brewing industry. My first experiments were carried out in the famous Old Carlsberg Brewery at Copenhagen. When I commenced work in this direction, the yeast question was everywhere a perfect enigma; it was the weakest point in brewing. When difficulties occurred, a change of yeast was introduced from another brewery, and frequently the yeasts from several breweries were mixed. Sometimes a good result was obtained in this way, sometimes also a bad one, and often the result was worse than that which induced the brewer to try a change of yeast. In all cues he was working completely in the dark; in short, he did not know in the least what he was introducing into the wort. At that time it was not possible to do more than at most detect whether the pitching yeast was contaminated with bacteria and mold fungi; it was, however, very frequently found that such an examination led to the assumption that a yeast was good, but which nevertheless gave a bad result. I was thus naturally led to the opinion that the secret must lie in the yeast cells themselves, and that these apparently similar cells might possibly belong to different species. It was from this starting point that my investigations on the **Saccharomyces** gradually developed. The practical results to which they led were in the first place **a new analytical method, and the certain demonstration that some of the commonest and most serious disease: of beer, such as yeast turbidity and objectionable changes in flavor, were caused not by bacteria but by certain species of yeast.**

It was only after this had been proved by exact experiments, both in the laboratory and on a large scale in the brewery, that it was evident that it was not sufficient to purify the yeast from bacteria and mold fungi, but that the question must be treated from a quite different point of view. And when I further showed that there are also different species of good brewery yeast which produce beers of different character, it also became clear that **the pitching yeast should consist only of a single species, namely, that best suited to the brewery in question.**

A short preliminary account of how this reform was effected and might be carried further, was published in the "**Zeitschrift für das gesamte Brauwesen**" for 1884. The first detailed description, however, appeared in 1888, in the first German edition of this work. In some respects it is as well that it was not published earlier; I had in the meantime gained greater experience, and was thereby in a position to summarize the most important publications which had appeared in the interval, and which threw light on my work, partly in support of it and partly against it. It would be out of place here to quote these treatises; they are published in the journals relating to the fermentation industries, and especially in the brewing journals, all the leading zymotechnologists having added their contributions. A list of the most

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important authors will be found in the first German editions of this book.

It was only to be expected that my work would meet with marked opposition. This was especially the cue at three different points. The first and severest struggle which I had to encounter was with the founder of the laboratory, the late Captain J. C. Jacobsen. He, in fact, regarded my efforts as misdirected, and for a time tried to oppose them. But as soon as he recognized that my discoveries might become of great importance, he at once decided to recommend them, although not always in a manner which I could approve (1883-1884). The experiments which I conducted at that time, in order to convince Jacobsen, became of fundamental importance for the full development of the question, and they will therefore be described in detail in the following pages.

Shortly afterwards the dispute arose with Prof. Delbrück and his followers in Berlin. My opponents started with the view that a botanical treatment of the yeast question could not lead to the desired end, and that my methods were not suited to the practical conditions of the brewery. They did not content themselves, however, with a verbal dispute, but experimented themselves. By means of these investigations, and some new ones published by myself and my associates, my opponents gradually changed their views, and they have also openly stated so. In 1889 my colleagues at the Berlin Station recognized and adopted my methods in their program, and awarded me their diploma as honorary member. Amongst the marks of honor accorded to my work, I valued this especially, as showing that the dispute had been carried on without personal animosity.

The third important attack was from the French school, at the head of which are the brewer Velten of Marseilles, well known through Pasteur's "*Études sur la bière,*" and Prof. Duclaux, Member of the Institute of France, and Director of Pasteur's Institute at Paris. Some other pupils of the French school sided with the above. Velten commenced his attack in 1886, and this was taken up by Duclaux in 1889. (I am glad to be able to add that Pasteur took no part in these attacks; on the contrary, he publicly recognized my work, in that at the end of 1886 he caused the award to be made to it of the gold medal of the Société d'Encouragement pour l'Industrie Nationale.) The dispute has not yet terminated. Velten constantly maintains that I was completely wrong in introducing into the brewery a pitching yeast consisting only of a single race. A refutation of his assertions will be found in the following pages. Prof. Duclaux, whose sympathies were at first in the same direction as Velten's, subsequently abandoned in some degree the latter's views, and at the Brewers' Congress at Lille in 1890, he even emphasized the fact that the yeast question had, as regards low fermentation, been solved by my investigations. With regard to high fermentation, he will still have nothing to do with my system, and argues in favor of the introduction of the old methods of Pasteur. The future will show that he is wrong.

L. Aubry, the director of the scientific station for brewing at Munich, has expressed himself as follows on this question ("La Gazette du Brasseur," 1890, p. 79): "Although we have to deal mainly with the preparation of pure cultures of bottom yeasts, it does not infrequently happen that we are asked for pure top yeasts, and we have already introduced pure cultures of such

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yeasts into practice and with good results. The method which we have employed, in the case of both top yeast and bottom yeast, has always been Hansen's method; we never make use of Pasteur's method. In the present position of science, no one who is concerned with the preparation of pure yeast will employ any other than Hansen's method. The hostility which Hansen's efforts have aroused is much to be deplored. We all recognize, and Hansen no less than any, what Pasteur has accomplished in connection with the yeast question, but this does not prevent us from perceiving that a new direction has been given to the question by Hansen's researches, and in our opinion this marks a great advance, which we joyfully admit."

At first I naturally had to take up the dispute single handed, but was subsequently supported by pupils and colleagues, and I was then better able by new and elaborate investigations to devote myself to the elucidation of the question from different sides. This is, indeed, the only way in which an investigator who belongs to a small nation will be able in the long run to maintain his standpoint against his powerful adversaries abroad. Politics, unfortunately, also still play their part in science. Most of the authors have taken my side, and the vigorous attacks have been gradually silenced. Feeble attempts are, however, still frequently made to minimize the importance of my work, and often the same arguments are brought forward which have previously been completely refuted. I have thus learned that the same truth must be repeated again and again if we wish it to prevail in the end. In most cases, however, I have purposely avoided directly "addressing my replies and corrections to the respective critics, as personal disputes were always distasteful to me.

2. My methods pure cultivation

The methods for the preparation of pure cultures which were in vogue when, in 1830, I undertook experiments expressly in this direction, were far from giving a sufficient degree of certainty. In 1878 Lister had indeed stated the manner in which he had obtained a pure culture of a lactic acid bacterium by means of a dilution method. By counting, he determined the number of bacteria present in a drop of the liquid under examination, and he then diluted this with a sufficient quantity of sterilized water, so that, in accordance with the calculation, each drop of the mixture should contain on an average less than one bacterium. After inoculating a number of flasks containing nutrient liquid, each "with one drop of this mixture, it was found that some remained sterile, and Lister then assumed that each of the remaining flasks contained a pure culture. The counting was carried out in ordinary microscopic preparations. The same method was subsequently employed by Nägeli and by Fitz. There is, however, no certainty about this method, and it is found that, in spite of the calculation, several of the inoculated flasks received more than one germ each; but since an absolute guarantee is only attained with a single cell culture, the problem which I set myself was to carry out this principle. In the case of yeast cells, I succeeded in my object towards the end of 1881. (My first publication in connection with this appeared in February 1882, in the "**Compte-rendu des travaux du laboratoire de Carlsberg,**" Copenhagen, 1 vol., 4 livr. p. 212. A detailed description of my methods is given in the same journal, 2 vol. 2 livr.)

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The method of counting which I adopted differed from that employed by Lister, in that I made use partly of the haematiometer, and partly of a special cover-glass divided into squares for the purpose (Fig. 1). One drop of the liquid containing the yeast cells is placed on the cover-glass, and within the limits of the large square, the small squares having merely the object of assisting in the counting.

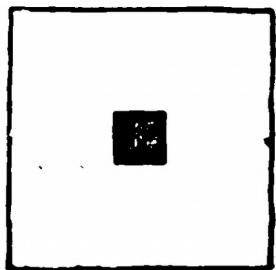


FIG. 1.

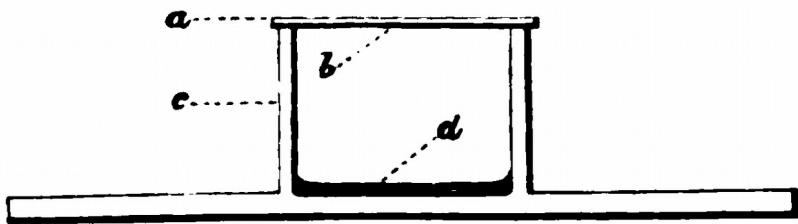


FIG. 2.

The cover glass with the drop on its under surface is fixed to the ring of the moist chamber (Fig. 2). If we now find that the drop contains, e.g. 20 cells, and we then introduce it into 40 cc. of sterilized water, we shall have assuming that we are working with average samples only one cell in every two cubic centimeters of the mixture; but this result is not always attained, even with the accurate method of counting which I adopted. The dilute yeast was introduced into flasks containing sterilized wort, the degree of dilution being such that only a small proportion of the flasks became infected. ***The new point about my method was, in the main, that I discovered a means by which I was enabled to distinguish between the flash: which received only one cell and those which received more than one***, this result being dependent upon the observation that after the cells had been well distributed by agitation in the nutrient liquid, each settled to the bottom and gave rise to a separate yeast speck. Only those cultures which contained a single yeast speck were assumed to be pure cultivations. By means of the divided cover-glass it is evidently also possible to inoculate directly from a single cell. ***An exact dilution method was thus for the first time achieved***. By its aid I prepared pure cultures of the six ***Saccharomycetes***, the description of which I published in 1883, and also of several brewery yeasts. I also made use of this method in my investigations on the conditions affecting spore formation, on the temperature curves for the development of the spores of the six species alluded to, and on the diseases in beer caused by ***Saccharomycetes***. A brief account of these researches was published in 1882 (Le, pp. 206 and 216), and formed the foundation of my yeast studies. ***All my experiments at that time were carried out with liquids, for the important advance-plate culture by means of nutrient gelatin which we owe to Koch, had not then been made.***

This historical account is given mainly as a reply to attempts which were made to show that my studies had their origin in Koch's method of plate cultivation; we have seen, however, that this was impossible.

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The basis for pure cultivation on a solid substratum was given by Schroter in 1872, and, as was pointed out above, the method was materially improved by Koch. The latter at first prepared his pure cultures in a somewhat imperfect manner by means of streak cultures in nutrient gelatin (1881). In 1883 this method was still generally employed in his laboratory, and Hueppe also made use of it in his investigations on the decomposition of milk by microorganisms (1884). In 1883 Koch published his improved method of plate culture, in accordance with which the pure culture is prepared by introducing some of the microorganisms into fluid, germ free, nutrient gelatin. After the cells have been distributed in this mixture as equally as possible by agitation, it is poured on to a horizontal glass plate, also free from germs. This is finally covered over with a moist bell-jar, and preserved in this manner at a suitable temperature. It is assumed that each separate growth speck which appears contains a pure culture. This is, however, by no means always the case, for it is more difficult to separate the cells from each other when they are in gelatin than when they are in a liquid. ***In order to obtain an absolutely pure culture with certainty, it is necessary, even when the gelatin method is employed, to start from a single cell.*** Hence I elaborated my new method by means of the moist chamber (1883).

A little of the nutrient gelatin containing the yeast cells is placed on the under surface of the cover-glass of the moist chamber (Fig. 2). Those cells are then accurately marked,* the position of which is such that the colonies to which they give rise can grow to their full size without coming into contact with other colonies. For the subsequent inoculation in a nutrient liquid medium, I only employed those colonies which were proved by direct microscopic examination to have developed from single cells. The correctness of my results has been confirmed by other investigators. Thus, Miquel found 134 different kinds of bacteria in 100 colonies prepared by Koch's plate method. In Holm's experiments with yeast cultures and wort gelatin, the average number obtained was that 100 colonies had sprung from 108 cells, whilst in the most unfavorable case 100 colonies were produced from 135 cells.†

* The object-marker of Klinne and Müller, of Berlin may be recommended for this purpose.

† Just. Chr. Holm, "Sur les méthodes de culture pure et spécialement sur la culture sur plaques de M. Koch, et la limite des erreurs de cette méthode" ("Compte rendu du Laboratoire de Carlsberg," 3 vol. I livr., 1891). In this treatise a historical account of the matter and an exact bibliography is to be found. See also Jörgensen's "Micro-organisms and Fermentation," new edition, London, 1893, p. 32.

Koch's method of plate culture is essentially in agreement with the dilution methods of Lister and myself; it is the same except that in one case gelatin, and in the other liquids, are employed. The various methods of obtaining pure cultures, which were elaborated in the course of time, can only be regarded as modifications of one another; there was no sudden development. The reason why the dilution methods with liquids are now but seldom made use of, is that working in this manner is more tedious and difficult than working with gelatin.

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In the case of yeast there are no special difficulties in carrying out the principle of pure cultivation from one cell, and it is indeed generally conceded that my exact method should be followed in the case of purely scientific experiments; on the other hand, there are still some zymotechnologists who are disposed to take the matter more easily when it is a question of preparing a pure yeast for the brewery. They do not consider the risk which is thus incurred of introducing a dangerous disease yeast together with the good brewery yeast. My warning here, as always, is: ***The best is not too good for practice.***

The differences between Koch's and my methods are at present no doubt acknowledged by most experts; my opponents have therefore of late turned to Brefeld. He himself has also claimed the priority of the gelatin method, the honor of which we are accustomed to attribute, and justly, to Koch. Brefeld's methods were elaborated with regard to morphological researches, especially of mold fungi, and for that purpose they are good; ***whilst they are unsuited for producing mass-cultures of bacteria and yeast cells*** (see Holm's above-named treatise, and Brefeld's publications, quoted therein).

Whatever method of pure culture we may employ, ***it is and remain: nothing more than a technical expedient***, which only becomes of importance when it is brought into the service of a scientific idea. In some journals the matter ha of late been misrepresented, in that my single cell culture is described as the essential part of the reform to which my yeast studies have given rise in the fermentation, industry. This is, however, an error. ***The essential point is the new principle the selection of a certain species or race.*** Consequently the commonly used terms "pure yeast," "pure cultivation," do not give a correct idea of the subject.

3. The contributions of previous investigators.

Just as every investigation is based upon the researches of previous workers, so also was this the case with my researches. In the last section we have become acquainted with the standpoint of our subject at the time when I commenced my studies in connection with the technique of the methods of pure culture; before proceeding further it will be useful to briefly consider also the previous investigations on the yeasts and their relationship to the brewing industry.

The foundations of the science of the micro-organisms of fermentation were laid about sixty years ago, and more especially by the discoveries of Cagniard Latour, Schwann, Turpin and Kützing. The experiments of Cagniard Latour and Schwann proved that yeast consists of living cells, and that it is these which bring about alcoholic fermentation. In 1765 Spallanzani opposed the doctrine of spontaneous generation, according to which, living organisms were able to develop out of dead nature without eggs, seeds or germs. The experiments of Schwann and his followers showed that various substances which readily ferment and putrefy, can be preserved unchanged when they are boiled, and care taken that the air with which they subsequently come into contact is freed from its germs. Thus the technique of

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sterilization was established both as regards practice and science. In 1839 Schwann showed that yeast cells perish when they are acted upon by certain chemical compounds, and he thus laid the foundation for the doctrine of antiseptics, which subsequently became of such importance. When the acetic acid bacteria were then discovered by Kützing, Turpin, in consequence of this and of his own yet very imperfect investigations, gave utterance to the following doctrine: "No decomposition of sugar, no fermentation without the physiological activity of vegetation."

These fertile ideas were followed up by Pasteur, and it was mainly due to his epoch-making researches that the new doctrine gained general recognition. On this occasion we have only to deal with his "*Études sur la bière,*" which appeared in 1876. In this he points out the disagreeable changes (diseases) to which beer may become exposed when it is attacked by bacteria, and since these are readily distinguishable by their form from yeast cells, he recommended the employment of the microscope in breweries; he further advised that the fermentation be conducted in such a manner that organisms could not gain admission from without. The groundwork was thus laid for important improvements in the brewing industry. The reason why they could not then be carried out was that the yeast question still awaited solution, and in the end everything depended upon this solution. According to Pasteur's experiments, it was the bacteria which gave rise to the diseases of beer, and he accordingly commenced experiments on the purification of yeast. Of the various means which he made use of for this purpose, he and his associates, Duclaux and Velten especially, recommended treatment with a solution of cane sugar to which a little tartaric acid had been added. There was thus no question here of a true pure culture, but merely a suppression of the bacteria present in the yeast. My experiments have shown, however, that **this method is unsuitable for tile purification of brewery yeast, as it favors the development of the disease yeasts.** The result attained is thus the opposite of what was desired (see p. 130). That Pasteur should recommend such a method is readily explained, for at that time there was no evidence as to what a good brewery yeast and what a disease yeast is. Under these circumstances it is evident that his method could not secure an introduction into practice.

Several writers before Pasteur often expressed the opinion that there are different species and varieties of yeast which produce different fermentations and impart different characters to the beer. Similar views occur in the writings of the earlier zymotechnologists, e.g. Bail in 1857. The most common view was that the brewery yeast could rapidly change its character, and even merely through employment in different breweries; some, indeed, even stated that it could change into mucor yeast and into a number of mold forms. Another view, advocated especially by Reess in 1870, was that the yeasts have a very limited sphere of development, and in accordance with this, species were named merely from the form of the cells: the sausage-shaped cells being named *Saccharomyces Pastorianus*, the small oval cells, *Saccharomyces ellipsoideus*, and the larger oval cells, *Saccharomyces cerevisiae*, &c. We now know, however, that this view is also quite incorrect, and that Reess's system has no true basis. In short, opinions were thrown out in all directions, but nothing was proved, and the truth still remained undiscovered.

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Pasteur got essentially no further in this field. This was due not only to the position of the science at the time, but to the fact that a botanical treatment of the problem did not occur to him. Nowhere in his work does he make an attempt to distinguish between the **Saccharomycetes** and the non-**Saccharomycetes**, all yeast cells exhibiting the ordinary bud "formation and capable of producing a fairly pronounced alcoholic fermentation, were grouped together and described sometimes 3 **Saccharomycetes**, sometimes as levures, ferment: alcooliques, &c.; he had not any exact methods of pure cultivation, and sharp characteristics did not occur to him. Under these circumstances there could consequently be no question either of analysis or of a systematic selection of species. Where Pasteur speaks of yeast, all possibilities are discussed, but no definite scientific standpoint was arrived at. As an example, it will suffice to mention his statements with reference to the transformation of high brewery yeast into low yeast, and vice versa. Whilst his observation mentioned on p. 189 seems to indicate that this does not take place, he comes to the conclusion later on (p. 213) that such may occur, and in the note on p. 333 he gives instructions as to what brewers must do if they wish to guard against their low yeasts becoming converted into high yeast; and it must be pointed out that he is here not speaking of a temporary transformation. I have elsewhere proved that such a change as that assumed by Pasteur does not really occur.

The above criticisms do not apply to Pasteur's justly famous work, but to his pupils who still cling to the vague standpoints, and with partiality revive obscure points in order to find in them that which is not there, and which could scarcely have been there, considering the time when the book was published.

The investigations of Nägeli, published during 1877-1879, gave rise to discussions on the degeneration and transformation which occur in brewery yeast. A great deal was talked and written about the degeneration of yeast, and the difficulties met with in breweries were to a great extent attributed to this cause: no experiments were undertaken, however, upon which this view was based. Attention was thus diverted further and further from the possibility that these misfortunes might also perhaps arise from the presence of foreign species which had gained admittance into the brewery. This was the standpoint when I commenced my studies in this field.

In the above account of the publications of previous workers, I have only laid stress upon the most important points; several of the questions relating to this subject are discussed partly in the section headed "What is the pure yeast of Pasteur," p. 130, and partly in the historical account commencing on p. 157. In these chapters I have also given an accurate summary of the literature. Those readers who are interested in the matter will also find at the end of the first-named section a statement of the main points in which my doctrine differs from Pasteur's.

4. The practical results which have been achieved.

When in 1883 I applied to the late Captain J. C. Jacobsen, the owner of Old Carlsberg, for permission to carry out my experiments on a large scale in the brewery, he had no confidence

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in my scheme, as was mentioned above. One of the main objections which he raised was that a pure culture of a brewery yeast could not give a satisfactory after-fermentation, but that for this the wild species which I had excluded would probably be necessary. There are also hints in the works of Reess and Pasteur which may bear this interpretation.

Shortly before I applied to him, some of my experiments had led to a striking result in practice, and this tended in a high degree to support the correctness of my doctrine concerning the **Saccharomycetes**. In a treatise on the diseases of beer, I had explained the cause of the malady, namely yeast turbidity, which had produced such disastrous results for two years in the Tuborg brewery at Copenhagen, and as matters rapidly mended when the explanation given was acted upon, substantial proof was afforded of the practical value of my discoveries. At about the same time a difficulty occurred in Jacobsen's brewery, namely, the beer began to acquire a disagreeable bitter taste and an unpleasant odor. In accordance with the views then prevailing, he naturally attributed the malady to bacterial contamination," and when it was found that bacteria could not be detected, he assumed that the wort and especially the hops were at fault. Here again, however, nothing wrong could be discovered. In a treatise published in 1882, I incidentally gave an account of the investigation of a **Saccharomycetes** which gave a similar beer to that complained of in Old Carlsberg. I at once expressed the opinion that wild yeasts can produce equally serious disturbances in the fermentation industry as are caused by bacteria. It is natural that no great attention was paid to my doctrine at that time, for it was not then sufficiently established. In 1883, however, I made another step in advance, and it was through the success which I had met with in the Tuborg brewery that the desired permission was granted me. The experiments were, therefore, carried out on a large scale in the brewery, and irrefutable proofs were gradually furnished.

From the impure yeasts I separated four **Saccharomycetes**, and on experimenting partly with each separately and partly with mixtures, it was found that only one of them gave a normal beer of good flavor and odor. This is the species which is now generally known in the brewing world as **Carlsberg bottom yeast No. 1**. That this species is in itself able to effect the whole fermentation in a desirable manner, both primary and after-fermentation, has been proved by the production of large and small quantities of beer by means of absolutely pure cultures in sterilized wort. Amongst the other species was the one with which I conducted my experiments in 1882, and of which I gave a detailed description in one of my papers published in 1883; it is there described under the provisional name **Saccharomyces Pastorianus I**. It was this species alone which produced the disease. Further, it was found that the beer was excellent which had been produced with the help of the selected brewery yeast alone, whilst an addition of **Saccharomyces Pastorianus I**. gave rise to the dreaded bitter taste and disagreeable odor. This result was of great importance, for Jacobsen had now to acknowledge that a reform was really at hand, and he admitted its introduction throughout his extensive brewery. At first he proceeded even more rapidly than I myself desired. The struggle for this matter in Old Carlsberg was thus ended. This occurred in 1884. I have here laid stress upon the observations in the two breweries named, partly because they show how the matter developed, and partly because they speak in a striking manner for the reform which I wish to become disseminated as widely as possible.

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The experiments made both in the brewery and in the laboratory, showed that Jacobsen's fears with reference to the after-fermentation were groundless. In recent years, indeed, it is only with regard to high fermentation in English breweries, that the objection has been raised that a pure culture of a single species is unable to effect the desired after-fermentation. Experiments which were conducted in the brewery have shown, however, that also in this case the objection is without foundation. This question will be discussed more in detail in a subsequent chapter which deals with the employment of pure yeast culture in high fermentation breweries. Influenced by these objections, which were raised in England against pure culture in its simple form, Professor Delbrück, of Berlin, was also led to the view that, in the case of the low fermentation beers of Germany, which undergo a vigorous after fermentation and which have a high reputation for stability, a single species of yeast would not be able to effect the whole fermentation, but that one or more special secondary yeasts would be necessary. At his suggestion, Dr. P. Lindner and Dr. Schonfeld undertook to visit various breweries in order to settle this question. "After that," he says, "I had to acknowledge that this question was answered in the negative. "His two associates visited breweries where beers of various ages four weeks, eight weeks, three months, and even six months old were placed at their disposal, and samples were taken at all these different stages in order to ascertain "the point at which the new fungus develops." It was proved, however, that there are breweries where a "pure" after-fermentation occurs in spite of its being prolonged, and where the whole fermentation is effected by the primary yeast. Delbrück concludes his remarks as follows: "The assumption which I made, that eventually we should also have to furnish pure cultures of secondary yeasts, thus falls through at any rate in the case of low-fermentation beer." On a subsequent occasion he stated that single yeast fermentation is also successfully adopted in top fermentation breweries in Germany. *

* Wochenschrift f. Brauerei, 1894, p. 759, and 1895, p. 2.

It was especially due to Jacobsen's influence that my practical experiments became known in a very short time both in my own country and abroad; they were, however, for the most part regarded with mistrust, even by intelligent brewers. This opposition would have been sufficient to delay progress for several years had not Aubry worked with vigor and ability from the very commencement. To him is due the honor of having introduced the reform into Germany.

In Bohemia the beginning was made by Bélohoubek, and in Norway by Hejberg. In Denmark my efforts were supported by A. ö and Grönlund, and considerable activity was displayed especially in Jørgensen's laboratory. In France the matter was first taken up by Louis Marx, and afterwards by Kokosinski, Petit, Monal and others; in England by Wilson, Miller and Hyde; in America by Wahl and Henius; in Australia by Peschka. During the last years I have found several able fellow-workers amongst the foreign chemists and botanists who have studied in my laboratory.

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From the above it will be evident that my system carries with it important practical advantages, which may be described as follows:

1. A definite result and rational working are secured where formerly everything depended more or less upon chance.
2. Maladies in the beer, causing great losses, are guarded against.
3. A yeast is obtained having a higher commercial value than the ordinary impure yeast.
4. Finally, it helps to raise the industry, and this must be a point of great interest to the intelligent brewer.

A further advantage is that there is no great expense connected with the reform, which is therefore within the reach also of small breweries. In this it therefore differs from some other improvements, such as the introduction of ice machines into breweries. There is also another difference which was overlooked by those who compared the reform brought about by my work with the advantages gained through the introduction of ice machines. In the latter case the gain is no more than that which the introduction of ice machines brings to other industries; the mode of working in the brewery remains the same as before; our ideas are not enlarged. On the other hand, by the adoption of pure culture, new aspects and a far reaching improvement were introduced. From a hygienic point of view, pure yeast likewise gives greater security than ordinary impure pitching yeast.

That my work would in many places be misunderstood and meet with failure was easy to foresee. This also occurred, and I have therefore directed special attention to this side of the question, and have carefully noted every case which came to my knowledge. In the lecture which I delivered on June 12, 1887, at the General Meeting of the Austrian Brewers' Union, held at Gratz, I dealt in detail with this matter. The following is an abstract of this lecture, with some additions and a few alterations. We shall thus have to speak of the mistakes and failures in order if possible to make an end of them.

It is a mistake to assume that pure yeast can do everything. On the contrary, it is necessary to point out that the requirements as regards the preparation of the malt, wort, &c., are the same as formerly. If there are defects here, the beer will also be faulty even though pure yeast has been employed. On the other hand, the doctrine more or less openly expressed by some zymotechnologists, even quite recently, is incorrect, namely, that the fermentation is in the main dependent only on the composition of the wort, so that a good malt always gives satisfactory fermentations, favors the development of the desired species of brewery yeast and checks the disease germs, whilst a bad malt on the contrary favors the development of the latter. The main result of such a doctrine would be that so long as we take care to have a good wort we do not require to trouble much about the character of the yeast. This, however, like a great deal more that has been written on the manufacture of beer, even by distinguished authors, is only conjecture lacking all foundation. *Likewise we must not forget*

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that we have no definite chemical knowledge as to what we are to understand by a good or an inferior malt, by a "good or an inferior wort.

A pure culture which has once been introduced into a brewery will not for ever remain sufficiently pure. The wort from the open coolers brings contamination with bacteria and wild yeasts, especially in the summer and autumn, and this is also the case with the more or less impure air in the fermenting cellars, especially where the air is not purified and no ice machines are used; infection is also readily introduced with the utensils and by the workmen themselves. Even if a pure yeast can be safely used for a longer time than an impure yeast, other conditions being the same, a point is always reached where it is necessary to introduce a fresh pure culture. The time when this must be done can only be determined "by analysis. Local conditions and also the time of the year play an important part in this connection; no fixed rule can be laid down for all breweries; it must also be borne in mind that different species of low-fermentation yeast do not possess the same resistive power to contamination.

My opponents have taken up this point and have exaggerated its importance. It has been stated, for instance, that when the pure yeast has been added to the wort from the cooler, it at once becomes reduced to its former impure state, and that pure culture is therefore of no use. It is true that it does again become infected, but it is not true that as a rule it soon becomes contaminated to such an extent that it cannot be used. Even under very unfavorable circumstances the employment of pure yeast is always of advantage, and it must always be preferred to impure yeast. As an example, I may here state that the yeast which in Old Carlsberg we call No. 1, keeps sufficiently pure for six to eight months in the brewery mentioned, and the No. 2 yeast (formerly used) which is less resistive, two to four months. This was found to be the case at the time when the whole of the wort was cooled on the open coolers. In fact the conditions here referred to were such as obtain in the great majority of breweries; in the following pages, however, forms of apparatus and plant will be mentioned by means of which all infection, so to speak, is excluded.

An objection which has been frequently raised against my efforts is the following: A pure culture was introduced by way of trial into a brewery at a time when all went well with the old impure yeast, and a careful comparison was made between the beers obtained with the two sorts of yeast. As a result it was found that nothing had been gained by the employment of the new yeast. Why not continue then with the old method? By all means if it really were a method; but this is exactly what is wanting. It affords brewers no certainty, but exposes them to great variations and to unknown dangers. The only novelty which pure yeast introduces is certainty and rational working. It is a complete mistake to imagine that a better product will result than the brewer can obtain under the most favorable circumstances with his old impure yeast. When the pure yeast has been properly selected, the latter is the result which it gives, and, contrary to the impure yeast, it gives it always, provided it is always cultivated under the same conditions.

One and the same yeast does not suit all breweries. As was previously mentioned, it has been found, namely, not only that there are several species or races of culture yeast, but also

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that these give beers dissimilar in their character, in their stability and in their flavor, and which also behave differently in the fermenting and lager cellars. ***Every brewer therefore must select, according to a definite plan, a species which suits his brewery, and one of the most essential advances which my investigations have brought about is that this can now be carried out with certainty.*** That there are species which are suitable for a large number of breweries has already been shown by experience. Such species are especially adapted for preservation as stock forms in zymotechnic laboratories. It would be going too far, should it come about that every brewery wished to have its own species of yeast. At the present time there is a tendency in this direction, and my object therefore is to warn against this.

A pure cultivated yeast separated from an ordinary impure brewery yeast, and which will therefore, as a rule, have sprung from a mixture of several different species, will not give exactly the same product as the mixture. In the taste especially there is almost always a small and sometimes even a very noticeable difference. It is consequently a mistake made by several brewers who have imagined that pure cultivated yeast would give them a beer having exactly the same taste as the beer obtained with the impure yeast from which the pure culture was originally separated. They obtain a finer and above all a constant product, but one differing somewhat from their former beer. This fact cannot be too strongly emphasized. A brewer, therefore, commits a great practical error when he suddenly introduces pure yeast throughout the whole brewery. In such a case it may readily happen that the beer will suddenly assume a different character, which may be displeasing to many customers. The change must be introduced by degrees, and then nothing different occurs from that which has formerly always occurred in every brewery. When it is thoroughly introduced a great advantage has been gained. Captain Jacobsen, the late owner of Old Carlsberg, on several occasions clearly and forcibly emphasized the conditions affecting this matter; nevertheless misunderstandings and mistakes still occur every day.

The question might perhaps be asked whether it would not be possible to impart a particular flavor to the beer by the employment of mixtures of yeast of known composition, and thus produce differences in the taste which might find favor with the public. That this could now be rationally carried out is self-evident. The culture yeast could for instance be mixed with a wild yeast which, like my ***Saccharomyces Pastorianus II.***, produces no sickness. In some cases the admixture could be made with the pitching yeast, and in others it could be introduced at the end of the primary fermentation. We are in a position to prepare such mixtures of different species of brewery yeasts. In some few breweries two species of culture yeast are employed separately, and the resulting beers are then mixed at the end of the primary fermentation. ***I can, however, by no means recommend such mixtures,*** not even if they are guaranteed by trustworthy analyses. The ideal in every manufacture is to conduct it in as simple and certain a manner as possible, and it is a recognized fact that it is easier to regulate one than several factors, even when they are all well known. In several cases the mixture will even give rise to a less satisfactory fermentation. Finally, it must be remembered that the ratio originally existing between the species in the mixture will be changed after a short time. Such a yeast will therefore give varying results, whilst this will not be the case when a pure yeast of single

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race is employed.

The method given in the first German edition of this book, which appeared in 1888, is still followed in the different countries. The pure yeast propagating apparatus, devised by Captain Kühle and myself, has, however, acquired no small practical importance in recent years. By its means we are in fact enabled to introduce at short intervals large quantities of pure cultivated yeast into the brewery. A detailed description of this apparatus will be given in the next section. An improvement which is also closely related to the employment of my pure cultivated yeasts was introduced into Old Carlsberg in 1885, by the late J. C. Jacobsen. This consisted of an apparatus in which the boiling hot wort from the copper could be cooled and aerated without coming into contact with the micro-organisms present in atmospheric dust. The idea of employing closed sterilized vessels in the place of the open coolers originated, 3 already stated, with Pasteur; subsequently such an apparatus was constructed by Velten; ***hitherto, however, it acquired no really practical importance, because what was most essential was wanting. Thus, of what use could it be to have sterile wort when pitching yeast was contaminated with disease germs?*** Moreover, the construction of Velten's apparatus was in several respects defective; this was especially the case with the method adopted for sterilizing the air, which was effected by heat in accordance with Schwann's method. The present director of the brewery, Captain Kühle, introduced cotton-wool filters, in accordance with my suggestion; and through his experiments, extending over several years, the Old Carlsberg apparatus has been gradually brought to its present highly practical form.* It was only after the yeast question had been solved that the question of the abolition of open coolers was justified, and it was only then that Jacobsen introduced the apparatus mentioned above. An apparatus constructed on the same principle, but differing in several respects from the above, has been erected in New Carlsberg by C. Jacobsen. In the second German edition of this book I expressed myself as follows in connection with this subject: "The time has now come, and there is therefore no doubt that this improvement will gradually find its way into several of the breweries where pure yeast has stood the test, and it will thus serve to perfect the new system." This has also been fulfilled; the Old Carlsberg apparatus has served 3 a model for a number of imitations and modifications brought out especially in Germany. A. Bergh's centrifugal machine has also recently been adopted for the same purpose. †

* A description of this apparatus and its employment will be found in I. C. Hollm's paper, "Die Vorrichtungen in der Brauerei zur Kuhlung und Luftung der Wurze" (Zeitschr. f. das ges. Brauw., 1887, No. 20), and in "Einige Bemerkungen über den Lufilen der Wurze," by A. Petelsen (ibid., 1888, No. 3).

† See the paper by Jörgensen and P. Poulsen, in Zeitschr. f. d. ges. Brauw., 1890.

In the foregoing account I have in the main only referred to low-fermentation breweries. That my work, however, with suitable modifications is also profitably adapted to the requirements of high fermentation breweries and the other branches of the fermentation industry, will be seen from the review given in Chapter VII.

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5. The preparation or pure cultivated yeasts on a large scale.

PRELIMINARY WORK.

We naturally take our starting-point in the brewery itself. If the yeast employed works satisfactorily as regards both principal and secondary fermentations, and, what is of most importance, gives a product having the desired properties, the brewer naturally wishes to retain it. In many, and possibly in most cases, the main bulk of such a yeast consists of one culture species, with only an insignificant admixture of other micro-organisms. Under these conditions the result will also be essentially due to this species, and this will alone give the desired product.

Quite at the commencement of my practical studies on low-fermentation yeasts and wild yeasts, I noticed that when both were present in admixture in the pitching yeast, the upper layers of the fermenting wort contained at the commencement of the primary fermentation a much smaller number of wild cells in proportion to culture cells than at the end of the primary fermentation. Before pure yeast was introduced, I had frequent opportunities of noticing this in the fermenting vessels of both Old and New Carlsberg. The same result was subsequently obtained from systematic experiments made partly in small rounds in the fermenting cellar, and partly in flasks in the laboratory with ordinary wort, and with definite yeast mixtures of both **Carlsberg bottom yeast No. 1 and No. 2**, and the three disease yeasts **Saccharomyces Pastorianus I., Saccharomyces Pastorianus III., and Saccharomyces ellipsoideus II.** That the above frequently occurs in the brewery is therefore proved, and there is much to show that it is possibly even the rule. What has been stated above explains why in breweries it is at least in many cases advisable to use wort in which fermentation has just been started as pitching yeast, instead of waiting until the end of the primary fermentation, as is usually done, and using only the sedimentary yeast. It is evident, however, that a true, pure culture cannot be obtained in this way.* In consequence of these observations, I already stated in the first German edition of this book that the sample to be tested for wild yeast is best taken at the end of the primary fermentation, and the sample for the preparation of the pure culture of the brewery yeast at the commencement of the primary fermentation, and in both cases from the upper layer of the wort. This point has become of no small practical importance.

* See also J. Vuylsteke's "**Ein Beitrag zur Entwicklungsgeschichte der Mischsaaten von Saccharomyceten,**" Zeitschr. f. d. ges. Brauwesen, 1839, No. 1.

When the brewery yeast, from which it is desired to prepare a pure culture, is moderately pure, the growth taken at an early stage of the fermentation will thus approximate more or less to a pure culture. For our experiments it is therefore advisable to take a sample from the

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surface of the wort as soon as this has become covered with a slight foam. From this it is comparatively easy to prepare a pure culture of the brewery yeast which predominates, and, in accordance with the foregoing considerations, this is what we require.

If the brewery is at a distance from the laboratory, it will, as a rule, be best to start with the ordinary sedimentary yeast, and, if this is not enfeebled, it can be used as it is; otherwise, it is introduced into a flask of sterilized wort, and, as soon as fermentation commences, a little of the liquid is decanted and made use of for the preparation of the pure culture. The object here is not only to obtain a growth in which the desired species preponderates, but also to obtain young and vigorous cells. The latter is of importance in reference to the treatment to which they are subjected in the course of the preparation of the pure culture. The method for this has already been described (see p. 5). *

* The flasks and apparatus which are used in my laboratory can be obtained from F. C. Jacob, Hauserplads 14. Copenhagen, and from Dr. Rohrbeck. Karlstrasse 24, Berlin.

In the foregoing, the case was mentioned in which a single species of yeast preponderated in the mixture with which we started, and effected, practically, the whole fermentation, and determined the character of the product. Cases also occur, however, in breweries where a single species does not predominate, but where there are several species which operate together to produce the result. Under such conditions, a pure culture of one of the species will, as a rule, give a product having very different properties from that yielded by the mixture. The different species contained in the mixture can certainly be separated from each other, and it is, therefore, possible at any time to make up the mixture, and with some difficulty even to introduce the different species in the desired proportions; but as we are not able to regulate these for any length of time, those species which are most vigorous will gain the upper hand in the commencing struggle, and will thus give rise to irregularities. Under these conditions a constant product can only be attained with great difficulty, and pure culture has, therefore, no practical value in this case. If, however, we wished to attempt this, each portion of the pitching yeast would have to be prepared separately, or the fermentation with each species of yeast would have to be carried out separately, and the resulting beers mixed at the end of the primary fermentation. With the methods of working now in vogue in breweries, I cannot regard the adoption of such a complicated method as practical, and I therefore advised against it even in the first German edition (1888) of this book.

Amongst the culture yeasts separated from such a mixture, several species may often be found which are of use in practice.

Nevertheless, nothing can be stated as regards their properties until they have been investigated (see also Chapter II). It must also be remembered that laboratory experiments with small quantities give but imperfect information in this direction; the trial must be made in the brewery, and under the conditions obtaining in practice. In such a case, therefore, it will be several months before we can obtain the result of the trials. *

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* The different laboratories in which pure yeast culture is undertaken on my system possess large collection: of yeasts which have been tested in practice, and are able to furnish at once a suitable pitching yeast. It is, therefore, only in the rarest cases that there is any occasion to undertake the tedious examination mentioned above

It will be remembered that we generally employ wort gelatin in the preparation of our pure cultures, and that these are grown at a temperature of 25° C; under these circumstances, varieties may sometimes be produced, and although these are only of a temporary nature, they may, nevertheless, now and then give rise to difficulties. Fortunately, this is only rarely the case, and, when it does occur, my earlier method of preparing pure cultures must be adopted that is to say, the single cells must be introduced directly into flasks containing wort, and not into gelatin; and the growth must take place at low-fermentation temperatures. Under these conditions it is also advisable to introduce several pure cultures of the same species into the same flask. In this way a pure culture is obtained of a single species which, however, is not derived, as is ordinarily the case, from one, but from several individual cells, and it will, therefore, already in the first stage of its development, possess all the peculiarities of the species. There are only very few species and races which show an exceptional tendency to form varieties, and which, therefore, require this elaborate treatment. In most cases the ordinary single-cell culture will at once give a normal fermentation.

Assuming that we have succeeded with our pure culture, and that we have a vigorous growth of the selected species in a flask, we must in the first place secure the preservation of this for future use. As a precaution, it is not sufficient to keep merely a single sample, but a reserve flask should also be kept, so that we may be certain that we always have a living and a pure yeast. Most of the *Saccharomycetes* can be preserved in flasks containing wort for a whole year, or even longer, if kept at the ordinary room-temperature, and protected from direct sunlight. In some cases, however, I have found that they have perished in less than a year, and when exposed even for a short time only to direct sunlight, and therefore also to a high temperature, they perish much sooner. It is necessary, therefore, to preserve these cultures in a dark cupboard, or, at any rate, to protect them from the direct rays of the sun; this also applies when other liquids have been employed for the cultivation. From some investigations of Duclaux on the duration of the life of yeast cells in beer wort, it might be assumed that this liquid leaves nothing to be desired in this respect. As we have seen, however, this is not fully supported by my experiments. The best liquid for the preservation of yeast which I know of, when it is a question of preserving it for several years, is not wort, but an aqueous solution of cane sugar; and for this purpose I generally employ a 10 per cent. solution. I have flasks containing this solution into which brewery yeasts and wine yeasts were introduced fourteen years ago, and all the species contained in them are still living. Many yeasts which lived in the sugar solution for over ten years, were found to perish in beer wort in from one to three years.

The method which I have described for preserving yeast in sterilized filter paper (see my treatise on ascospore-formation in "*Compte-rendu des travaux du laboratoire de*

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Carlsberg." 1883, p. 29) is very useful for sending small samples of yeast, and is, in fact, generally made use of for sending samples from breweries to the laboratories where the pure cultivation of brewery yeasts is undertaken. As a result of my experiments up to the spring of 1883, I stated (lac. cit.) that yeast cells can be preserved in filter paper in some cases for twenty months and in others for only five months. From more recent experiments I have found that the cells do not completely perish in less than about five months, and that most species do not survive after two years under the conditions mentioned; only one sample was still alive after two and a half years. The experiments were made with young vigorous cells, and the samples were kept in a drawer at the ordinary room-temperature. It must be remembered that with this method of preservation the yeast is introduced into an envelope of filter paper. In this manner a pure culture cannot, however, be preserved with certainty. When it is desired to make use of the same principle, it is best to employ the flasks containing cotton wool, and which will be described below; under these conditions several species often form spores, and these will live longer than the ordinary cells.

For the reasons mentioned, flasks containing a solution of cane sugar afford in general the best means for the preservation of yeast, and it is thus seen that there are no difficulties in the way of preparing and keeping a whole series of tested culture yeasts in zymotechnic laboratories, so that breweries, distilleries, &c., can at any time be provided with the species which they require. In Jörgensen's laboratory, for instance, there is a collection of about 200 culture yeasts, and, according to his verbal statement, none of these cultures in cane sugar have died, although several of them are nine years old; they are kept in a room at the ordinary temperature which, especially in the summer, is fairly high. From these flasks the yeasts, both high and low, desired by his clients are propagated in beer wort. There was never any question of preserving these yeasts in ice. Mr. Petersen at Old Carlsberg, and Professor Grönlund at New Carlsberg, have likewise successfully made use of the same method. Jörgensen states that he made some experiments in order to ascertain whether he could obtain a better pitching yeast by propagating not from the cane sugar flasks, but from wort cultures prepared for this purpose. The growth obtained from the wort cultures, after being rejuvenated frequently and at short intervals at the ordinary temperature, gave a pitching yeast which was inferior to that obtained directly from his culture in cane sugar solution,"and which was several years old. Preservation of streak cultures in wort gelatin likewise gave no satisfactory result. Thus the experience gained up to the present time indicates that the cane sugar solution affords a good medium for the preservation of brewery yeasts at the ordinary room-temperature; this holds good in all probability to the same extent for the other yeasts which are employed in the fermentation industry.

MY OLD METHOD.

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Having procured our pure culture for future use, we proceed with the preparation of the yeast on a large scale for the brewery. For this purpose the following apparatus is made use of: Four or five Pasteur flasks of about 1 1/4 liter capacity, four copper vessels of the form shown in Fig. 5 or 6, each of 10 liters capacity.

The form of the Pasteur flask is represented in Fig. 3, which shows a slight modification consisting of a bulb blown in the bent tube, the object of which is to ensure greater security against infection. Our pure culture is contained in a flask of this form, and of 125cc capacity. The form of the larger flasks of 1 1/4 liter capacity, which are next made use of, is the same. The flask stands on a cork base; the straight tube is fitted with a rubber tube which is closed by a glass stopper; the end of the bent tube is plugged with asbestos.

The metal vessel (Figs. 5 and 6) is made of tinned copper. At first I used Pasteur's model (Fig. 4), but when I discovered its imperfections, and the great difficulty of working with it with safety, I abandoned it. This vessel is closed at the top with a rubber stopper, through which pass a short straight tube for introducing the yeast, and a long bent tube for the exit of the carbonic acid gas; the top of the vessel is also provided with two windows. Nearly at the bottom is a tap for drawing off the beer and yeast. It is especially at the windows and tap that infection from without occurs after the vessel has been used a few times. In some laboratories, however, it is still employed, and I have therefore introduced the accompanying sketch. In the course of years my assistants and I have contrived the vessels shown in Figs. 5 and 6; the form shown in Fig. 6 is used in the Carlsberg laboratory, and I now especially recommend it, but Fig. 5 has the advantage of being cheaper. The name Carlsberg vessel is generally applied to them; the difference between them and Pasteur's vessel is distinctly shown in the figures.

Over the short tubes **a** and **b** (Fig. 5) are fitted rubber tubes, and these are closed with glass stoppers in the usual manner; the lower tube **b** is also provided with a pinch-cock. The metal part of it must be as short as possible, and the pinch-cock closing the rubber should be as close as possible to the mouth of the former. The bent tube passing from the top of the vessel is made in two pieces, which are joined at **c** with India-rubber tubing. The end **a'** of the tube is closed by a plug of cotton-wool, tightly packed in



FIG. 3.



FIG. 4.

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a glass capsule; at **e** there is the enlargement in the tube mentioned above, to ensure against infection occurring, especially shortly after sterilization. In place of the cotton-wool filter we now generally employ asbestos; this is contained in a brass cylinder which is screwed tightly on to the tube, and is provided with a loosely fitting cover (Fig. 6).

In the form shown in Fig. 5, the bent tube is fixed to the can. It may, however, also be fastened by means of a screw union as suggested by my assistant, Mr. Poulsen; this is shown in Fig. 6, whilst Fig. 7 shows the screw union in detail. The diameter of the cone of the union (shown **d** by the dotted line) is 28 mm. at the larger, and 25 mm. at the smaller end; its length is 18 mm.; it must, of course, no. 5. be well ground in. This arrangement admits of the whole tube being detached when the vessel requires cleaning, and there is, therefore, no need for the rubber connecting tube (Fig. 5, **c**). The cleaning is, however, readily effected in both cases; if the union is well made this form is to be preferred. The chief point in connection with these and similar vessels is that **they must be perfectly tight so that air can only enter through the bent tube.**

The nutrient solution employed is ordinary hopped wort as prepared in lager beer breweries;* the glass flasks are two-thirds filled with it. The simplest way of effecting sterilization is by boiling on the sand-bath. When the liquid has commenced to boil briskly, the glass stopper is inserted into the rubber tube and the steam is allowed to escape for a short-time through the bent tube; the flask is then placed on the cork stand and the asbestos plug immediately introduced. If desired, the sterilization can also, of course, be effected by steam. The method of boiling described does not always kill all the germs present. If, for instance, samples from a number of flasks which have been thus treated are introduced into a medium especially favorable for the development of bacteria, and if these are then placed in an incubator at 27-30° C., we shall find that living bacteria were present in some of our wort flasks. These are, however, unable to develop in the boiled wort, and as we have to deal with this liquid only, the fact mentioned is of no importance; practically, we may say we have a sterile liquid.

* If the produce of yeast is wanted for distilleries, wine manufacturing, &c, nutrient liquids suitable for the purpose are of course employed.

The sterilization of the wort in the large metal vessels (Figs. 5 and 6) is rather more troublesome than is the case with the small glass flasks. I have often heard complaints from

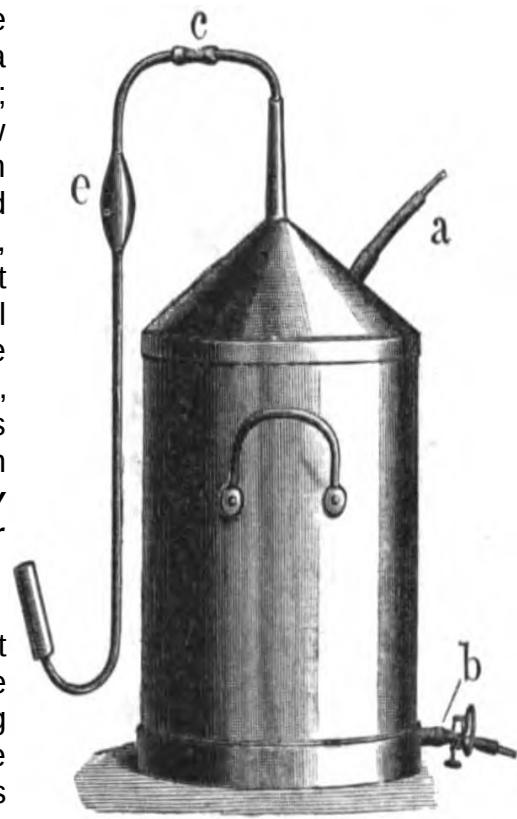


FIG. 5.

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pupils who have attended my lectures and exercises, that they could not work at all with these vessels. For this reason I will describe the method in detail. After the vessel has been well cleaned it is charged with about 5 liters of water; this is then boiled for an hour, both the tube a and the bent tube remaining open; the rubber tube at a is then closed by a glass stopper which is first sterilized in the flame, and the boiling is continued for 15 minutes during which the steam escapes through the bent tube; the gas flame is lowered somewhat during this time so that the pressure may not become too great. Shortly before the gas. is turned out, the tube b is opened and about 100 cc. of the boiling water run out. In this way the sterilization of this tube and its contents is secured. It is then closed as before with the pinch-cock and glass stopper; the latter being first rather strongly heated in the flame. The boiling is now finished, and all that remains to be done is to press the cotton-wool filter firmly over the end of the bent tube or to screw on the asbestos filter. Before introducing the wort, the water must, of course, be removed, or at any rate the greater part of it. A mixture of 7 liters of wort with j liter of water is a suitable proportion. Sterilization is effected in the same manner as in the cue of the water, but at the end greater precautions are taken. Whilst the 100cc of the boiling wort are being withdrawn through the tube b, and during the 15 minutes that the steam is escaping through the bent tube, the latter is strongly heated by means of a second gas flame, and the filter d is then attached with the greatest care. In short, every precaution must be taken that the large bulk of air which is drawn in during the cooling of the wort does not carry with it any living germs into the liquid. The vessels, after being treated as above, have stood for several months without the contents becoming attacked by micro-organisms. Before being used, samples of wort from each vessel must be drawn off at b, and whilst this is done the bent tube is heated in order that the air which passes into the vessel becomes sterilized.

As far as we can confine ourselves to comparatively small vessels we naturally make use of glass flasks. In spite of the fragility of this material, these vessels possess material advantages since, on account of their transparency, a check can be kept on the liquid within them. If this control is to have any importance the flasks may only be of such a size that every portion of the liquid and its sediment may be well examined from without. This limit is reached with a capacity of 1-2 liters. If the glass flasks are larger than this it may happen that the liquid contains small colonies of different micro-organisms which may escape detection, although of such a size that they would be at once noticed in the smaller flasks. Only when the colonies of the microorganisms present have attained large proportions will it be possible to detect them in the large flasks; but under such circumstances we shall also be able to detect them in the samples withdrawn from the metal vessels. A glass vessel of 10 or more liters capacity is, therefore, no more serviceable in this respect than a metal vessel of the same size; and as the latter is stronger and more convenient to handle, it is naturally to be preferred.

Having prepared our flasks and vessels of sterilized wort, it is best to set them aside for a time in order that the wort may take up oxygen from the air through the bent tube. It has been found, for instance, in some experiments that the yeast which had been cultivated in aerated wort gave a satisfactory normal result as regards clarification from the commencement, whilst the same yeast which had been grown in non-aerated wort was unsatisfactory in this respect and only became normal after several fermentations. Similar observations have also been

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made by Aubry and A. Jörgensen.* This phenomenon is deserving of further study. In accordance with our present experience it is, therefore, advisable to work with aerated wort; and this is of great importance also for another reason if it is desired to obtain a compact sediment of yeast in the vessel. This applies especially to species like **Carlsberg bottom yeast No. 1**. That the wort in the flasks and metal vessels does take up oxygen from the air on standing at the ordinary room-temperature was very easy to prove. In fact, after four months, the sterilized wort was found to contain more free oxygen than the normally aerated wort from the open coolers. Some experiments with **Carlsberg bottom yeast No. 1**, which under normal conditions is not a good clarifying yeast, have shown that it is of especial importance to **thoroughly aerate the wort whilst still hot**.

* *Zeitschr. f. d. ges. Brauwesen 1890.*

In most laboratories the Carlsberg vessels are treated in the manner described above, and thus no special arrangements are made for the aeration of the wort. In my laboratory it is customary, however, to pass a considerable volume of sterilized air into the wort whilst it is still boiling. This air passes from a holder in which it is contained under a pressure of 3-4 atmospheres, and is purified from all germs by means of the cotton-wool filter described on p. 66. For greater security against infection, the bent tube through which the steam escapes is heated in a flame. A sterile glass tube is inserted into the rubber tube near the bottom of the vessel and is connected with the cotton-wool filter; the pinch-cock is then opened and the air-cock turned on at the same time. As soon "as aeration has thus commenced the flame under the vessel is turned out, and a few minutes later also the lamp employed for heating the bent tube. Aeration is continued until the temperature of the wort has sunk to 30-35° C.; if carried further, froth may readily be carried over into the bent tube. My assistant Mr. Nielsen found that 60 liters of air were used in the aeration of the wort contained in one Carlsberg vessel, the time occupied by this operation being 5-6 hours. Prior attaches a self acting aerating apparatus to the vessels. *

* *Bayer, Brauer-Journal, 1891.*

Four or five of the previously mentioned glass flasks are next inoculated with a vigorous growth of our pure culture; strictly speaking, we require only four, the fifth serving as a reserve in case of accident. These are set aside at the ordinary room-temperature, and in the course of a week or less an abundant sediment of yeast will have formed. The greater part of the beer is then decanted, only enough being retained to detach the yeast. This is then introduced into four of the Carlsberg vessels, each of the latter being inoculated from one flask; the yeast is introduced through the tube a, Fig. 5. It is evident that all these operations must be performed in such a manner that no infection can occur from without. For this some special knowledge and no little practice are required, and these cannot be acquired from a description. A distinct fermentation sets in by the following day, and it is then advisable to remove the filter d. If it is desired to hasten the fermentation, the bent tube should be heated and a portion of the carbonic acid gas expelled by agitation. After about seven days as much yeast will generally have formed as can be produced, and the four vessels then contain

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sufficient yeast to pitch about a hectoliter of wort.

The above completes the work in the laboratory, the further operations being conducted in the fermenting cellar. For this purpose, a vessel of about 1 1/2 hectoliters capacity is fitted up. This must be thoroughly cleaned, recently varnished and covered over with a loose lid to allow the escape of the carbonic acid gas. The wort must be aerated, and in most cases we must, therefore, be satisfied with the ordinary wort of the brewery. After passing a flame over the surface of the four vessels containing the pure culture, these are shaken up and the contents poured into the above wort.

If it is not desired to add the partly fermented wort, or if the yeast is not to be transferred directly from the laboratory to the fermenting vessel, but has to be packed up for sending to a distance, it is advisable to let the vessels stand a few days longer in order that the yeast may form a compact layer at the bottom. When this is the cue the liquid may be drawn off through the tube 5, Fig. 5. It is evident that whilst this is being done, the precaution must be taken of sterilizing the air which passes in through the bent tube.

As soon as a vigorous fermentation ha set in, and the first signs of a head have appeared, the whole may be added to 3-4 hectoliters of wort. In this manner we pass rapidly from the small scale to the normal scale of the brewery. The same result can also be attained by adding the yeast to a hectoliter of wort contained in a larger vessel, e.g. of a capacity of about 3} hectoliters, and introducing an equal quantity of wort as soon as fermentation has set in", when this is well fermenting and a head has formed, another hectoliter of wort is added. At the commencement the temperature of the wort should be a shade higher than that of the fermenting cellar, and this is especially the case when the vessel is a small one. If it is desired, each fermentation can also be carried to the end in the ordinary manner, and the sedimentary yeast collected at the end of the primary fermentation; this is then weighed and added to a suitable quantity of wort. It should be pointed out that such a pure culture frequently, though by no means always, attenuates somewhat lower in the first fermentations, and the clarification is also less satisfactory at first. Many brewers have been alarmed by this, although without cause.

Until the introduction of the large pure yeast apparatus described later on, the above method of working with my pure cultivated yeasts has been adopted in breweries, and is still made use of in many places at home and abroad. It has rendered great service and will also do so in the future.

THE PURE YEAST APPARATUS.

It was mentioned above that some beer yeasts were less resistant than others to competing organisms, and **Carlsberg bottom yeast No. 2** was mentioned as an example. In working with species like this, the danger of the disease germs gaining the upper hand is comparatively great. It is, therefore, of special importance to introduce large quantities of

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absolutely pure yeast throughout the fermenting cellars in the shortest possible time, and thus to displace the older contaminated yeast. With my old method described above, a good deal of work was involved in furnishing the brewery twice a month with pure pitching yeast for 1 hectoliter of wort; and as this is not sufficient in all cases where absolute certainty is required I naturally desired to go further. I applied to Capt. Kühle, the Director of the Old Carlsberg brewery, and in 1885 we commenced working jointly to devise an apparatus for the continual production in quantity of absolutely pure yeast. After some experiments we met with success, and the credit for this lies mainly with Capt. Kühle. I gave a short account of the apparatus at the meeting of the Austrian Brewers' Association in Gratz on June 12, 1887.

In the following descriptions, my object has been to make them clear to every intelligent reader, who need have no special knowledge. I have also endeavored to give such full and accurate details that the practical brewer will find every reasonable question answered, so that he may set up the apparatus and work with it without loss of time or money. In writing a work which is to be of use in practice, it is not sufficient to confine oneself merely to outlines, but it is necessary to elaborate the details; many matters which in theory appear trifles have in such a case a special importance. In accordance with this, everything that is stated here is to be regarded as the result of several years' experience and trials in several breweries.

In the following, the form of apparatus which is first described is that in which neither the fermenting cylinder nor the wort cylinder is provided with water-caps. The former cylinder is covered with an insulating material such as wooden laths. In this form it is intended to be fitted up in the fermenting cellar. The construction of the apparatus is next described when intended to be placed in a room above ground or under conditions which necessitate the regulation of the temperature of the "fermenting cylinder. Finally instructions are given for using the apparatus.

As shown in Fig. 8, the apparatus consists of three main portions and the connecting tubes, namely:

- (1) the air pump **A** for aerating the wort, and the air-holder **B**;
- (2) the fermenting cylinder **C**; and
- (3) the wort cylinder **D**.

The pump **A** is driven by machinery and draws the air through a filter in order to effect a preliminary purification. The air-holder **B** is provided with a pressure-gauge and a safety valve. It is charged with air under a pressure of 1-4 atmospheres. The pipes must be fitted with cocks at suitable points for removing the water which collects in them. This is of especial importance in the case of the pipe between the air-holder **B** and the filters **g** and **m**. These are best united by metal tubes with the air pipes. If rubber is employed for this purpose, it must be very strong in order to withstand the pressure. If metal tubes are used, they should naturally possess some degree of elasticity and must be so arranged that the filters can be readily fitted and disconnected.

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At the side of the fermenting cylinder **C** are two windows placed at an angle to each other. I have added these windows as some attach importance to them. In the figure they are shown as rectangular, but recent experiments show that they are best circular. According to my own experience, however, they are advantageously omitted. Through the top passes a stirrer **b**, the lower end of which is fitted with two blades, one carrying a sheet of rubber cut in such a way that when rotated it comes into contact with both the bottom and the sides of the cylinder. From the top there passes a doubly bent tube **c** and by opening its cock, connection is made with the inside of the cylinder. The lower free end of the tube dips under water in the vessel **d**. This tube can be fitted to either side of the cylinder where it is most convenient, but care must, of course, be taken that the water cannot splash into the vessel which is placed under the cock whilst the yeast is being withdrawn in the manner described later on. A little below the top is a horizontal tube **e** provided with a cock, and by means of which the inside of the cylinder is connected with the vertical glass tube **f**. This is connected at its upper end with the filter **g** and at its lower end with a second cock and similar horizontal tube **h** to that described above.

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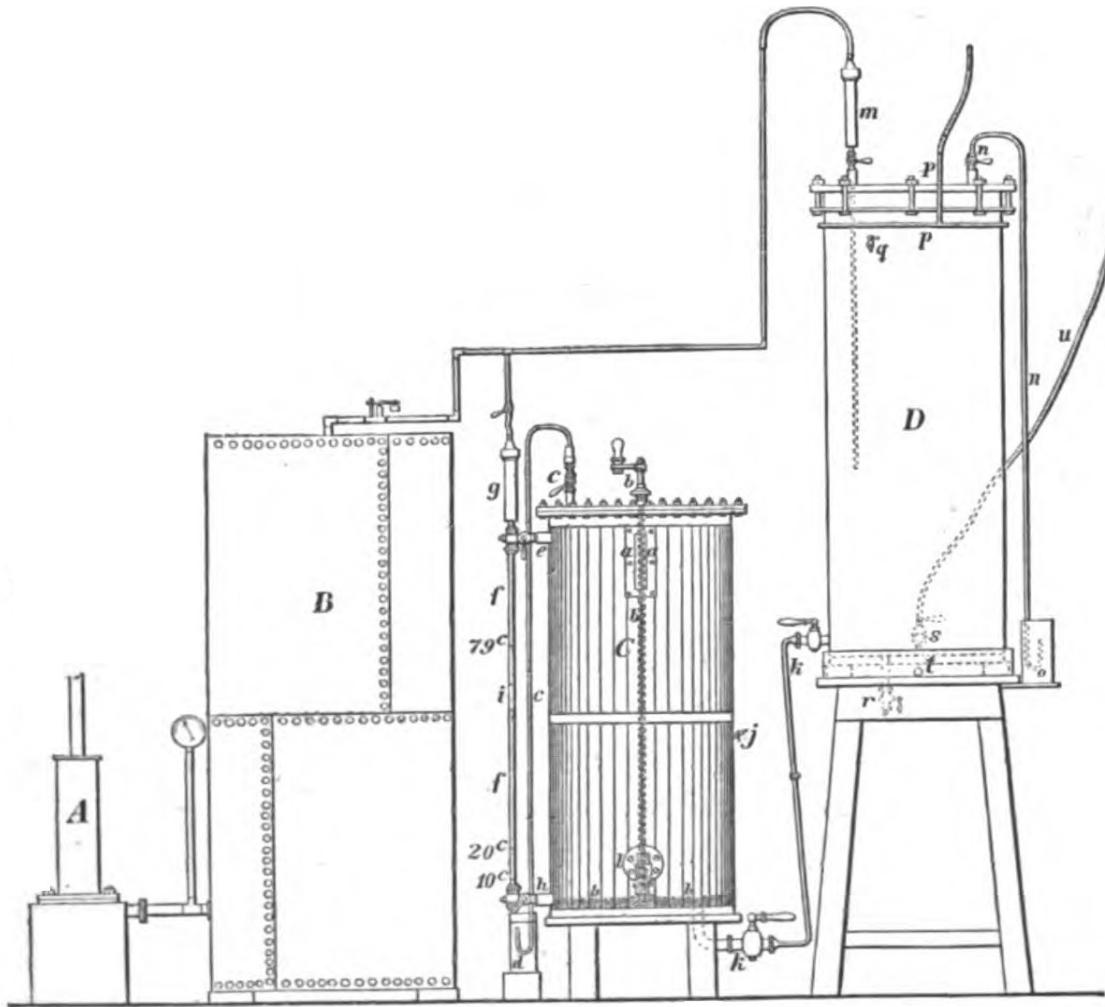


Fig. 8.

The top mark on the glass tube is 79 cm. from the bottom of the cylinder, the next 20 cm. and the lowest 10 cm. from the bottom of the cylinder. When filled to the top mark, the cylinder holds about 170 liters. The glass tube is fixed into the cocks **e** and **h** by a packing of hemp or cotton-wool with Vaseline; rubber is not suitable as it is hardened by steam.

The filter **g** consists of a metal capsule containing a tightly packed plug of cotton-wool 22 cm. long and 3 cm. in diameter. This plug consists of at least 35 grams of cotton-wool; the addition of a few more grams is immaterial. If firmly pressed in, the capsule will hold 50 grams and more, but this is not necessary. The filter is closed above by means of a cover which is screwed on and which is connected with the tube from the air-holder. Before the filter is screwed on, it is sterilized by heating it for two hours at a temperature of about 150° C. The filtration of the air will be described below.

At the opposite side of the cylinder there is a small tube **j** scarcely 1.5 cm. long and fitted with

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rubber tubing, the latter being closed by means of a pinch-cock and a glass stopper. Passing from the bottom of the cylinder is a tube **k** through which connection can be made with the wort cylinder **D**; this tube is made in two pieces to prevent too great rigidity, and in addition to the two large cocks shown, it is provided with two smaller ones which are made use of during the process of steaming described below, partly for running off the condensed water and partly for introducing the steam.

The cock shown at **I** is for withdrawing the beer and the yeast. The construction of this cock is shown in Fig. 9, and the direction in which the liquid passes through it is indicated by the arrows. The valve is screwed down in opening the cock and is screwed up when this is closed. In the figure it is closed. Its construction prevents infection from occurring whilst the liquid is being drawn off, as the liquid cleanses the cock on passing through it. The pipe carrying the cock is carried through the side of the cylinder and is bent towards the bottom, its end being 3.5 cm. above the latter (see Fig. 10, **C, I**). It is, in short, so arranged that no air from without can enter the cylinder whilst the contents are being drawn off.

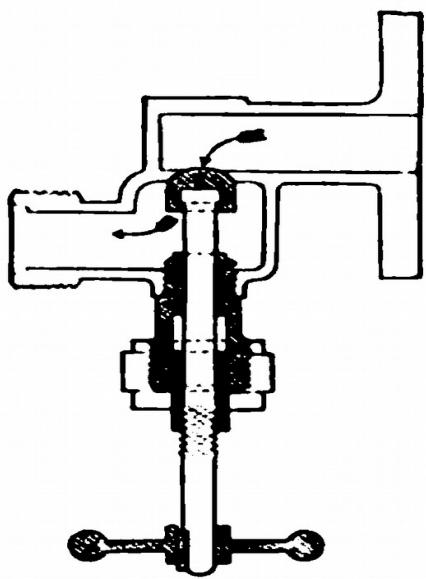


FIG. 9.

When the cylinder is fitted up in the fermenting cellar, it may be covered with laths as shown in Fig. 8, **C**. The cylinder with water-cap will be referred to later on.

The wort cylinder **D**, as is shown in Fig. 8, must be raised somewhat above the level of the fermenting cylinder. (The wort can, of course, also be forced into the fermenting cylinder by means of compressed air, but in this case the wort cylinder must be provided with a safety valve.) Its height is also greater than that of the latter, but its diameter is the same. At the top is a filter **m** exactly as at **g**, and connected with it is a pipe (indicated by the dotted lines) passing inside the cylinder. The lower closed end of this pipe has some small perforations through which the air finds an exit after passing through the filter. The tube **n** corresponds with the tube **c** of the first cylinder, and like the latter its open end dips into a vessel of water **o**. In the case of the wort cylinder it is very important that the bore of the tube **l**, and of its cock, should not be too small, in order that they may not become choked by hops or other matter; a suitable diameter for the tube is 1.3 cm. Around the upper portion of the cylinder, a little below the top, there is a pipe in the form of a ring **p**, the inner side of which is provided with small perforations. One end of this pipe is closed and the other is connected with a cold-water tap. In addition to the cocks on the connecting pipe **k** between the two cylinders, the wort cylinder has three others **g, r, s**. The cock **s** is for the introduction of the wort, and is put in connection with the wort main **u** between the copper and the cooler. The cylinder stands in a shallow tray provided with an outlet **I** for the water which flows over the sides of the cylinder, whilst the latter is being cooled. The dotted lines at **I** show the bars on which the cylinder rests, and also the ring-like portion and bottom of the cylinder.

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When, at the beginning of 1886, the apparatus was about to be introduced in New Carlsberg, some modifications were made in connection with the fermenting cylinder, for owing to its being placed in a room above ground it was subject to appreciable changes in temperature, and especially during the summer to too high a temperature. The necessary modifications were made in an "excellent and very practical manner by the chief inspector, Mr. Henningsen. The main point was to arrange the fermenting cylinder in such a manner that the temperature of the liquid contained in it could always be controlled, and that it could be lowered when desired. This is done by means of the jacket, shown in Fig. 10, **C**, which surrounds not only the sides but also the bottom of the cylinder; the bottom of the jacket is fixed with screws and can without much difficulty be removed when it requires cleaning. For the introduction of a thermometer there is a tubular aperture through the jacket and the side of the cylinder. The jacket is provided with a tap near the bottom, forming the inlet for the cold water, and another near the top and on the opposite side for its exit; a third tap at the bottom serves for removing the sediment which is gradually deposited by the water.

The wort cylinder (Fig. 10, **D**) is here also provided with a jacket, which, however, can very well be omitted as the perforated ring (Fig. 8, **p**) serves the same purpose sufficiently well. Nevertheless the jacket has the advantage that it encloses the water from the ring so that the operator is not liable to be splashed. It adds, however, considerably to the cost of the cylinder, and it makes it less simple to manipulate.

In Fig. 10, **C**, **x**, is shown, an improved, and therefore also a somewhat more costly construction of the cover than that represented in Fig. 8. The middle portion is made of copper and is provided with a brass flange with Fm H twelve bolt holes. Between the the cover and the collar of the cylinder a rubber washer is inserted and fits into a groove; a perfectly air-tight joint is thus ensured.

The letters in Fig. 10 otherwise correspond with those in Fig. 8, and the above description therefore applies to both.

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The arrangement of the stirrer is seen better in Fig. 10 than in Fig. 8. In order to prevent its being raised out of its bed at the bottom of the cylinder whilst in use, a ball-socket is provided. As shown in Fig. 11 the axis ends in a ball which rests in a hemispherical socket, and two pieces accurately fitting the upper portion of the ball are bolted on; the axis can be rotated but cannot be raised from its socket.

FIG. 10.

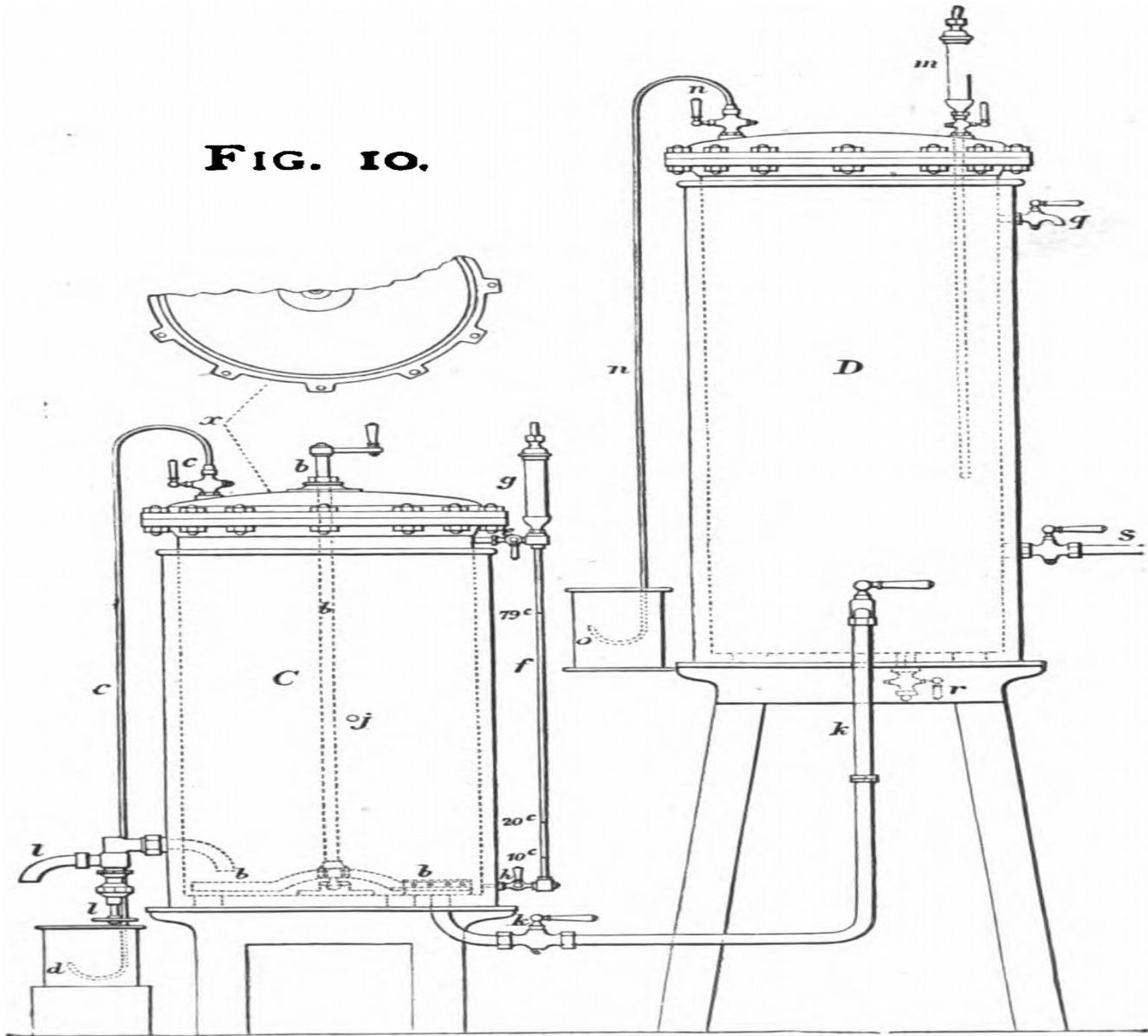


Fig. 12 shows the arrangement of the whole apparatus in the form in which it is at the present time generally fitted up.

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With regard to the tinning of the cylinder, it must be pointed out that the tin should not contain an appreciable amount of lead. If this is the case, the yeast grown in the apparatus will, according to Prior, be unsatisfactory.

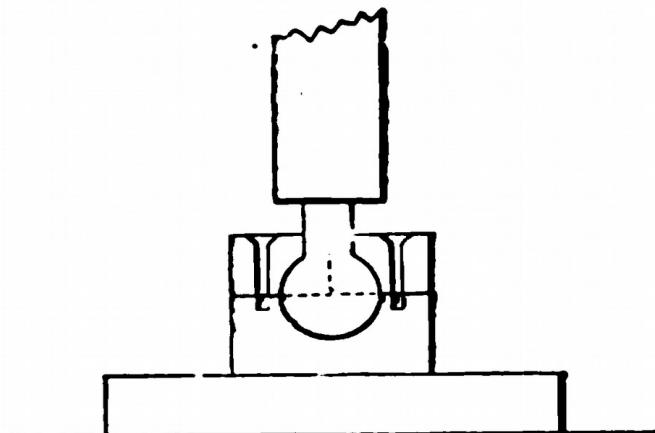


FIG. 11.

A careful study of our apparatus will show that it is constructed on the principle of the flasks employed in the laboratory in experimenting with micro-organisms, and that especially Pasteur's two-necked flasks have served as the model. The principles involved and the apparatus necessary for effecting sterilization were discovered by Pasteur's predecessors, and the discoveries of Schwann (1837) in particular were of fundamental importance in this connection. They are discoveries which were made before I was born, and I can therefore lay no claim to priority with regard to them, and I have, indeed, never thought of doing so. The attacks in the "Berliner Wochenschrift," which

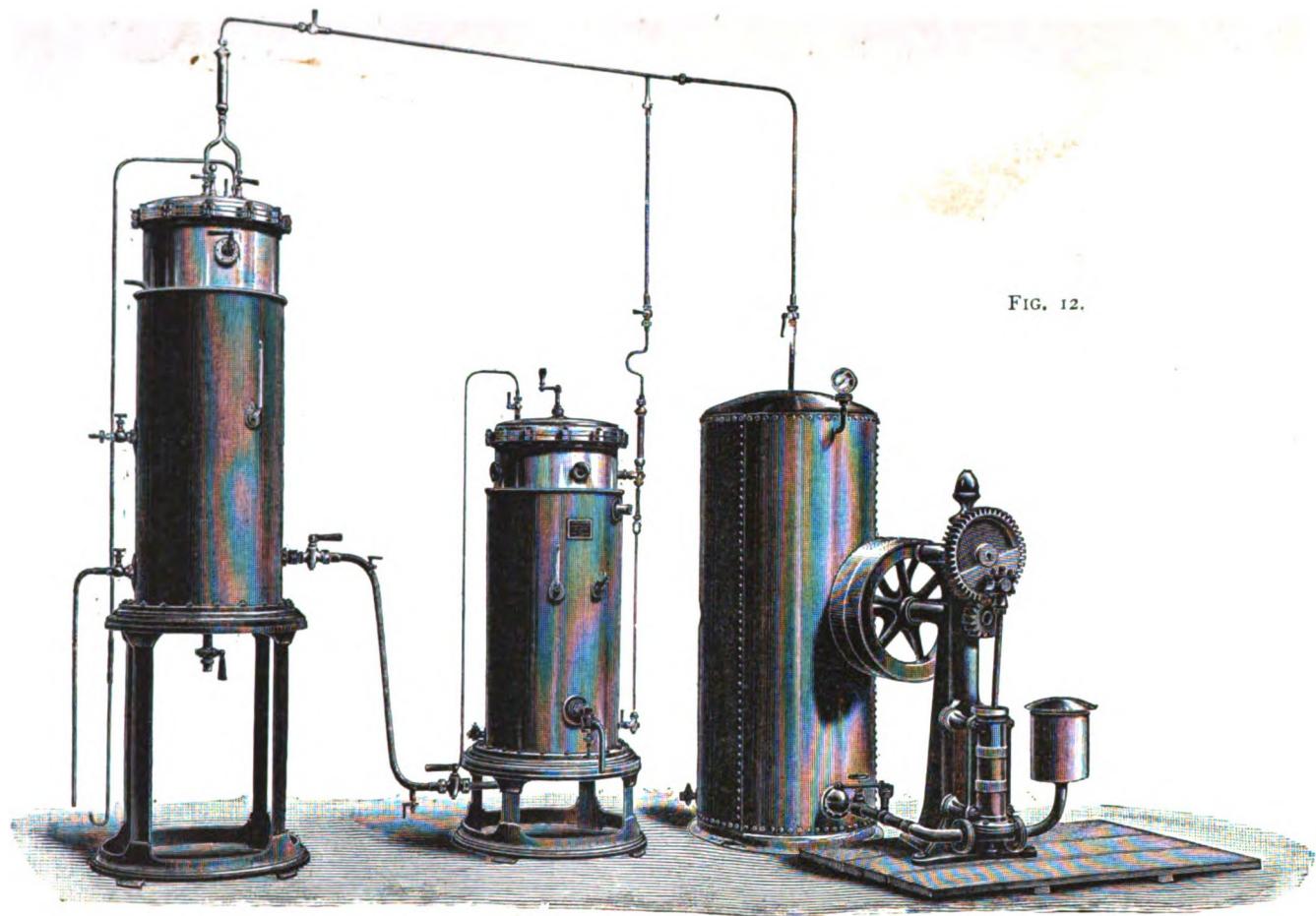


FIG. 12.

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have been directed against me in connection with this are therefore perfectly groundless. (See further the historical account, p. 157)

In putting up the apparatus, it ought above all to be borne in mind that it should remain in its position undisturbed. When possible, it will generally be best to place it in the fermenting cellar. There is then, as a rule, no trouble with regard to regulating the temperature, and in drawing off the beer and the yeast there will also be less work involved, for those occupied can, in the interval, do other work close at hand. If the temperature of the fermenting cellar is below 6° C. it is advisable to have the fermenting cylinder jacketed. In putting up the apparatus it is, of course, necessary to at once consider whether one or two fermenting cylinders are to be employed; in any case a single wort cylinder will suffice.

The apparatus having been fixed, it is necessary in the first place to test whether the cylinder is tight. To do this, steam is cautiously introduced through **k**, whilst the other cocks are closed; water-pressure may also be employed. It is evident that some care must be adopted, especially at first. It is of importance that whoever is to use the apparatus shall have previously made himself familiar with its construction and use. The first trials are best made with water. The rule should be made that one man only has charge of the apparatus. Experience has shown me that especially at the beginning this is often forgotten; but when several are in charge, matters do not work satisfactorily.

Before the apparatus is set working it is necessary to thoroughly sterilize the two cylinders, the pipe which unites them, and also the pipe through which the wort passes on its way to the wort cylinder. This is done by blowing a strong current of steam through the whole. The filters are sterilized as already mentioned in a sterilizing oven. The fermenting cylinder is sterilized by steam, admitted through one of the cocks on the pipe **k** in Figs. 8 and 10. Whilst the high tension steam is passing, the different cocks are opened from time to time, so that it can escape through these as well as by the bent tube **c**; this operation takes half an hour. Shortly before this the filter is screwed on, and then all the cocks are closed except that on the bent tube. Simultaneously the cock of the filter is opened in order that air may pass through the filter **g** and the tube **k** into the cylinder. The latter cools down as the air enters and the steam is gradually turned off. In short, the cooling is effected by the current of air, which mixed with the steam escapes through the bent tube **c**. So long as a current of steam is seen to escape, the vessel of water **d** is not required; this is only required as an indicator at a later period. If the steam were shut off suddenly, there would be a danger of the filter not admitting a sufficient volume of air to prevent a diminution of the pressure due to cooling, and the result would be either that impure air would be drawn into the cylinder, or the latter might collapse from the external pressure of the atmosphere. Under the conditions mentioned and at the ordinary temperature of the fermenting cellar, the cooling takes about two hours. If the cylinder is provided with windows these must not of course be screwed down tight during the steaming, as they would then crack; they should be screwed down cautiously and gradually when the operation is nearly finished.

With regard to the small vessels of water **d** and **o** at the bottom of the bent tubes, it may be

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stated once for all that their only object is to indicate the direction of the air current, whether outwards or inwards.

The wort cylinder and its two pipes **s** **u** and **k** are sterilized in the same manner, but the process of cooling is here omitted. When the steaming is nearly finished, the cock of the air-filter is opened and the wort is admitted. The wort employed is the ordinary hopped lager beer wort, which has been sterilized by boiling in the copper, and is run as hot as possible through the pipe **u** (shown in Fig. 8 only) and the cock **s** into the cylinder. Shortly before the steaming is finished the pumping of the boiling wort on to the cooler is commenced, and ten minutes later the cock **s** is opened. The wort is allowed to run into the cylinder until it reaches the upper cock **q** and the cock, **s**, is then closed. It is advisable to place a small bucket under the cock **q** to catch the wort which runs out, and when this occurs the cylinder is known to contain the desired volume of wort. The hot steam and air escape partly through **q** and partly through the bent tube **n**. It is advisable to run off the first small quantity of wort which enters the cylinder by means of the cock **r**, as it is mixed with water from condensed steam, which gives it a disagreeable taste. When the desired quantity of wort is in the cylinder the cocks **q** and **s** are closed. Air, sterilized by passing through the filter, is now forced through the hot wort for an hour before the cooling is commenced, and the aeration is also continued during the process of cooling. Generally, a pressure of from 1 to 2 atmospheres in the air-holder suffices. It is merely necessary that the sterile air in the cylinder should always exert a slight pressure in excess of the atmospheric pressure, and thus prevent any impure air being drawn in, and ensure the full amount of oxygen being taken up by the wort. It is evident that the operator must not forget to first open the cock **n**. If this is not done, there is a risk of injuring the apparatus.

As soon as the wort is ready for cooling, the perforated ring **p** is connected with a water tap and the sprinkler allowed to play against the sides of the cylinder until the temperature of the wort is reduced to about 10° C. In an ordinary fermenting cellar this takes about an hour; the further cooling must be effected by means of iced water. The air is passed through the liquid continuously, and in escaping through the bent tube carries some of the wort with it; the rousing of the wort produces a good deal of foam, but this never gives rise to contamination. The aeration must not, however, be very vigorous or there may be too great a loss of wort. It is only when the wort has cooled to about 11° C. that the foam comes through the tube; this is rendered less troublesome by introducing warm water into the vessel **o**. The wort, now ready for undergoing fermentation, is run through the pipe **k** into the fermenting cylinder.

In order to avoid rousing the wort by the aeration whilst it is passing into the fermenting cylinder, the filter may be connected with a forked tube, one limb of which is a continuation of the air-tube mentioned above, whilst the other only just passes through the top of the cylinder without coming into contact with the liquid. These two limbs must be so arranged that either can be opened or closed by a cock. The air admitted whilst the wort is being run off has, of course, to pass through the last-mentioned limb. This arrangement is not, however, essential; some of the cylinders (Fig. 12) manufactured by Mr. W. E. Jensen have this modification, whilst others are without it.

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If it is thought desirable that the wort should deposit its sediment, an hour can be allowed for this to settle. To guard against impure air being drawn in, the filter must not be completely closed, the current of air being merely checked. There is, however, no objection to the sediment remaining in the wort, which may therefore be transferred to the fermenting cylinder as soon as it is cooled. By this time a very considerable sediment will have formed, and as the mouth of the pipe *k* is at a moderate height above the bottom of the wort cylinder, only a small portion of the sediment is carried through.

The wort at first introduced should not reach above the small tube *j*, through which the yeast is introduced. The yeast is previously collected in large two-necked glass flasks, and in the transferring operation a spirit lamp may be made use of if a gas flame is not at hand. Particulars regarding the preparation of the yeast and its introduction into the cylinder will be given later on.

The stirring apparatus is now set in motion and the yeast well mixed with the wort. As soon as this is done the remainder of the wort is added until its level rises to the upper mark on the glass tube *f*, the volume then measuring about 170 liters. The column of liquid in this tube is forced by the pressure of the air passing through the filter into the cylinder, the cock on the upper horizontal tube *e* being closed, and the cock on the lower tube *l* opened. When it is not desired to continue the aeration during the fermentation, the latter cock is of course also closed, but only after the cock above the filter has been closed.

After about ten days the desired portion of the newly formed yeast can be drawn off. It is here assumed that the cylinder has been exposed to the ordinary temperature of the fermenting cellar; if the temperature has been higher, the yeast will naturally be ready for removal in a shorter time. The beer is run off at the cock *I*, and when froth appears this is closed. Some wort from the wort cylinder which by this time has been re-charged with wort for a new fermentation is now passed in until the level rises to the second mark from the bottom on the glass tube *f*. The yeast is now well stirred up by means of the stirring apparatus, and the mixture of yeast and wort is drawn off into a perfectly clean vessel (cleansed with hot water and then steamed). When the level of the liquid has sunk to the lowest mark on the glass tube, the cock is closed and wort again run in to the second mark. The yeast is again stirred up and drawn off to the lowest mark; the amount withdrawn now measures about 50 liters. The portion remaining behind is sufficient to start a new growth.

It is advisable to have two marks in the vessel into which the yeast is drawn off, one indicating 25 liters and the other 50 liters. Great accuracy is not required in these measurements.

The yeast obtained is sufficient to pitch 8 hectoliters of wort, and a new fermentation is started as soon as possible in an ordinary and well-cleaned fermenting vessel. If this cannot be done at once, the vessel containing the yeast must be covered over and set aside in a cool and clean place.

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Whilst the wort and the beer are being drawn off from the two cylinders, care must naturally be taken that sufficient air is continuously passing through the filters. Otherwise the liquids will not run freely and air will be drawn in from without. As soon as the yeast has been withdrawn from the fermenting cylinder, wort is run in until it reaches the top mark on the glass tube; the contents of the cylinder are mixed by means of the stirrer, and the new growth then commences.

In the above I have described the mode of working adopted in Old and New Carlsberg. In both of these breweries the fermentation is allowed to proceed to the end of the primary fermentation, and the sedimentary yeast is then removed and employed as pitching yeast. It is evident, however, that instead of proceeding as above, a portion of the fermenting wort may be withdrawn as soon as the yeast cells have multiplied to a sufficient extent (which, under the conditions given would take about 4.0 hours). This is a more rapid method of procedure than the former, and is adopted with good results in some breweries. ***Whether the sedimentary yeast or the fermenting wort should be made use of must be decided by local conditions and by the nature of the yeast.*** *

* Mr. Joe. Peska informs me that during his stay at Melbourne, as director of the low-fermentation brewery of the Foster Company, the pure yeast culture for a long time gave rise to various difficulties, until it occurred to him to let the yeast become very old; he accordingly took it out of the fermenting cylinder only once a month. All the earlier difficulties then disappeared, and he got a very satisfactory fermentation.

The mouths of the cocks I and h are carefully cleaned to remove the beer and wort remaining in them, as otherwise a growth of bacteria, yeast and mold may develop. For this purpose a steam-hose is best made use of, its end being fitted with a tubular nozzle so that it can be used like a spray. The warm water which first comes out after turning on the steam washes the cock, and the steam which follows will effect its sterilization. When this is done, the mouths of the cocks are closed by means of clean metal caps which are screwed over them.

Whilst fermentation is proceeding, the carbonic acid which is produced prevents any air from being drawn in, and consequently it is not necessary to pass a current of air through the vessel; fermentation and yeast production also take place satisfactorily without aeration. If, however, it is considered desirable to employ aeration in order to be able to influence the progress of the fermentation, only a small quantity of air is as a rule passed through the liquid. Its admission is regulated by the cock above the filter, so that a little air passed out through the bent tube and through the water in **d** every few minutes. The slow intermittent bubbling of the air through the wort can also be heard when the ear is placed against the cylinder. When the latter is jacketed, the current of air can only be observed by means of the water indicator **d**; it is evident that the air may also be passed over the fermenting wort through the tube **e**. With regard to the influence of aeration, no rules of general application in practice can as yet be given.

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By means of the windows *a* previously mentioned, it is possible to observe the surface of the liquid. No sufficient information can, however, be gained in this manner, and as the windows are liable to crack during steaming and may become a source of infection through not being perfectly air-tight, they are preferably omitted altogether. The arrangement of the apparatus readily permits also of the withdrawal of samples for controlling the fermentation, large samples being drawn off at *I*, and small ones at *j*. Experience, however, soon teaches how the fermentation is proceeding, and the right time for withdrawing the yeast can then be determined with sufficient precision without the previous examination of samples. We must recollect that our object is merely the cultivation of pitching yeast, and not the production of beer of a definite character. The resulting beer is, of course, not lost, but is mixed with beer in the brewery.

When the propagating apparatus was introduced into the Old Carlsberg brewery by Capt. Kühle and myself, the question at once arose in our minds whether, after long usage of the fermenting cylinder, the beer and the yeast might not gradually assimilate bitter principles as, if such were the case, a frequent cleansing of the apparatus would be necessary. It was found, however, that this did not occur, and an experience extending over several years has since fully confirmed this result. In Old Carlsberg, the fermenting cylinder is kept in use for a whole year. The whole apparatus is cleaned before it is again set working. *I would emphasize this in view of the incorrect opinions prevailing in some breweries, namely that the fermenting cylinder should be very frequently cleaned;* this is a complete mistake. There is, indeed, nothing to prevent the fermenting cylinder from being used for a much longer time than is the case in Old Carlsberg; yet, in my opinion, it is as well to clean the apparatus once a year. With proper care the yeast can be transferred to the wort cylinder, and kept there until the fermenting cylinder has been cleaned and sterilized. The arrangement of the apparatus is, in short, such that the cylinder may be cleaned at any time, and as often as required, without losing the pure yeast culture. If we have a pure yeast which works well in practice, it should be employed as long as it keeps pure, i.e. free from infection. It is a great mistake to imagine that a new pure culture should be introduced into the fermenting cylinder at least once a year. When the apparatus is handled with skill and no accident is encountered whilst working with it, the yeast will continually retain its purity. At New Carlsberg, the fermenting cylinder at one time contained a pure culture which was introduced into it more than five years previously. As soon as any infection manifests itself, the yeast will, of course, have to be renewed. As a rule, the brewer will not be in a position to carry out the necessary analysis of the yeast, and laboratories have, therefore, been appointed in which this class of work is made a specialty.

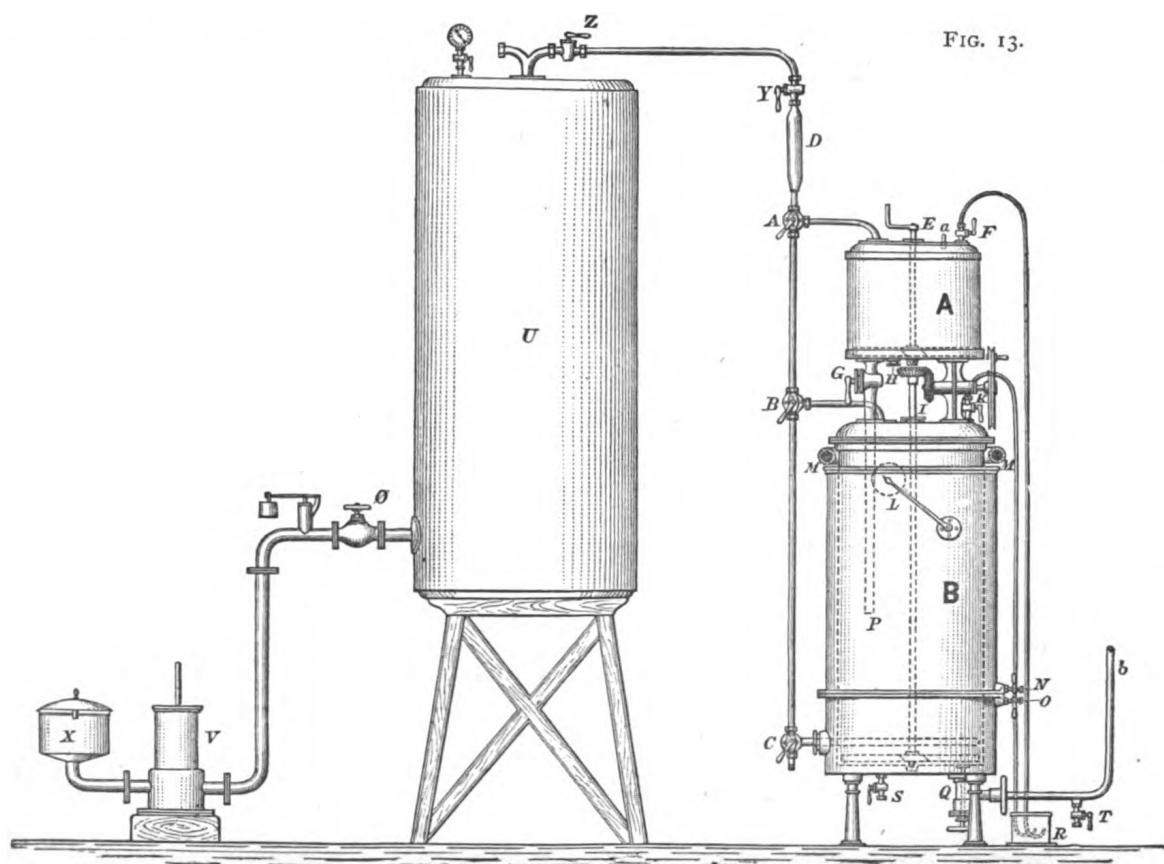
In using the apparatus, attention must be paid to two main points: (1) *that the steaming is sufficient, so that thorough sterilization is effected;* and (2) *that during the process of cooling and whilst the contents of the cylinder are being drawn off the pressure exerted by the sterile air within the latter is in excess of the external atmospheric pressure.* When these two conditions are fulfilled, no infection and no back-suction of impure air can occur. That the different operations must further be executed with care cannot be too strongly enforced. If the above instructions are closely and intelligently followed, no difficulties

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will be encountered. During the years in which the apparatus ha been in use in the fermenting cellar of Old Carlsberg, it was occasionally submitted to a thorough examination, but it was always found to be in order.

It has been mentioned that the apparatus as fitted up at New Carlsberg, requires to be modified in certain directions to suit local conditions. Another modification subsequently introduced by Dr. Elion, consists essentially in the addition of a sterilizer. In the above description it was assumed that the boiling-hot, and, therefore, sterile wort was taken from the brewery main before it reached the cooler. This is, indeed, the most practical way of charging the wort cylinder with sterile wort, and this method will, therefore, be selected even in those breweries as for example the Tuborg brewery at Copenhagen where the pipe for this purpose has to be made rather long. There are, in fact, not many breweries in which this arrangement cannot be adopted. When, however, local circumstances do not readily permit of this, the wort must be sterilized by boiling after it has been run into the cylinder, and it is then cooled and aerated in the manner previously described. Working in this manner is more troublesome, and it also takes more time, but the difficulties are not insurmountable. With the view to sterilize the wort in the cylinder, Dr. Elion has surrounded the latter with a steam-jacket. Mr. W. E. Jensen, on the other hand, employs a spiral steam pipe, which he places inside the wort cylinder.

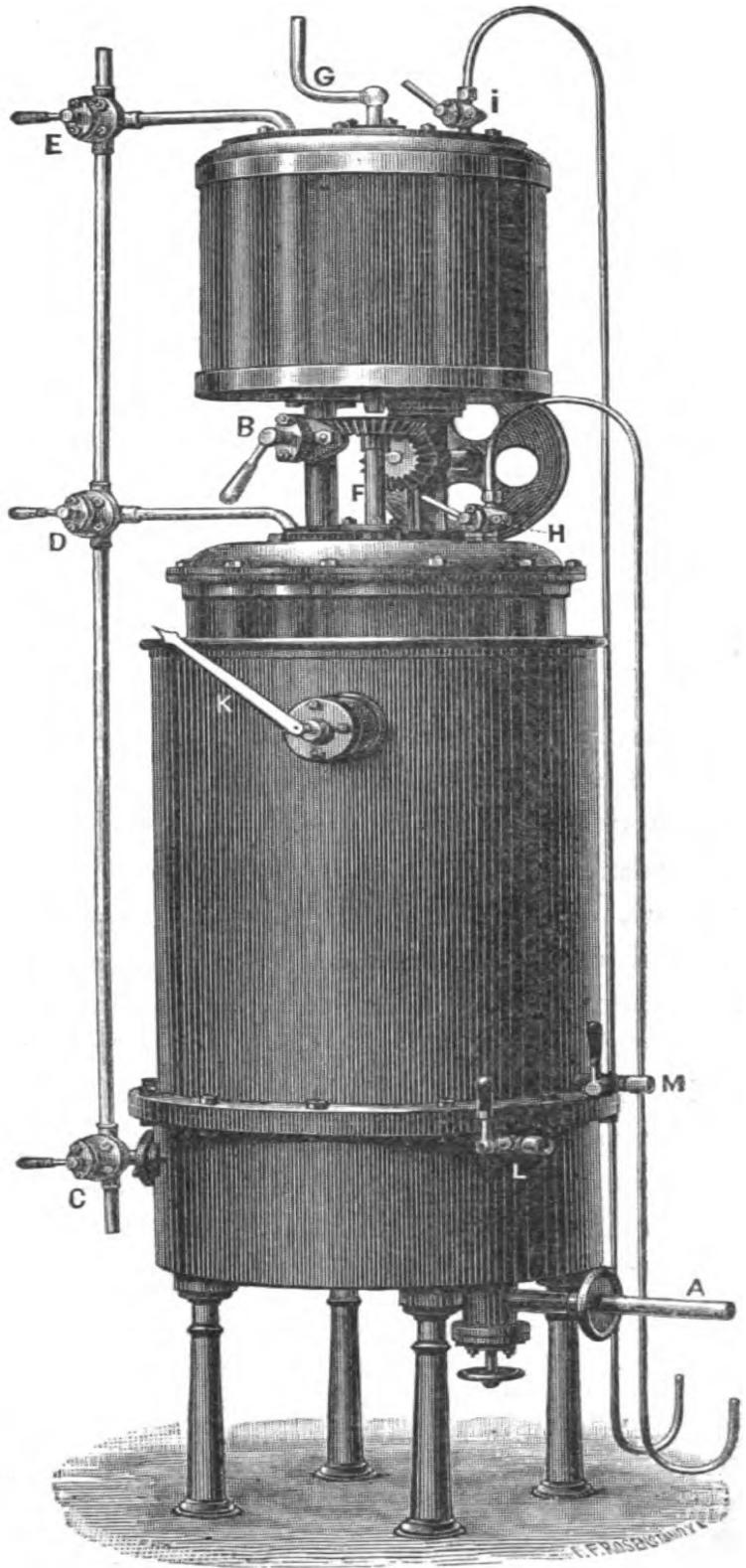
FIG. 13.



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Somewhat different from the above is the pure culture apparatus devised by Louis Marx, which, however, is so constructed as to furnish only sufficient yeast for 1 hectoliter of wort. Several other forms were subsequently described by P. Lindner, Brown and Morris, Wichmann, Thausing, the Chicago Experimental Station, and others. Velten, as long ago as 1878, had patented his so-called "Generator." For the purpose of purifying the air he employed heat in accordance with Schwann's method, and as far as can be judged from the imperfect description of his apparatus, the construction of the latter is altogether faulty. It was, indeed, never employed outside his brewery at Marseilles, and the main reason for this is that what was **most essential was wanting namely, pure yeast itself** (see p. 136).

The different forms of apparatus mentioned above are only very imperfectly known to me, and I have never tested them. On the other hand, I have had an opportunity of testing the apparatus shown in Figs. 13 and 14. It is manufactured by a Danish firm of repute (Burmeister and Wain, of Copenhagen), and I therefore recommend it to those who prefer this form. In the most essential points the two forms of apparatus are identical. The new apparatus was devised by Messrs. Bergh and Jørgensen who have obtained patent rights both at home and abroad. Fig. 13 shows the whole apparatus complete with its adjuncts. **X** is a filter through which the air passes on its way to the pump; **is the air-pump with its pipe Φ leading to the air holder **U**, and from this the pipe **Z Y** leads to the filter **D**, and**



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thence to the propagating apparatus. The filtered sterile air passes into this through the three side tubes **A**, **B** and **C**, provided with three-way cocks. (These cocks require very careful and accurate manipulation, as otherwise serious mistakes may easily be made.) The two cylinders **A** and **B** are made of copper, but with brass bottoms. **A** has a capacity of about 48 liters, and **B** a capacity of 160 liters. The former is provided with a stirrer **E**, and a tube **a** for introducing the yeast and for withdrawing small samples, this last being effected by the aid of compressed air. The bent tube **F** provides an exit for the carbonic acid gas; **G P** is a wider tube uniting the two cylinders **A** and **B**, and this connecting pipe may be opened or closed by the cock **G**. **H** is an outlet for the water used in cleaning the apparatus. The cylinder **B** is surrounded by a cast-iron jacket made in two parts; through the top passes a closed tube for introducing a thermometer into the cylinder. The upper portion of the jacket is for the cold water used in cooling the wort, and it can also be used for regulating the temperature during fermentation. The lower portion forms a steam-jacket, and is provided with two cocks **O** and **S** for the admission and outlet of the steam. **M** is a tube in the form of a ring, and provided with small holes through which cold water is passed for cooling the wort, the water passing out through **N**. The cylinder **B** has its own stirring apparatus **I** worked by toothed wheels, and is also provided with a gauge **L** consisting of a float, and an arc and pointer. (In adjusting this, care must be taken on the one hand that the packing is sufficiently tight to prevent any liquid from coming through, and on the other hand, that the pointer can turn with sufficient freedom.) From the top passes the bent tube **K**. At the bottom is a cock **Q** in connection with the pipe **b**, the latter being also provided FIG. 14. with a cock **T**. **R** is a small vessel containing water into which the ends of the bent tubes dip.

Fig. 14 shows the two cylinders in perspective, and requires no further explanation.

When the apparatus has been sterilized, the wort is introduced into the cylinder (Fig. 13) **B**, and if necessary it is then heated by passing steam through the steam-jacket. After the wort has been sufficiently aerated and then cooled, the pure yeast is introduced into the upper cylinder **A** through the tube **a**. In order to wash down the whole of the yeast, a little wort is forced by means of compressed air from **B** into **A**, and when the whole of the yeast has been brought into **B**, the cock **G** is closed. When a vigorous fermentation has set in the liquid is stirred up and a portion is forced up into **A** to be used for the next fermentation in **B**; the rest is withdrawn and used as pitching yeast. In this method the fermentation is not allowed to go so far that the greater part of the yeast becomes deposited as a firm sediment, and the same cylinder **B** is used alternately as fermenting and wort cylinder. When used for the latter purpose the yeast is, as stated, in the upper cylinder **A**. That the operations mentioned can be carried out during the different stages of the fermentation is evident, and likewise that it is possible to modify the manner of using the apparatus in several ways.

In the above, only one method has been described, which, however, as the apparatus is constructed, may certainly be regarded as practical in most cases.

With regard to the employment of the apparatus, the reader is referred for further particulars to the directions given on pp. 49-58, and especially all that was stated with regard to the

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filtration of the air, the sterilization of the cylinder, and the aeration and cooling of the wort fully applies also to the new apparatus.

For high fermentation, Kokosinski, Wilson and Jensen, have introduced some slight modifications in the Old Carlsberg fermenting cylinder, in that they have made it somewhat higher, and have arranged for the wort to enter at the upper portion of the cylinder, within which is fixed a pipe in the form of a ring and provided with small holes; the wort passing out of these holes washes down the sides of the cylinder, so that any yeast or froth which may have collected from the previous fermentation is carried down into the fermenting wort, and permanent accumulations are thus prevented. *

* Captain Kühle and I have not patented our apparatus, and anyone is, therefore, at liberty to make it. To those who wish to use it, however, we recommend the cylinders made by Mr. W. E. Jensen, of Copenhagen, as he has taken part in the construction of the apparatus, and is thoroughly familiar with it. Below is given Mr. Jensen's price list:

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	<i>£ s. d.</i>
Wort cylinder, with water-cap and ring-sprinkler, complete (Fig. 10, D)	38 10 0
Wort cylinder as above, but with shallow vessel in place of water-cap	27 10 0
Fermenting cylinder with water-cap, complete (Fig. 10, C)	41 5 0
Fermenting cylinder as above, but without water-cap ..	30 5 0
Spiral tube described for sterilising wort	3 5 0
Larger fermenting cylinder for <i>high fermentation</i> , with water-cap, &c.	44 5 0
Ditto, without water-cap	33 5 0
Packing and insurance	2 0 0

The cylinders are all made with the cover shown in Fig. 10; they are made of tinned copper, and parts are nickelated.

The prices charged by Messrs. Burmeister and Wain, of Copenhagen, are as follows:—

The apparatus devised by Capt. Kühle and myself.

	<i>£ s. d.</i>
Wort cylinder, with water-cap and ring-sprinkler, complete (Fig. 10, D)	42 0 0
Fermenting cylinder, with water-cap, complete (Fig. 10, C)	47 0 0

The new apparatus of Messrs. Bergh and Jörgensen.

(See Figs. 13 and 14, and the description given) 75 0 0

The apparatuses supplied by Messrs. Burmeister and Wain are tested by Mr. Jörgensen.

The above prices are valid, February 1895.

The air-pump and air-holder can be obtained anywhere. A suitable air-pump costs about £22; an air-holder with a capacity of $\frac{1}{2}$ cubic meter and tested to five atmospheres, costs about £28.

To prevent misunderstanding, it may be pointed out that neither Capt. Kühle nor myself have any control over the manufacture of the above apparatus, and therefore no responsibility rests with us. Neither can we undertake to answer any questions, or to execute any orders for the apparatus. It is requested that *all* enquiries should be sent direct to the manufacturer with whom it is intended to deal.

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The pure yeast propagating apparatus is now used in more than 160 of the largest and most important breweries, and it has recently been introduced into some distilleries. Just as the reform has spread with enormous rapidity, so the time will soon come when a highly contaminated brewery yeast will be uncommon. A thorough comprehension of the employment of the propagating apparatus will constitute an active means towards this end. On pp. 234, 240 and 252 will be found a list of the breweries, distilleries, &c., where the pure yeast propagating apparatus has been introduced.

It must not be forgotten, however, that neither this apparatus nor that which as my system becomes more and more widely adopted is now taking the place of the old cooler, plays more than a secondary part in my system; they do not constitute, and they can never become the essentials of the system, and it is a gross mistake to regard them as such. ***The absolutely pure culture of the systematically selected species of yeast is, and always remains, the main point, and it is this only which give: importance to these forms of apparatus.*** Good results are obtained when pure yeast is used in accordance with my old method, but not when impure or badly selected yeast is cultivated in the apparatus.

FILTERS.

We should attain our object more quickly and simply if it were possible to employ the cooled and aerated wort from the cooler, and by filtration to purify it from the microorganisms which it always contains. The wort cylinder would then be superfluous, and the whole process would be simplified. Experiments which were made in this direction did not, however, give any satisfactory result.

The filter to be employed for this purpose must not only satisfy the condition that the filtrate shall be absolutely free from germs, but also the time required for the filtration must not be too great, and finally, the composition of the liquid must not be materially altered by the filtration. The Chamberland filter, according to data to hand, best fulfills these conditions, and the new model which was introduced in 1886 was, therefore, selected for the experiments. My assistant, Mr. Poulsen, carried out the experiments under my supervision, and the following results were obtained: A filter composed of five tubes delivered 10 liters of clear hopped wort (13.5 per cent. Ball.) in 2 1/2 hours, when the suction was 6/7 atmosphere and the filter tubes were cleaned externally by brushing them every ten minutes. In order to obtain a hectoliter of wort in the same time, so tubes would consequently be required. Under these circumstances the cleaning would be a somewhat difficult matter, and it might easily happen that first one and then another of so many fragile tubes would get a knock, resulting in larger or smaller cracks. In such a case, the wort entering the fermenting cylinder would be infected, and the operator would be deceived. The most serious fault, however, was that during filtration the wort lost half of the oxygen which it had taken up during aeration on the cooler, and which is necessary for a normal fermentation.

It is, therefore, not advantageous to employ filtration for sterilizing the wort for the fermenting

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cylinder. In the course of these experiments, however, I perceived that the Chamberland filters would be of great service in preparing different sterile liquids for laboratory experiments. If an absolutely germ-free filtrate is required, the tubes must be sterilized at short intervals; they must not be kept in action for an indefinite period, as was formerly imagined, for the bacteria pass through the walls after a longer or shorter period.

We have already learned that the Chamberland filters yield sterile liquids; and it is, therefore, also evident that they will yield sterile air. Difficult as it is to obtain sterile liquids by filtration, so it is equally easy to obtain germ-free air. This fact has long been known, and the cotton-wool filters employed by Schröder and Dusch (1854) in their famous experiments in connection with spontaneous generation have, as is well known, led to an extended and varied application in practice. In order to determine the most practical form of cotton-wool filter for the propagating apparatus already described, I requested my assistant, Mr. Poulsen, to carry out a Series of Experiments (1887), and these gave the following results: When it was found that metal tubes like those shown in Figs. 8 and 10 (g and m) were suitable in form and size, the experiments were made with them. As previously stated, these tubes will hold a column of cotton-wool 22 cm. long and 3 cm. in diameter. At the lower end there is a short tube about 1 cm. in diameter. The other end is open, but is provided with a mouth-piece, which screws on to it and terminates above in a short tube. The cotton-wool is introduced in small portions, and is rammed tight with the help of a cylindrical rod. Before the mouth-piece is screwed on, some cotton-wool is put into it to catch the coarser impurities. It is important to note that no cotton-wool must be pressed into the tube of the mouth-piece. The air is led through this into the filter; the opposite end, through which it passes out, is closed with a tight plug of cotton-wool before the filter is sterilized. The sterilization is readily effected by heating the filter for two hours at about 150° C. in an ordinary sterilizing oven. As there might be some doubt, from statements made by Klein, whether such cotton-wool filters become sterilized right through to the middle, experiments were also made to decide this point. They proved that the middle portions of the filter were sterile. In the trials with a filter sterilized as above, a considerable volume of air was forced through under a pressure of 3-4 atmospheres, and then passed into flasks containing sterilized yeast-water, a liquid very favorable to the development of bacteria. The flasks were exposed to a temperature of 30° C. for at least fourteen days. In order to test for mold and yeast fungi, similar experiments were made with sterilized beer-wort. In all cases the experiments were so conducted that had any germs been present in the air they must have been taken up by the liquid. In addition to this, portions of the cotton-wool from the upper end of the tube were afterwards introduced a quickly as possible, and with the necessary precautions, into flasks containing the sterilized liquids mentioned. As was to be expected, it was found that the latter always contained living organisms. This was also the case with the air passing through the filter if this were too loosely packed and contained only 25 grams or less cotton-wool; when, on the other hand, the filter was more tightly packed, and contained 35 grams or more cotton-wool, the air after being forced through, even under great pressure, was always found to have been freed from all germs and to be perfectly sterile. With force, 50 grams of cotton-wool can be pressed into the tube with moderate ease, but this quantity is not necessary. In the above experiments, 16 liters of air were on the average passed through the liquid in each flask.

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For its employment in practice it is important to ascertain how often the filter must be sterilized. It might, for instance, be assumed that the micro-organisms retained by the cotton-wool at the upper end might be able to multiply, and if the filter became moist, to penetrate through the cotton-wool and thus render the filter useless. In order to determine whether this were so, some of the filters used in the earlier experiments, and which contained micro-organisms at their upper ends, were set aside in the laboratory for six months. They were then tested in the same manner as formerly, with the result that they were always found to yield sterile air. Tests of a more severe character were then made as follows: The cotton-wool in the cover of the filter was dipped in nutrient liquids containing vigorous growths of bacteria, yeasts and *Penicillium glaucum*. Air was then forced through this infected cotton-wool under a pressure of 3 atmospheres, and for a period of two hours, the test flasks used being of the same nature as previously. This **moist** and **strongly infected filter** was set aside in the laboratory for three weeks and then tested as before. The result was the same in both cases: the air passing through the filter carried with it none of the micro-organisms present in the upper portion of the cotton-wool it was, in fact, sterile. In agreement with this, it was also found that cotton-wool taken from the lower portion of the filter yielded no growth either in yeast-water or in wort; on the other hand, when the samples were taken from the upper layer, vigorous growths were obtained.

It follows from these analyses that such cotton-wool filters can be used for several months without being freshly sterilized; no definite period applicable in all cases can be given, but it is advisable to re-sterilize the filters occasionally. Neither must it be forgotten that the filter of the wort cylinder, when detached and put away for future use, should be immediately plugged at its lower end with sterile cotton-wool. Before it is again used, the plug must be withdrawn and the mouth of the tube passed through a flame. It is also advisable to occasionally remove the cover, and to replace the upper contaminated layer by fresh sterilized cotton-wool.

It must not, however, be concluded from the above experiments that liquids can be sterilized by means of these filters. It has, in fact, already been mentioned that this is not the case. When the cotton-wool becomes saturated with liquid, it loses its property of sterilizing. It is, therefore, important that the pipe between the air-holder and the filters should contain no water.

The last Series of Experiments which Poulsen carried out at my suggestion had for their object the determination of the different quantities of air which pass through the filter in an hour, and under varying pressure. For measuring the volume of air passing, a very accurate gas-meter was employed. By way of comparison, a Chamberland filter, a porcelain tube marked with the letter F, was also examined. The results obtained were as follows:

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I. Chamberland filter with one tube—

At $1-1\frac{1}{2}$ atm. pressure, 20 cubic feet passed in one hour.

„	$\frac{1}{2}-\frac{3}{4}$	„	14	„
„	about $\frac{1}{2}$	„	10	„
„	„ $\frac{1}{2}$	„	5	„

II. Filter with 50 grams cotton-wool (about .32 gram per cc.)—

At 3 atm. pressure, 120 cubic feet passed in one hour.

„	I	„	15	„
„	$\frac{1}{2}-1$	„	14	„
„	$\frac{1}{3}-\frac{1}{2}$	„	10	„
„	about $\frac{1}{2}$	„	about 7	„

III. Filter with 35 grams cotton-wool (about .22 gram per cc.)—

At $\frac{1}{2}$ atm. pressure 27 cubic feet passed in one hour.

„	$\frac{1}{2}$	„	19	„
„	$\frac{1}{4}$	„	12	„

The reason that the above table does not contain more results is that the measuring apparatus was only for a short time at Mr. Poulsen's disposal. The numbers obtained, however, sufficiently indicate the capabilities of the two kinds of filters. On comparing these numbers it is seen that the Chamberland filter and the filter containing so grams of cotton-wool are about equal in their filtering capacity, but that the filter containing 35 grams of cotton-wool considerably excels them both in this respect. These experiments were, of course, all carried out with the same measuring apparatus.

The air filters described above are not only used in connection with the pure yeast propagating apparatus, but also in the centrifugal apparatus of Bergh, and in Ritter's apparatus for collecting the yeast. In a slightly modified form they are also used in the Old Carlsberg apparatus, and in the similar closed vessels which have been introduced during the last few years in the place of the open coolers.

THE INTRODUCTION OF TIRE YEAST INTO THE PROPAGATING APPARATUS, AND ITS TRANSPORT.

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The yeast which is to be employed to produce a normal fermentation of the wort (about 170 liters) contained in the fermenting cylinder, is grown in the four metal vessels (Figs. 5 or 6) previously described. When as much sedimentary yeast as possible has been produced in these in the manner described, all the beer is drawn off. From half to two-thirds of a liter of sterilized water is then run into each vessel, and these are then shaken to loosen the yeast. The yeast thus diluted is now transferred to sterilized two-necked glass flasks of a liter capacity. Four of these will rarely be sufficient, and, as a rule, six will be required. Their contents are finally transferred to the fermenting cylinder through the tube *j*, Figs. 8 and 10, or *a*, Fig. 13. All these operations must, of course, be conducted in such a manner that the pure culture receives no contamination.

It is not possible to transfer the yeast direct from the metal vessels if the tube *j*, in Figs. 8 and 10, through which it is introduced is in the side of the cylinder. The position of this tube was selected with a view to render it possible to withdraw samples into sterilized flasks so that the culture in the apparatus may be tested with reference to its purity or otherwise whenever it is thought desirable.*

* In Bergh and Jörgensen's apparatus (Fig. 13a) the construction is different, and the above does not apply

In order to be able to analyze the yeast and to prepare the pure culture, some special knowledge and great experience are necessary, and this can only be acquired by long continued work in a properly equipped laboratory. My different treatises on this subject, and especially the present work, will be of assistance. Their object, with reference to the practical man, is to point out to him the importance of the new reform, so that he may be induced to introduce it in a rational manner into his brewery. If, however, this treatise is written as I desired, it will convince him not only that he himself is not in a position to carry out these difficult operations, but that he must have the assistance of a specialist.

It is now not difficult to obtain the necessary assistance. Some breweries have fitted up laboratories of their own; but usually assistance is obtained from independent institutions, and in most cases this is perhaps preferable. My methods are carried out in the following laboratories: A. Jörgensen laboratory, Frydendalsvej, Copenhagen; Detlefsen and Meyer's laboratory, Copenhagen; Hiepe and Miller's laboratory in Manchester; Ancker and Bergh's zymotechnic laboratory, Stockholm; the Versuchsstationen in Weihenstephan, Berlin, Munich, Nuremberg, Augsburg, Hohenheim, Prague, Odessa, and Vienna; the practical school of brewing at Munich; Doemen's Brewing Academy at Munich; the school of brewing at Worms; Ehrich's Versuchsstation for brewing at Worms; Eckenroth's laboratory at Ludwigshafen on the Rhine; Kokosinski's laboratory at Lille; Laboratoire de Brasserie de la Faculté des Sciences at Nancy; Wahl and Henius' laboratory at Chicago and Brewing Academy at Milwaukee. There are, perhaps, several others unknown to me, and my omissions must, therefore, not be regarded as being due to want of consideration on my part. In addition to those mentioned above, some laboratories and institutes have recently been established, most of them in Germany, which deal especially with the requirements of the distillery,

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pressed yeast, and wine industries. I would ask those firms which desire aid in the direction mentioned to apply to the institutions and laboratories named. ***The Carlsberg laboratory, as a scientific institution, is engaged in other directions, and cannot undertake this work.***

I will also take this opportunity to state that, for this reason, I can only exceptionally receive pupils, and ***these must have had previous scientific training (physiologists, botanists, chemists).*** At the Carlsberg laboratory no fees are taken. Correspondents who are unknown to me are requested to enclose testimonials with their applications. Popular elementary courses are held at Mr. Jørgensen's laboratory, at Hiepe and Miller's laboratory, and in most of the laboratories and institutions named above. For the more advanced students opportunity will also be afforded for research work in these laboratories.

Those who wish to obtain the No. 1 yeast used at the Old Carlsberg brewery should apply to Captain Kühle.

The brewer must himself take charge of his apparatus, and if there is no zymotechnic laboratory in the neighborhood, he will, as a rule, also have to introduce the yeast into the fermenting cylinder. He will often be compelled to procure his yeast from a distance, and, having regard to this, I have elaborated the following method. The object I had in view was that the transport of the yeast might be effected with the greatest security possible, and yet that everything should be easily carried out; the brewer should have nothing more to do than to shake the flask containing yeast, and then connect it with the small tube *j* at the side of the fermenting cylinder (Figs. 8 and 10) or with the tube *a* shown in Fig. 13. The whole of the yeast is then easily poured into the latter. For these reasons I employ a strong, but moderately small flask constructed in accordance with the following description:

It has a capacity of 1.5 - 2 liters, is made of thick glass, and has a flat bottom (Fig. 15), so that no support is required. Fig. 16 shows a longitudinal section of the straight tube; *c* is a glass tube, the end of which is provided with a slightly raised collar; *b* is a tightly fitting rubber stopper; *a* is a stout India rubber cap stretched over *c*, and bound at *d* with copper wire (the binding is shown in Fig. 15). The rubber stopper (Fig. 16, *b*) must not only fit very accurately, but it should also be moderately easily withdrawn when the cap *a* is removed. An India rubber tube (Fig. 15, *c*) fitted at its lower end to *a*. glass tube *c* is fixed to the bent neck of the flask *d*. The rubber tube *b* must be strong, and should be bound with wire over the two glass tubes; near the mouth of the bent neck is a pinch-cock, by means of which

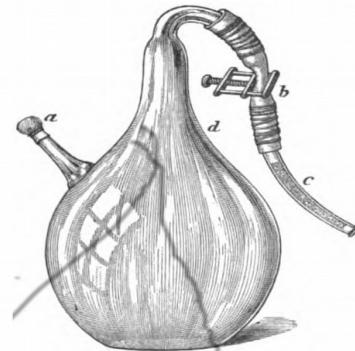


FIG. 15.

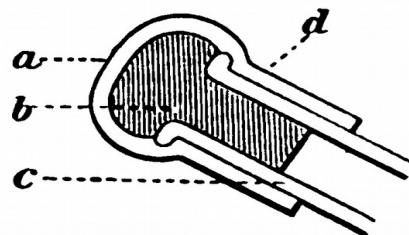


FIG. 16.

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the rubber can be completely closed. The glass tube **b**, which becomes somewhat narrower towards its lower end, contains a cotton-wool filter.

The cotton-wool pressed into this tube should not be in excess of that required to prevent atmospheric germs from entering the flask whilst its contents are being poured out through the straight tube **a**, Fig. 15. The object of the filter is to obviate the use of a flame. The parts made of rubber are sterilized by boiling in water or by heating in steam, the rest by heating for two hours at about 150° C., the ends of the two tubes being first plugged with cotton-wool. As the flask is made of thick glass, it is necessary to be very cautious in heating it; it should be placed upon a cork block, which, again, rests on a sheet of asbestos, and care must be taken that there is no great difference in the temperature at the top and bottom of the sterilizing oven. When cold, the cotton-wool plug is removed from **b**, Fig. 15, and the rubber tube, pinch-cock, and filter-tube are joined together as quickly as possible.

Into this flask is introduced the yeast from one of the 10-liter metal vessels (Fig. 5 or 6), and sterilized water is then added until the flask is about three-quarters filled. Finally, the stopper **b** (Fig. 16) is inserted, the cap a placed over it and bound with wire at **d**, and the rubber tube closed by means of the pinch-cock. The flask is now ready to be packed, and this must be done in such a manner that, when unpacked, the flask must present a sterile surface; this can be effected by wrapping the flask in cotton-wool, or other substances, which has been previously heated for a few hours at 150° C. Full and distinct instructions should be sent from the laboratory from which the yeast is forwarded, and the most important directions should be specially emphasized.

At my request, Mr. Jörgensen kindly tested this method by sending some samples abroad from his laboratory at Copenhagen, and in all cases a favorable result was obtained. A metal flask has, of course, greater strength, but by the employment of such a vessel we lose the special advantages possessed by the glass flask.

The yeast in such a flask is sufficient to start the fermentation of 50 liters of wort. This can be measured with sufficient accuracy by means of the vertical glass tube **f**, Figs. 8 and 10. If the temperature of the room in which the fermenting cylinder has been placed is about 8° C., fresh wort may be added after 4 to 5 days until it reaches the highest mark on the glass tube **f**, but if the development proceeds slowly, more time should be allowed.

For sending pure cultivated yeast in an absolutely pure condition and in such a manner that its multiplication can be effected easily and with certainty, I have likewise made use of the following method, which has also given good results: To the small cylindrical flasks generally known as Freudenberg flasks, I have added a side-tube (see Fig. 17). The tube **a** on the hood is, as usual, filled with cotton-wool; a firm layer of cotton-wool is placed at the bottom **e** of the flask, and a plug is inserted in the neck **b**. The side-tube is also plugged with cotton-wool, and the flask is then sterilized by heating it for two hours at 150° C. When it has cooled, the tube is joined to the rubber of a two-necked flask in which the yeast has been grown, and a drop of the fairly thick yeast is poured on to the layer of cotton-wool **e**. The tube is then

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closed by a stopper **d** of asbestos card previously sterilized in a flame, and the stopper is then coated over with a layer of sealing-wax **c**.

The main point which I had in view in this arrangement was to preserve the yeast from infection without hermetically closing the flask. The layer of cotton-wool **e** must therefore be firmly pressed to the bottom, so that it will remain in its place, and for this reason also no more liquid should be poured in with the yeast than is absolutely necessary. Should a little of the liquid, however, run out of the cotton-wool when the flask is turned upside down, it would not escape, as the side-tube is perfectly closed, and it could not pass the neck **b**, but would be absorbed by the cotton-wool. When, subsequently, the yeast is going to be used, the sealing-wax **c** must be scraped off, and the side-tube and the whole surface of the flask, passed through a flame; the asbestos stopper is either pushed into the flask or withdrawn from it by means of a pair of forceps, and the tube is then introduced as quickly as possible into fl ~ the rubber tube of a Pasteur flask containing nutrient liquid. If we do not wish to use this flask, the sterile nutrient liquid can very easily be introduced through the side-tube by means of a pipette. In the place of the cotton-wool we may also have a layer of gelatin; but as it is difficult to sterilize this substance without running the risk of its losing its property of again solidifying, and as other disadvantages are also met with, I have always preferred cotton-wool.

The method just described has become of great practical importance in affording an excellent means of sending pure yeast cultures to the tropics. It has been employed with success by Jörgensen in sending samples of yeast from Copenhagen to South America, Asia and Australia Grönlund has also used it successfully for sending yeast from Copenhagen to Ecuador. It is important that the flasks should be kept in a dry place, as otherwise molds will readily grow through the cotton-wool plug at **a**; it is evident, therefore, that this should be perfectly dry after sterilization. If it is only a question of a short time e.g. a few months, the tube may, perhaps, be closed with sealing-wax, and in that case it will be perfectly secure.

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CHAPTER II. Researches on yeasts.

1. Characteristics of the *Saccharomyces*.

THE method of pure yeast culture described in the last chapter is founded upon the view that the *Saccharomycetes* occur as definite species, and that the characters which I discovered are suitable for distinguishing them. Should these organisms readily change, one into the other, and the boundary lines thus disappear, as some investigators are inclined to assume, my investigations would, as regards their application to practice, lose most of their significance. This was, therefore, the point upon which my opponents based one of their strongest attacks. The following account deals mainly with brewery yeasts, but in order to make it intelligible, I have had to include also some sort of survey of my most important yeast studies.

It is evident that a systematic examination of the yeasts must be of an experimental nature, and that it must start with endospore formation; thus it was from this point of view that I commenced my studies in this field. These not only proved that there are different species of the *Saccharomycetes*, but they also gave the first distinguishing characters for them. It was found, namely, that the temperature curves for the development of spores have in the main the same form, but that the cardinal points, especially those representing the maximum and minimum temperatures, afford definite characteristics. It was also found that there were differences in the behavior of the species towards different media at various temperatures; for instance, it was shown that the species when heated in distilled water perished at different temperatures. Differences were also found with respect to bud-formation, fermentation activity, film-formation, &c.

When yeasts are cultivated under identical conditions, the forms of the cells may afford characters for their division into groups, and sometimes also species; this applies both to sedimentary growths and to film-forms, and not only to cultures grown in liquid media, but likewise to cultures on solid substrata. It is true that almost all *Saccharomycetes* can assume the same forms, and that at least most, if not all species can in the course of their development assume all the forms mentioned by Reess as characterizing different species. Nevertheless, the same forms do not occur under the same conditions in the case of the different species. Therefore the character does not rest merely with the form alone, as assumed by Reess and his followers, but is at the same time dependent upon external circumstances.

There are also distinct differences in the behavior of the yeasts towards the carbohydrates, especially towards maltose, and in the chemical changes which they bring about in nutrient liquids. In connection with this, the fact may be mentioned that whilst some species can be made use of in the fermentation industry, others cannot, and some others even produce diseases in beer.

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Differences, although only of a slight nature, were also found when different stainings were applied.

Of greater importance, at any rate as regards the practical analysis, is the difference which, under certain conditions of culture, is noticeable in the contents of the plasma of the spores of culture yeasts compared with that of the spores of wild yeasts. If we confine ourselves merely to the microscopic appearance, the difference noticeable is that the spores of culture yeasts contain a less dense and less refractive plasma: as a rule they contain vacuoles, and often have the appearance of being empty, in which case their walls are sharply defined. The spores of the wild yeasts, on the other hand, are completely filled with a uniform, strongly refractive plasma. A short time after I had drawn attention to this in the lecture mentioned below, communications reached me from other investigators, who stated that they had made similar observations. It was found, however, as is usually the case when a question is submitted to a thorough experimental examination, that the subject was more complicated than at first appeared, and that a long series of studies is necessary for its solution. Apart from the difficulties which the microscopic examination itself offers when this is intended to give definite information with regard to the said structure, the matter is further complicated in other respects. Namely, low-fermentation brewery yeasts can also, under certain conditions, give bright spores filled with a dense plasma, and inversely, the wild yeasts can give spores having a structure like that mentioned as belonging to the culture yeasts. Whilst discussing the differences between species, it will be of interest here to point out that whilst some species e.g. *Saccharomyces Pastorianus I.* can very easily be made to yield spores having the appearance of being empty, this is, on the other hand, more difficult in the case of others e.g. *Saccharomyces ellipsoideus II.* That there is a peculiar condition of structure which has its practical significance in the analysis of beer yeast is evident from all investigations which I have hitherto carried out.

Whilst the spores of most species are round, oval or kidney-shaped, those of others are, on the other hand, hat shaped. There are, likewise, morphological differences noticeable with reference to their germination, but these only seldom occur. Differences between the species may also be observed with regard to the budding-systems which precede the development of the spores.*

* The above investigations were published in "*Compte-rendu des travaux du laboratoire de Carlsberg.*" Copenhagen, 1882, 1883, 1886, 1888 and 1891. A German translation of the French résumés was given by the editor of the "*Zeitschr. f. d. ges. Brauwesen,*" and appeared in that journal for the corresponding years. The behavior of yeast cells on solid media, and the above mentioned difference in the structure of spores were discussed in my lecture delivered at Cut: on June 12, 1887 (see "*Zeitschr. f. Bierbrauerei,*" Vienna, 1887, p. 518, and "*Centralbl. f. Bacteriologie und Parasitenkunde,*" 1887, ii. p. 118).

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The terms "culture yeast," "culture species," "brewery yeast," &c., do not imply that these species have been produced by cultivation for as yet nothing is known with regard to this but merely that they are employed in the industry. All other **Saccharomyces** are, on the contrary, spoken of as "wild yeasts," "wild species." So far as experiments indicate, we may assume that not only the so-called wild yeasts, but also the culture yeasts occur in nature.

It is self-evident that species cannot, in all cases, be distinguished from one another by means of one of the characters mentioned, but that several characters must frequently be made use of for this purpose. The characters afforded by the development of the spores are of especial importance; I have made use of these as the foundation of my method for the analysis of low-fermentation brewery yeast, and the more so because they enable us without first preparing a pure culture to make a direct analysis when it is a question of determining whether disease yeasts are present or not.* This analytical method is rendered still more sensitive by the employment of tartaric acid, as described on p. 151.

* See Just. Chr. Holm et S. V. Poulsen: "Jusqu'à quelle limite peut-on, par la méthode de M. Hansen, consister une infection de "levure sauvage" dans une masse de levure basse de *Saccharomyces cerevisiae*? **"Compte-rendu des travaux du laboratoire de Carlsberg, Copenague,"** vol. ii. livr. iv. 1886, et livr. v. 1888. A German translation appeared in the **"Zeitschr. f. d. ges. Brauwesen,"** for the corresponding years

A series of **Saccharomyces** has thus been found which are differently affected by external influences. That the observed characters are not quite of the same kind as those with which we are acquainted in the case of the higher organisms, is not to be wondered at when we bear in mind that the organisms under discussion consist of only a single cell. If we are to regard them, however, as species, the differences found should be constant. In order to ascertain how far this is the case, it again became necessary to make special experiments, and to expose the cells, which for the time being we have assumed to represent different species, to different conditions for some length of time, partly each separately in the form of a pure culture and partly several mixed together, and therefore under the influence of competition. In the course of the last twelve years I have carried out a large number of such systematic experiments, especially with the six species which I described in 1883, and subsequently also with some others, including also brewery yeasts. The results obtained showed that it was comparatively easy to produce temporary, and in some respects even great variations, but by suitable cultivation these again disappeared, and the respective species returned to their original condition; new species or varieties could only be obtained by a certain treatment continued for some length of time; some particulars concerning this will be given subsequently. It is of practical interest that species which were cultivated uninterruptedly for several years in wort were subject to only slight modification; this has been confirmed not only by laboratory experiments, but also by an experience extending over nearly eleven years in different breweries in which pure yeast culture has been thoroughly carried out. In short, my experience indicates that we have as much right to assume the

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existence of species in the case of these lower fungi as in that of the higher.*

* It has quite recently been pointed out by Takamine that the *Aspergillus Orza* employed in the preparation of Japanese sake develops yeast-cells which produce a very vigorous alcoholic fermentation. Juhler obtained the same result with his cultures, and he states further that these yeast cells formed endospores, and that in their properties they agreed with the *Saccharomyces*. Juhler's statements led to the revival of the view held by Bail, Hoffman and others nearly fifty years ago, and which has ever since had some advocates namely, that the *Saccharomyces* originate from the ordinary mold fungi. The correctness of Juhler's observations was confirmed by Jörgensen, who afterwards stated that he had made similar observations in the case of other species of Arptrgillur, and also in the case of Dematium ("Centralbl. f. Bakteriologie," Zweite Abt. 1895, pp. 16, 65, 321, and 326). Klöcker and Schiönnung repeated the experiments with *aspergillus oryzae*, but without being able to observe any development of yeast cells. The whole question must at present still be regarded as an open one.

These investigations have, of course, hitherto had no effect with reference to the systematic analysis of yeast. For this it is also necessary to make experiments with the yeast cells themselves, and in such a way that we may be able to identify the corresponding parent form in every case. I have frequently laid stress upon the importance of experiments of this kind, and several years ago I made the first positive contributions in this direction in that I proved that typical *Saccharomycetes* can develop a mycelium with distinct septa, and also form: similar to Oidium and Dematium (see my figures given in "Zopf's Handbuch der Pilze," 1890, p. 703; also the plates in "Compte-rendu des travaux du laboratoire de Carlsberg," 1886).

It is necessary to refer to these results here, since, as mentioned above, they form the groundwork of my practical studies.

2. High and low yeasts

As is known, widely different views prevail as to whether the high and low yeast of breweries consist of one or several species. Reess distinctly expresses the opinion that they constitute two varieties of the same species *Saccharomyces cerevisiae* and that the one can be transformed into the other; he especially emphasizes that high ale-yeast becomes transformed into a typical low yeast after a few days' cultivation in wort at 4 - 6° C.

Pasteur, as previously pointed out, takes no definite standpoint on the questions relating to the *Saccharomycetes*; he confines himself to the discussion of different possibilities; nevertheless, he is in the main inclined to assume that low brewery yeast can be readily

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transformed into high yeast, and that this transformation likewise occurs even in breweries.

Other writers have also occupied themselves with this question, but no conclusive experiments have been made; true pure cultures were not made use of, and in most cases it was not even ascertained whether the yeasts employed belonged to the *Saccharomycetes*: or not. The question must, in fact, formerly have been a difficult one to treat, when we remember that the ordinary low-fermentation yeast of breweries frequently contains high yeasts and *vice versa*. Under these circumstances, the conclusions drawn from observations made in the brewery itself are naturally valueless. At most, different possibilities could be suggested, and in the literature of the subject, and even in an important work like Pasteur's "*Études sur la bière*," we thus find contradictory views expressed without any possibility of ascertaining which is correct.

Since the commencement of 1884 I have carried out systematic experiments bearing on these questions. Absolutely pure cultures were made use of in all cases, and these were grown in Pasteur flasks containing sterile wort. The low-fermentation yeasts with which I experimented were *Saccharomyces Pastorianus I.*, *Saccharomyces ellipsoideus I.*, *Saccharomyces ellipsoideus II.*, *Carlsberg bottom yeasts* No. 1 and No. 2, and also some other low-fermentation yeasts which had been tested in practice. The experiments were made at the ordinary room temperature, and the wort was frequently renewed, so that numerous generations were produced at the temperature employed in high fermentation, and at intervals the cultures were grown at a still higher temperature namely, 25° C. high fermentation phenomena did not, however, manifest themselves, and the forms of the low-fermentation yeasts remained constant; in the case of some species these experiments were continued over eleven years.

Similarly, since the beginning of 1884, I have cultivated at the low-fermentation temperature 5° C. two yeasts *Saccharomyces cerevisiae*. and *Saccharomyces Pastorianus III.* both of which exhibit high fermentation phenomena in a high degree. In these experiments the nutrient liquid was renewed every fortnight. So long as the flasks were exposed to the low temperature mentioned, the fermentation was very feeble, especially in the case of the first-named yeast, and there were, therefore, no indications of high fermentation phenomena; these appeared, however, as soon as the cultivation was carried out at the ordinary room-temperature, or at 25° C., and the yeasts always behaved in the same manner, even when they were examined the last time after 10 years' cultivation.

When it is desired to revive an enfeebled high yeast so that it will again manifest high fermentation phenomena, this can be more readily effected when, in addition to growing it at a favorable temperature, the liquid medium is also vigorously aerated.

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For the reasons stated above, the experiments just described are the only ones hitherto carried out which afford any proof, and the results which they have yielded show that the transformation of high into low yeasts and vice versa, cannot, as some believe, be brought about under the conditions mentioned. That the low-fermentation yeasts cannot, as Pasteur appears to assume, develop forms of high-fermentation yeast by means of their film growths, has been proved in my treatise on film-formation in the **Saccharomycetes**. In this treatise I have also stated how, after a certain treatment, low fermentation yeasts are able to exhibit high fermentation phenomena during a few fermentations, after which they resume their normal properties. We can bring about temporary changes, but as yet we cannot effect permanent transformations. Whether we might, however, attain this by varying the experiments, and exposing the cells to the same action for a longer period than in the experiments described above, is another question; facts alone are dealt with here, and these, at all events, show that such transformations cannot, within a measurable time, take place in the brewery. The only explanation that can be offered with reference to the experiments of Reess and Pasteur is, that these investigators must have been dealing with mixtures of high and low yeasts, which may easily have been the case, considering the time when their experiments were made.

The high fermentation yeast mentioned, **Saccharomyces cerevisiae**, was, in 1882, the chief constituent of a yeast largely employed in Edinburgh and London breweries; it is not improbable that this yeast still plays an important part in those breweries, but I have not since made any exact experiments on this point. Besides this species there are other high fermentation culture yeasts in English and Scotch breweries, and different species are also employed in Danish breweries.

3. Investigations on low fermentation yeasts which have been tested in practice.

From the way in which my above investigations developed, it became necessary to examine first the wild yeasts, and especially those which produce sickness in beer, and then the low-fermentation culture yeasts. On account of the small importance of high fermentation in Denmark, and in most" other brewing countries, less attention was paid to the yeasts employed in this branch of the industry, and only in the case" of **Saccharomyces cerevisiae** I. was a thorough systematic investigation carried out.

In 1881 I expressed the view in one of my papers that the low-fermentation yeast employed in breweries consisted of only one species **Saccharomyces cerevisiae**; at that time this was the general opinion. The differences which the yeast exhibited were attributed essentially to local circumstances, and it was thought that these differences were easily interchangeable, and could again disappear. It gradually became the custom to speak of **Saccharomyces cerevisiae** as of a definite and well-known quantity, and this was also done in the publications from the Carlsberg Laboratory up to the end of 1881. I was then for the first time able to submit the question to an experimental treatment. I thereby soon gained a very different insight into the matter, and the main result proved that **the view which had been held with**

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regard to the systematic name *Saccharomyces cerevisiae* (low-fermentation form) was incorrect; for under this name are included not one, but several forms, differing both morphologically and physiologically, and to which we are equally justified in applying different specific names, as in the case of numerous other well-characterized micro-organisms. The same holds good of the other species of Reess. With regard to the different species and varieties of wine-yeast (*Saccharomyces ellipsoideus*) which have been tested in practice, see Chapter VII.

I have carried out these experiments in accordance with the above principles with **Carlsberg yeasts No. 1 and No. 2**. The first name has been applied to a definite species namely, the first pure cultivated yeast which was introduced into the brewing industry; the name **Carlsberg yeast No. 2**, on the other hand, applies to different species which were tried at Old Carlsberg in the course of time. Some zymotechnologists have completely misunderstood me in thinking that with these names I wished to imply that all low-fermentation brewery yeasts could be grouped around these two types. We have, as yet, no knowledge as to how many groups or types exist; the number would appear, however, to be considerable. Similar analyses of other brewery yeasts were subsequently made by A. Jørgensen, Will, P. Lindner, Holm, Poulsen, Windisch, Kukla, Irmisch, Reinke, Wichmann, Lasché, Prior, Bau, and Olsen, and they have obtained the same main result. In agreement herewith are also the investigations of Borgmann and Amthor. *

* The literature here referred to will be found partly in Jørgensen's "Microorganisms Ind Fermentation," New Edition, London, F. W. Lyon, 1893; partly in "**Compte-rendu des travaux du laboratoire de Carlsberg.**" 1882, 1883, 1886 and 1888; in the "Zeitschr. f. d. ges. Brauwesen." Munich, 1883-95; Fresenius' "Zeitschr. f. analyt. Chemie," xxv. Heft. iv. 1886; and "Zeitschr. f. physiolog. Chemie," xii. 1887.

It is seen that some species yield what practical brewers call good fermentations, a well-developed head and satisfactory clarification, whilst other species do not. There are also distinct differences with regard to the attenuation, the character of the sedimentary yeast, and likewise in the taste and odor of the finished beer, also in its stability and its power of keeping its head. Whilst some yeasts give a beer having a mild taste, and often with a milder flavor than that of the corresponding beer which has been fermented with impure yeast, others, on the other hand, give a product having a stronger taste, and sometimes a fruity, or a slightly bitter taste. By way of example, I will briefly describe the **Carlsberg yeast No. 2** and one of the yeasts which in the Old Carlsberg brewery has been called **No. 2**.

Fig. 18 represents cells of the **Carlsberg yeast No. 2** as

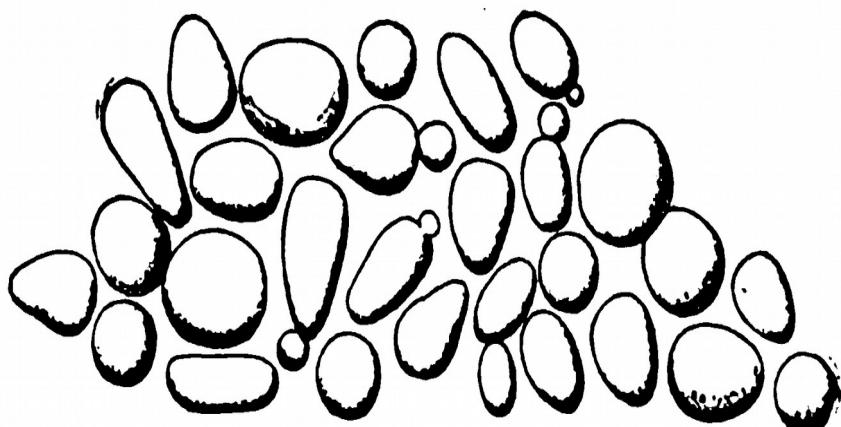


FIG. 18.

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they appear in the sediment of a wort culture which has stood a few days at the ordinary room temperature, or in the sedimentary yeast taken from a fermenting vessel of the brewery at the end of the primary fermentation. In addition to the round and oval cells, there are many egg shaped and a few short sausage shaped cells; it is especially the egg shaped, somewhat pointed form of cell which, under the conditions mentioned, characterizes this species. Under conditions which, in the case of other species hitherto examined, are favorable to spore formation, this species yields either very few spores or even none at all.

Fig. 19 shows a growth of **Carlsberg yeast No. 2**. The cells on the right hand of the figure are from a gelatin culture, and have formed spores; the remaining cells have been grown under conditions similar to those described in the case of the No. 1 yeast. The No. 2 yeast is distinguished from the latter by its cells being rounder, and by the presence of giant cells here and there. It gives abundant spore-formation.

When these two species are examined under the microscope with reference to their cell-contents, it is at once seen that the protoplasm of the No. 2 yeast is more uniform and less granular than in the case of the other species, and that the vacuoles are less pronounced. The colonies in wort gelatin given by both species have the ordinary appearance characteristic of the **Saccharomyces**. The differences observed in the growths of the sedimentary yeasts were also noticeable in the gelatin cultures; especially the presence of the giant cells mentioned gave a very definite character to the growth of **Carlsberg yeast No. 2**. In the cases mentioned it was, in short, an easy matter to distinguish the two species from each other by a simple microscopic examination. The figures do not show the differences in form and size as distinctly as they are actually seen, and the different appearance of the cell contents is not represented at all. Jörgensen has described similar observations in his "Micro Organisms and Fermentation" (New Edition, London, 1893). *

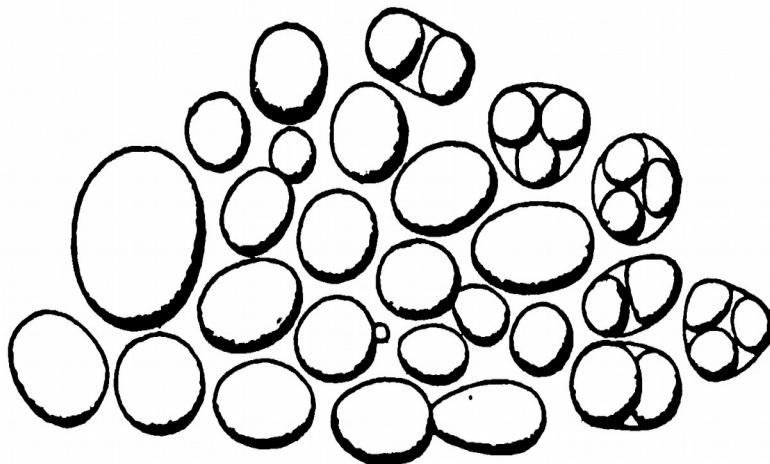


FIG. 19.

* It must not be imagined that the above differences can be regarded as general characters by means of which we are enabled to determine whether a certain species of yeast, of which nothing is known previously, will give a high or low attenuation, will clarify well or ill in short whether in these respects it resembles more closely the one or the other of the two species described, whether it gives a beer of this or that taste, &c. &c. To determine this requires much more than a simple microscopic examination. Cultures with giant cells like those of **Carlsberg yeast No. 2**, are found not only in the case of certain high

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fermentation yeasts, but also in species of *Torula* (see my figures in "**Compte-rendu des travaux du laboratoire de Carlsberg.**" 1883. p. 152); and low-fermentation yeast cells, the contents of which exhibit a microscopic appearance similar to that of **Carlsberg yeast No. 2**, may, however, give a strong attenuation and exhibit bad clarifying properties in the brewery.

Whilst I was chiefly engaged with such investigations I obtained from Mr. Jörgensen's laboratory six low-fermentation yeasts, the properties of which were well known through several years' trial in different breweries. Two of them gave a feeble attenuation, two gave a strong attenuation, and it was stated that, as regards fermentation, the last two occupied an intermediate position between the above. The differences between these species revealed by a careful microscopic examination were, however, ill-defined, and not of the nature which I have described in the case of the two **Carlsberg bottom yeasts**. Thus no rule of general application was found, and the result showed that species of low attenuating properties presented the same microscopic appearance as highly attenuative yeasts.

We all know that none of the theories of fermentation hitherto brought forward by Stahl, Liebig, Pasteur, Nägeli and others can explain the numerous and very varied phenomena with which we are now acquainted; the knowledge which we possess has proved these theories to be incorrect, but is not yet able to supply a true theory. Although these erroneous theories are, as a rule, still discussed even in the text-books written for practical brewers, the main reason is, no doubt, that the writers have thoughtlessly followed the same old beaten track. In practice nothing is learned from these speculations, and they are only of historical interest. There can be no doubt but that the study of protoplasm will lead to the solution of this important question, which will some day not only enrich science, but will also prove of service in practice. In my investigations on the behavior of the alcoholic ferments towards the carbohydrates, I referred to the yeast cell as a favorable subject for such investigations, and I have myself done some work in connection with this problem. But nothing will be gained by a simple microscopic examination like that mentioned above; the attack must be directed against the elementary parts of the protoplasm and the cell-wall; our object must be to find an expression for the functions in the structure and composition of the cell.

It is only in the most recent times that it has been possible, with the help of the highly-developed technique of modern science (microscopical, chemical and physical), to make even a beginning in such investigations, and so far we have not many results of general importance to chronicle in this field. With regard to the fundamental problems touched upon above, it must even be confessed that nothing has been accomplished. I refer here to the truly scientific investigations; if, however, we look through the half-scientific literature of some of the

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zymotechnic journals for the last ten years, we shall find not a few publications in which it is announced that these questions have been solved by the various authors. They state how they have been able, by a simple microscopic examination of the yeast cells, to foretell the behavior of the yeast in the brewery, and likewise the attenuation, the brightening, taste, stability of the beer, &c. &c. Investigators of this kind do not, in their ignorance, in the least perceive the difficulties which others continually encounter, and as they have no idea of the amount of work already accomplished by such investigators as Strassburger, Wicsner, Zacharias, Flemming, Zimmermann, F. Schwarz, Pfeifer, Schmitz, and several others, they ignore the whole of the literature and boldly bring forward their own "discoveries."

These half-scientific writings cause great confusion and do much harm. The above remarks are directed as words of warning against this class of literature.

We will now proceed to describe how the two yeasts behave in the brewery. The experiments were conducted under identical conditions in the Old Carlsberg Brewery, so as to permit of a comparison being made; and it was also with beers obtained with the help of these two species that Borgmann conducted the chemical investigations mentioned above.

No. 1 gives a rather thin head with low foam, a strong attenuation, and it clarifies slowly and rather badly; the sedimentary yeast is loose and rather slimy; in the lager cellar clarification also proceeds slowly.

No. 2 on the other hand, gives a strong head, high foam, feeble attenuation, quick and good clarification, a firm pasty sediment and rapid clarification in the lager cellar. The beer from both yeasts has a good and delicate taste, but the No. 2 yeast gives a beer which is fuller, contains more carbonic acid gas, and holds its head better; the beer from No. 1 yeast is, on the other hand, far more stable. When bottles of this beer were kept in a dark cupboard at the ordinary room temperature and examined after three weeks, it was found that no appreciable yeast sediment had formed; but when the beer from No. 2 yeast was similarly treated, the abundant formation of yeast which occurred often rendered it undrinkable even after ten days.

The fermentations produced by the **Carlsberg yeast No. 2** have always caused surprise to brewers who have visited the fermenting cellars of Old and New Carlsberg; this yeast is, in fact, one in which all the external characters looked for by the practical brewer in a good brewery yeast are wanting, and yet it gives a good and, in particular, an extremely stable product. This latter property is of great importance, especially when, as is the case in Denmark, most of the beer is sold in bottles.

That the difference in the stability of the two beers is not dependent solely upon the attenuation is shown by the fact that the beer from No. 2 yeast, after attaining as strong an attenuation by long storage as the beer from the

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No. 1 yeast, is still far inferior to the latter as regards stability.

In all the breweries where these two yeasts were tried, they always behaved in the manner described; the chief characters upon which stress has been laid were the same in all cases.

The term stability as here employed has reference only to the formation of yeast sediment, and not to bacterial disease. If we examine the matter more closely, we find that the yeast sediment may consist partly of the culture species, which alone has brought about the whole of the fermentation, or was in preponderance from the commencement, and partly of wild yeasts. We are not here considering cases in which several culture yeasts were present together. A yeast which is to yield a stable beer must be a species which multiplies only to a small extent in the finished lager beer, and which can keep competing organisms in check during the fermentation. With regard to the last point, the chief reason why certain species excel in this respect must be sought in some cases in the fact that they are better able to avail themselves of the conditions of nutrition, and especially the oxygen, than are the competing organisms, and, on the other hand, in other cases, that during their multiplication they secrete substances which act as poisons.

In making comparative tests on stability in the sense employed above, the beers must be bright, and should contain only a small number of yeast cells; strictly speaking, there should be the same number of cells in the different samples. There are low-fermentation yeasts which give a very stable beer, but in which the brightening in the lager cellar is slow, and inversely there are several species which yield unstable beers, but produce a rapid brightening in the lager cellar. At a given time, when the latter beers are already bright, the former will often be found to contain an infinite number of yeast cells, and this circumstance is sufficient to render these beers less stable than the others at this stage, although at the end of the storage period they are far more stable.

4. On variation.

The characters which we make use of for distinguishing different species of animals and plants have no absolute validity, but are constant only under certain conditions. It is especially to the epoch-making works of Darwin, that we are indebted for the doctrine of variation. The greatest and most difficult work, however, still remains to be accomplished, namely, the determination of the active factors with a view to the final elucidation of the laws regulating variation.

As long ago as 1883, I pointed out in some of my first studies on the *Saccharomyces* how varieties could be produced under different conditions, and by degrees I published a series of communications on the subject, more especially interwoven in my researches in the physiology and morphology of alcoholic ferments quoted above.

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The changes may be partly of a more or less temporary nature, and partly permanent; in the latter case, the properties being reproduced through endless generations and under various conditions of culture. In the following, I give examples of both kinds taken from my investigations, and I have selected such as may prove of interest not only to the theorist, but also to the practical zymotechnologists. Those readers who wish for further information, are referred to the original treatises.

If we wish to prepare a pitching yeast for industrial application, it is not sufficient that it is an absolutely pure culture of the desired race of yeast, but it must also be in such a condition that it will behave in a normal manner in practice, either at once, or at any rate after a very short time. In breweries, the question of attenuation and brightening plays an important part. Imperfect brightening and strong attenuation, in many cases at least, go together. According to the treatment to which a yeast is subjected in the laboratory, one and the same species may be made to clarify well or badly in the brewery; it can be made to behave somewhat differently according to the conditions of nutrition under which the numberless generations of cells of which it is composed have been cultivated. I will not, however, here enter into general considerations of interesting theoretical possibilities which speculation suggests, but I will briefly state some of the most important results to which my experiments have led.

When in 1883 I began my experiments in the brewery with the species named by me **Carlsberg yeast No. 2**, I at once found that a bad result was obtained when the yeast had been cultivated in a wort which had not been aerated. If the wort is not aerated directly after sterilization, the containing flasks must be set aside for a length of time, in order that the wort may become gradually aerated by the air which slowly gains access to it through the bent tubes or through the cotton-wool plugs. I have already pointed out how important it may be that the first portion of the yeast, even in the laboratory, is grown in well aerated wort. The following experiments were carried out in 1884, and were subsequently repeated in 1890. The yeasts employed were **Carlsberg yeast No. 2** and **Carlsberg yeast No. 2**, both in pure culture. In one Series of Experiments non-aerated, and in the other normal aerated wort was employed; it was sterilized, and was the same as that generally used in the manufacture of ordinary lager beer (13.5 per cent. Ball.). The wort was in Pasteur vessels, each of which contained 1.5 hectoliters, and these stood in a room, the temperature of which was 10-12° C. Each species of yeast was introduced into a separate vessel, and the amount employed was 0.5 kilogram of moderately thin yeast. In all cases the brightening was unsatisfactory, but the yeast produced behaved differently in the two Series of Experiments. The yeast which had been grown in wort aerated in the ordinary manner behaved normally as regards clarification and attenuation during the first or the second fermentation in the brewery; on the other hand, the yeast which had been grown in the non-aerated wort behaved in another manner altogether, and this was especially the case with **Carlsberg yeast No. 2**. Through a number of successive fermentations in the brewery this yeast behaved very unsatisfactorily as regards clarification, and the attenuation was abnormally strong. After a time the yeast certainly improved, but it did not return to its ordinary condition in which it existed at the commencement of the experiment. **Carlsberg yeast No. 2**, under similar conditions, likewise proved less satisfactory than usual (as regards clarification) at first, but in a short time (after

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two primary fermentations in the brewery) it returned to its normal condition, and behaved in the manner desired. The influence to which the cells were exposed in the two Series of Experiments, with aerated and non-aerated wort, thus acted in the same direction in the case of both species of yeast. The cells which were grown in the non-aerated wort lost, at least for a time, the power of acting normally under the condition: obtaining in the brewery. A specific difference was, however, distinctly noticeable, in that the **Carlsberg yeast No. 2** very quickly returned to its normal state, whilst the **Carlsberg yeast No. 2** did not. The latter species, in fact, not infrequently causes trouble with respect to the brightening of the beer.

In order to ensure a good result in the direction mentioned, it seems to be of considerable importance that the wort should be aerated, and especially at a high temperature. As I have mentioned elsewhere, no rules of general applicability can, however, as yet be laid down. My object was merely to show how changes of an undesirable character can occur in brewery yeasts when the wort in which the yeast is grown has not been properly aerated.

In this connection, it may be well to remind the reader that a yeast growth does not behave like a uniform chemical mass, but that, on the contrary, it consists of individuals; and these, as is the case with organisms in general, differ" more or less from one another. These individual differences may show themselves in the power of effecting clarification in a more or less satisfactory manner. Since we take the single cell as our starting point in the preparation of our pure cultures, the growths which we obtain represent the individual peculiarities occurring in the species. If we test these growths separately, we are often enabled to propagate a race which is especially characterized by its clarifying properties.

In the first German edition of this book (1888), I gave an account of some other individual peculiarities which may occur in low-fermentation brewery yeast. Here I will deal with those which manifest themselves in the form of the cells. "From this standpoint the question is one of practical importance when we have to deal with the preparation of pure cultures. In the method which I have described, the starting point is always a single cell. Let us assume that we have obtained an absolutely pure culture of **Carlsberg yeast No. 2**; I take this species because it was used in most of my experiments. Some cells of this pure culture are shaken up in wort gelatin, and a little of this is spread over the under surface of the cover glass of a moist chamber; those cells are then located the positions of which are such that the colonies to which they give rise will not grow into one another. The resulting colonies, each of which we consequently know for certainty to have sprung from a single cell *, are often very different, some consisting of cells which, on account of their elongated and sausage-shaped forms, would be described by Reess as **Saccharomyces Pastorianus**, whilst others have the form which we generally regard as belonging to **Saccharomyces cerevisiae**, and yet both belong to the same species, both have sprung from a single cell." When some flasks containing wort are inoculated from these colonies, so that each of one series only receives cells from a colony containing the **Saccharomyces Pastorianus** form, and each of a second series only cells from a colony with the **cerevisiae** form, it will be found that the growths obtained in the wort will exhibit the same difference; if, however, the cultivation is continued further, the difference between the two series will become less, the sausage-shaped cells gradually disappearing, so that finally all the growths will consist of oval cells. In one

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experiment, however, it was only after seven such cultivations that the oval cells predominated, the original culture consisting of sausage-shaped cells. The time during which this occurred was about two months. In these experiments, each new growth was obtained by inoculating with a very small sample of the sedimentary yeast from the preceding culture, precautions being, of course, taken to prevent the pure cultures from becoming contaminated. Whilst the sausage-shaped cells still preponderated, I introduced some of the yeast into the propagating apparatus in the fermenting cellar of Old Carlsberg. Even after ten days, the growth which had formed consisted mainly of oval cells, and when this was used as pitching yeast in a larger vessel, a typical yeast consisting of oval cells was at once obtained. The oval form was tested in a similar manner on the large scale, and here again it retained its oval form. Both gave beers of the same character, and were thus proved also to belong to the same species.

* I have in previous communications also pointed out that colonies in wort gelatin: grown from the same species of *Saccharomyces* may yet present a different appearance when examined with the naked eye or with a low power.

These experiments teach us that there is a difference in the indwelling properties of the individual cells, and therefore a microscopic examination of the yeast colonies in wort gelatin gives us as little information about the species in question as the first cultures in" wort. This is a new proof showing that little value can be attached to the microscopic examination alone when it is a question of the analysis of a sample of yeast. (Both in breweries, in yeast factories, and distilleries, too much weight is still attached to the microscopic appearance of the yeast cells.) The new experiments further show that if we wish to avail ourselves of the behavior of the cells under external influences for the purpose of characterizing species, we must never depend exclusively on the behavior of the single cell, but we must take the collective behavior of a number of them. Other low-fermentation yeasts give similar results under the conditions mentioned.

The following experiment may be taken as another illustration of the problem mentioned: When making plate cultures with wort-gelatin, it is frequently seen that two or more cells lie so close together that on budding they will soon grow together and form a single colony; such a colony cannot, of course, be made use of for the preparation of a pure culture. If, however, as in this case, we have started with a pure culture, it is evident that colonies like those mentioned contain pure cultures, just as those which have grown from a single cell, and that, in fact, all the colonies will consist of one species, provided that no foreign organisms have gained admission. If we confine ourselves to the colonies which have grown from several cells, we shall often find a number of them which appear very similar under the microscope, since they all consist of a mixture of oval and sausage-shaped cells; but if we inoculate a corresponding number of flasks, each from one colony, we may, however, obtain two series of fairly different growths, one containing a preponderance of *Pastorianus* cells and the other of *cerevisiae* forms. Since we know that each of the colonies .sown from was originally formed from several cells, the most probable explanation is that the two cell forms were present in different proportions in the portion introduced into the flasks, or if this were not the case, that

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during the struggle for the upper hand the one form was more vigorous than the other. In this case the difference was not so marked as in that first described. On further cultivation in wort it completely disappeared, the newly formed cells again taking the oval form.

In one of my papers (1883) referred to above, I described a still more pronounced change in the form of the cells of ordinary brewery yeast into long sausage-shaped cells which occurred under the influence of a certain temperature. As this process, however, is of no practical importance, it will not be described here.

There are several different ways in which it is possible to act upon yeast cells so that in one case they will produce more alcohol and in another case less than that yielded by the original parent cells. The following examples may suffice: By repeatedly cultivating different species on solid nutrient media (especially gelatin with yeast extract) growths were at length obtained, which produced considerably more alcohol than the original parent cells. On the other hand I have succeeded in transforming the low fermentation **Carlsberg yeast No. 2.** into a new variety, which produces less alcohol and gives a better clarification at the end of the principal fermentation than the primitive form. The process employed in that case was a prolonged cultivation in wort at the temperature of 32° C., each culture being left at rest. The practical bearings of these experiments will be understood without further explanation.

Saccharomyces Pastorianus I. is, as already mentioned, one of the disease yeasts of beer, to which it imparts an offensive odor and a disagreeable bitter taste. According to Mach and Portele's investigations, however, it gives a good wine; and my own experiments have shown that when this species is cultivated for a number of generations in a solution of cane-sugar in yeast water, a growth is obtained the cells of which have for the time lost the disagreeable properties referred to. From this it is seen that it is possible to act upon yeast cells in such a way that they can be made to impart to fermenting liquid a taste and odor different from that originally characteristic of the yeast. Experiments in this direction may acquire practical importance especially in the manufacture of wine.

Most of the changes mentioned above are of a somewhat temporary character. A more deeply seated change, and one of greater permanency was attained in the case of the species which I have named, **Saccharomyces Ludwigii** ("Centralblatt f. Bakteriologie und Parasitenkunde," 1889, p. 632; "**Zeitschr. f. d. ges. Brauw.**" 1889, p. 253). By the systematic selection of single cells, and cultivating each separately, I succeeded in obtaining three different forms of growth of this species. When these were cultivated separately in wort, and tested for spore formation in the ordinary manner, it was found that one variety yielded spores abundantly, the second only very sparingly, whilst the third gave no spores. * Numerous generations of this last form were cultivated in wort both at the ordinary room-temperature and at 25° C., and under conditions of nutrient which ordinarily favor the spore forming power of a yeast, but it still refused to yield spores either on moist gelatin or on moist gypsum blocks. Only after the cultivation in wort had been continued for a length of time were growths gradually obtained which again yielded spores, and then never in abundance. If, on the other hand, the cultivation were made in a 10 per cent. solution of dextrose in yeast water, new generations were at once obtained, whose cells again possessed the property of yielding

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spore in abundance. In order to bring back the cells to their normal function, the new nutritive liquid was required.

* I have obtained similar results also with brewery yeasts.

We will now consider some cases in which the change is permanent, and repeats itself again and again in new generations. In the treatise quoted above, I gave an account of the main results of my experiments on *Saccharomyces Pastorianus I.* It was found that when the cells of this species were cultivated for a length of time in aerated wort, and at a temperature approaching its maximum temperature, it completely lost its power of forming spores; and even after numerous generations of vegetative cells had been produced in new wort cultures at favorable temperatures, none were yet able to develop spores. In this case, **therefore, a property which is a very important one as regards morphology and classification has been entirely destroyed.** Simultaneously with the loss of this faculty, the power of film formation in old wort cultures also disappeared.

The treatment which the cells must undergo in order to bring about such a deep-seated transformation, produces, in fact, a revolution in their vital functions. That a long continuation of this treatment is necessary, is easy to understand when we recollect with what tenacity the cells of the *Saccharomyces* retain their power of producing spores, even under the conditions obtaining in breweries and distilleries where they can only reproduce themselves through numberless generations by the process of budding.

I afterwards obtained similar results with various other species ("Annales de Micrographie," Fevrier 1890; "Zeitsch. f. d. ges. Brauw." 1890, p. 145), and found that in beer wort one group of them gives a quicker and more abundant growth, but a slower fermentation than that produced by their progenitors. Amongst these were also some brewery yeasts e.g. the *Carlsberg yeast No. 2.* After this species had by the treatment mentioned above been brought to the condition in which it had completely lost the property of spore-formation and film formation, I made some comparative experiments with this variety, and with the original yeast from which it had sprung, under the conditions obtaining in the brewery. It was found that during the primary fermentation, the newly-formed variety attenuated more slowly and more feebly than the original yeast, but at the same time the brightening was better. After normal storage the difference in the attenuation gradually diminished, and almost disappeared. The beer produced by the variety, as a rule, brightened somewhat a lot better, but was always less stable (in the sense used above) than that obtained by means of the original yeast.

It is now several years since I **succeeded in producing the first varieties or possibly, even new species of Saccharomyces. Although they have since been cultivated under very different conditions, they have remained constant; the newly acquired properties have been perpetuated through numerous generations.** *

* In a special treatise on which I am at the present time engaged, I will give a

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detailed account of the variation phenomena and of the factors and laws which influence them.

On glancing at the results described in this chapter, it is seen that the following conditions determined the direction in which my studies had to be continued. In order to obtain a definite starting point, it was, in the first place, necessary to ensure the absolute purity of the cultures experimented with; I therefore elaborated the methods described on p. 5, and by means of which I was enabled to start always from the single cell. The next point was to investigate more closely whether there were differences in the cultures so obtained, and, if so, to determine further what the differences were. Since the characters given by previous workers for the recognition of species are false, it became necessary to discover new ones, and for this purpose I treated the question from a botanical standpoint. It was necessary to complete these studies before an experimental investigation of the complicated questions of variation could be successfully undertaken. Since 1882 I have from time to time made experiments in this direction, but it was only in 1888 that my investigations were sufficiently advanced to enable me to take up this new problem as a main point in my plan of work. This does not, however, in the least imply that I gave up my old line of study.

The above investigations on the question of variation again opened up new paths for the study of the ***Saccharomyces***. Though the results hitherto obtained are at present essentially of theoretical interest, yet some of them have already become directly applicable in practice. What has long been achieved in horticulture and agriculture will also be attained in this case, and, in fact, with a more thorough comprehension of the active factors; for the one-cell organism is a far more favorable object for such experiments than the more highly-developed flowering plants.

But even when we have got so far that we can with certainty determine the action of different chemical and physical factors, and are enabled to bring about the changes in the desired direction, we still know nothing as to what it is in the cells which effects the changes or which produces this or that result. These great problems again lead us into new paths of study, and point specially to the investigation of the protoplasm as the direction which will some day lead to their solution. Every problem gives rise to a greater and more difficult one, and science is never at a standstill.

5. Main results.

Both the theoretical investigations in the laboratory and those of a purely practical nature carried out in the brewery have thus ***shown that there are different species of Saccharomyces, and, in fact, not only of the so-called wild yeasts, but also well-characterized high and low yeast: which are employed in breweries.*** Exposed to different external influences, they may vary to a considerable extent, but in most cases they return to their original condition when they are cultivated for a length of time under normal conditions. New species or varieties can, however, be formed by proper treatment continued for a length

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of time, whose newly-acquired properties are perpetuated in the different cultures. *As long as the yeast: were cultivated under the condition: obtaining in the brewery their properties varied but :light{y." it thus follow: that in practice we can and must regard them as definite species, and we .r/could adapt our method according{y.*

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CHAPTER III. The practical examination of beer in the storage casks.

1888. *

The text-books relating to the manufacture of beer contain either no information at all on this subject, or at most very little. At first glance the question appears such a simple one as to require no special treatment; when we consider it more deeply, however, we soon perceive that it has several sides, and that we are, in fact, on uncertain ground.

The experiments described were carried out at the beginning of 1883, and therefore at a time when my pure cultivated yeasts had not been introduced into the brewery. The beers in question were low fermentation beers, and had in all cases been produced by means of impure yeast. A large portion of the observations was placed at my disposal by Captain Kühle, and at my request Professor Grönlund carried out some experiments at New Carlsberg similar to those made by myself at Old Carlsberg, the object of which was to determine whether the samples of beer were influenced by aeration or not, and what was the effect of exposing them to the ordinary room-temperature, or to a temperature of 25-27° C. My intention from the commencement was to publish the results in the present series of my investigations, to which from their character they also belong. But as their publication was delayed until 1888 on account of some other work, this was also the case with the investigations under discussion. Their utility, however, is still the same as at the time when they were made, for the question, as stated above, is still awaiting its solution.

When a brewer takes his samples of beer in the lager cellar, his object is to ascertain not only its present condition, but also how it will be after it has stood for a certain length of time. His examination is a purely practical one, and he does not avail himself of any scientific methods. The taste, odor, color and brightness of the beer are noted. Samples are drawn off into clean bottles of colorless glass, which are then well corked, and in order to ascertain how long the beer will keep, these are set aside in a dark cupboard at the ordinary room-temperature. It is then noted whether the beer remains bright and unchanged in color, and what length of time elapses before an appreciable sediment has formed; and further, the appearance of the sediment, whether on shaking it becomes readily diffused through the liquid, rendering the latter cloudy or turbid, or whether it forms flocks, which again soon sink to the bottom without appreciably affecting the brightness of the beer. These changes are to be attributed to the action of micro-organisms. If the beer gradually becomes cloudy and discolored without being shaken, there is bacterial disease. This, however, seldom occurs, and is a very rare occurrence in well-conducted low-fermentation breweries. On the other hand, a yeast sediment forms in the best beer after a longer or shorter time, and consists partly of culture yeast, partly of wild yeasts, and species are often present which produce diseases e.g. yeast turbidity and disastrous changes in the flavor. As stated, a yeast sediment will form in a variable length of time, according to the culture yeast employed and the degree of contamination with wild yeast. In the following the term stability has reference merely to the yeast sediment. In connection with the question of stability the reader is referred also to the

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last chapter.

The first point to which we must pay attention is that the small samples taken from the storage cellar for examination are average samples, otherwise we can naturally draw no conclusions as to the condition of the bulk from which they were taken. Here, however, we are at once met by great difficulties. Even if one section of the cellar is racked at the same time and in such a manner that all the casks are gradually filled with the same beer, their contents will still vary somewhat; it is, in fact, not possible to introduce the same amount each time, one cask receiving sometimes too much and sometimes too little; but since the contents of the fermenting vessels may vary somewhat during the length of time required for filling the casks, it naturally follows that there may also be differences in the cask contents. It is evident, therefore, that samples must be taken from every cask. The question then is whether average samples can be obtained under the conditions prevailing in the brewery, and in what manner they are best collected; it is self-evident that not every small sample drawn from a large cask can afford exact information with regard to the whole contents of the latter.

The method made use of was as follows: Some of the bottles mentioned above (each holding about 350 cc.) were filled in the lager cellar, a small cult sampler-tap being used for the purpose, and they were then well corked. Both the bottles and corks were previously sterilized, and the samples of beer were collected with care. As soon as they were taken to the laboratory, they were placed in a dark cupboard, where the temperature was, as a rule, 16-18° C. in the day-time and often only 10° C. at night.

I. Experiment

Seventy-two such samples were taken from 12 cask of export beer which had been stored for seven months, six samples being collected from each cask, two from the lower layer, two from the middle, and two from the upper portion of the cask. After 14 days there was a considerable sediment in 20 bottles from the upper layers.

20 bottles from the upper layers
7 „ „ middle „
3 „ „ lower „

The remaining 42 bottles contained only a slight sediment.

II. Experiment

Thirty samples were taken in the same manner from 5 casks containing export beer which

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had been stored for nine months. The result obtained differed from that of the preceding experiment; for only in the case of the beer from two of the c did a yeast sediment form more rapidly in the samples from the upper layers than in those from the lower layers. An opposite result was obtained in the beer from two of the casks, whilst the samples from the different layers of the fifth cask behaved alike.

III. Experiment

Sixty samples were taken from two casks of lager beer which had been stored for four months, and these were treated in the same manner as before. After 16 days there was a considerable sediment in only nine bottles, and all these contained samples from the upper layers of the casks.

Captain Kühle informed me that he had made a similar observation in the case of lager beer which had been stored for six months.

IV. Experiment

Thirty samples were taken from 5 casks of lager beer which had undergone three months' storage. The result in this case was that the samples from the three layers behaved essentially alike; as far as any difference could be detected, it was that the yeast sediment formed a little earlier in the samples from the lower layers than in those from the upper layers.

The main result was, therefore, that the upper layers of the lager cults in most cases developed a yeast sediment sooner than the lower layers, the opposite occurring but seldom. Samples taken from a single layer of the cask, as a rule, therefore, give no trustworthy information; in order to avoid chance results it is best to take a number of samples.

Since the samples were finished beer from the lager cellar, and in the case of the lager beer were even very old, it would have been expected that the upper layers would contain no yeast cells, and that the lower layers would in each case contain a larger number than the upper layers. It is possible that if the cells had been counted, this would indeed have been found to be the case; it must not, however, be forgotten that the result does not depend merely on the number of yeast cells, but also on the species and on their condition. It would be easy to suggest an explanation; but as I have made no experiments on which to support it, I prefer to merely state the facts.

This, then, was the point with reference to average samples. The experiments show that, as a rule, the brewer will not obtain fair samples when he draws them in the ordinary manner from the lower portion of the cask.

The next point to be mentioned is the method which we must employ in order to ascertain how long the beer will remain sound under the conditions to which it is exposed after bottling.

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We will here assume that it is properly handled both in the carriage casks and in bottle. In the case of ordinary lager beer, the temperature to which it is exposed is, as a rule, not higher than the ordinary room-temperature, at any rate under the conditions prevailing in Denmark. In the case of export beer, on the other hand, the matter is different, and this is furthermore required to remain sound for a much longer time. The brewer, therefore, is also in the habit of exposing his samples of lager beer to the ordinary room temperature, whilst he subjects the samples of export beer to a higher temperature e.g. 25° C.

In transferring the beer from the store casks to the transport casks, and from these again to the bottles, it becomes moderately freely aerated, and thus one active factor will be furnished for bringing about the multiplication of the yeast cells. When the beer is drawn off by means of compressed carbonic acid-gs, this will, of course, be avoided; but it is only exceptionally that this method is adopted.

The samples in our bottles are, in consequence of what has been stated above, less aerated than the beer occurring in commerce; that this influences the stability is proved by the following experiments.

Four of the bottles mentioned were filled as previously described from the lower portion of a store cask; two of these bottles were at once corked, and the contents of the other two were decanted into two similar empty bottles, and these were then corked; all four bottles were then set aside in a dark cupboard at the ordinary room-temperature. It was found that beer aerated in this manner in most cases gave a yent sediment sooner than the same beer which had not been aerated, and it was only exceptionally that the sediment formed in the same length of time in both cases; in no case did a sediment appear sooner in the non-aerated than in the aerated beer. The difference in the time amounted in some cues to several days. These experiments were made with export and lager beers from both Old and New Carlsberg breweries, and the samples employed were taken from 89 casks. The lager beer had been stored three months, and the export beer 7-12 months. They show, amongst other things, that beer keeps better when it is not aerated whilst it is being drawn off from one vessel into another.

A similar Series of Experiments was undertaken in order to determine whether, in the case of the non-aerated samples, the yeast sediment formed most readily at the ordinary room temperature, or at 25-27° C. It was found that the lower temperature favored the production of sediment. After the lapse of a month at the second temperature, most of the samples of lager beer were still free from all trace of yeast turbidity, whilst on the other hand, the corresponding samples which had been exposed to the ordinary room-temperature were all cloudy after 15-23 days. In the cases investigated the difference was most marked in this class of beer, and was less pronounced in the case of export beer. I again repeat that I am speaking only of the formation of yeast sediment; bacteria, on the contrary, will probably generally develop more rapidly at the higher temperature. Finally, some aerated and some non-aerated samples were placed in an incubator at 25-27° C., and again in this case the former became cloudy sooner than the latter.

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Although the results given here are founded upon a considerable number of experiments, we must not assume that they are of universal application; we can only conclude that they apply under the conditions described; before we can go further than this, similar "experiments must be made in several different breweries. I should be glad if other investigators would also make experiments in this direction. For my part, I must be content to have made a beginning.

A continuation of such observations should also be extended to beer which has been treated with shavings in the cask, and not only to the finished stored beer, but also to beer in the different stages of secondary fermentation. In the latter case the problem would be to determine rules for judging how the beer will subsequently behave with reference to stability namely, at the time when it will leave the lager cellar. These questions will be least complicated in breweries in which pure yeast is employed, and especially where only one well-known species is made use of.

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CHAPTER IV. The technical analysis of air and water for micro-organisms.

1892.

1. One of the first of the more extensive investigations which undertook on commencing my studies of the microorganisms was the Investigation of the organisms which occur in the atmosphere in and around Carlsberg at different times of the year, and which are capable of developing in beer wort. *

* *"Compte-rendu des travaux du laboratoire de Carlsberg."* 1 vol. 2 livr., 1879, and 4 livr., 1882, Copenhagen.

As the title indicates, these investigations throw light upon those species of atmospheric micro-organisms which are able to develop in wort, and upon their occurrence, both in the brewery and in the open air, at the different seasons of the year. Special attention was paid to the question of the habitat of these organisms. In this, as in several of my other investigations, theoretical and practical studies are closely united. Prominent amongst the former is the investigation of the cycle of alcoholic ferments in nature (see also Chapter VI.); the results obtained have, on the other hand, also a practical interest, especially in connection with the manufacture of beer, in that they throw light upon the greater or less danger of infection during the different seasons and under various conditions. To those portions of the treatises which are mainly of practical interest, belong the investigations of the micro-organisms which occur in brewers' "grains," and in the air of the different portions of a brewery.

As is known, "grain" contain numerous bacteria which give rise to acid fermentations which are readily noticeable by the odor produced. Should these bacteria be carried into the air with the vapors arising from the grains, the presence of the latter in the brewery yards would become very dangerous. It is, therefore, quite natural that these vapors have always been regarded with mistrust. Experiments which I made at Mr. Kogsbölle's suggestion showed, however, that these vapors did not carry away any bacteria. If, on the other hand, the grains are allowed to become so dry that the wind can blow them about as dust, they become highly dangerous. As a rule, however, they are left for so short a time that the whole mass remains moist, and danger only occurs when the main bulk is removed, and small quantities are left behind in the yard in thin layers. If these are not carefully swept up and removed, they may give rise to bacterial diseases. *

* I must not omit to call attention to the great danger in the drying machine for grains which have been employed in recent years, especially in Germany. In use: whee I had the opportunity of examining grains dried in this manner, it was found that the micro-organisms which they contained were by no means killed, and this was especially noticeable in the case of the bacteria. If brewers had studied with more attention than they have done my investigations on grains in the brewery yards referred to above, they would certainly have shown more

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hesitation than has often been the case before fitting up a drying machine. in such a manner that coolers and fermenting rooms are liable to become daily infected by the dust teeming with bacteria which such a machine will produce. In drying the grains, they are brought into the very condition in which they are highly dangerous for the fermentations in the brewery. The brewer ought to take all this into account when he is considering the question of putting up a grains drying machine.

A point of practical interest is shown especially in the different analytical results obtained at the same time, but in different parts of the Old Carlsberg brewery. The atmosphere in the fermenting room was found to be the purest. This is due not only to the strict order which is always maintained, but in a still higher degree to the fact that the air of this room is cooled by means of an ice-machine, and that it is submitted to a special purifying treatment by means of a shower of salt water. The air of the fermenting cellar of Old Carlsberg contained, on the average, 0-0006 germ in 1 cc. or 1 germ in 1591 cc. These numbers are given on the assumption that they may serve as a kind of standard in such analysis at least, until we obtain a better one.

Similar analyses were likewise made in the fermenting rooms of other breweries in which no purification of the atmosphere had been attempted, and the difference was most marked; the air not infrequently contained more than four times the number of germs that were found in the case mentioned above. Bacteria - Sarcina amongst others were also frequently found in the latter case, and also wild yeasts, which proved to be disease-producing yeasts. It was during these studies that the idea first occurred to me that some of the most common and most dangerous diseases of beer were caused not by bacteria, but by certain of the **Saccharomycetes**, and this idea was the starting point of my investigations in this direction.

The experiments naturally hold strictly good only for those places where they were made, and under the conditions there obtaining. Generally speaking, however, they will also hold good for other similar districts and for other breweries. Although carried out in 1878-80, they are still the most comprehensive experiments which "we have in this field, and the results obtained have lost nothing as regards their validity. With regard to the technique of these old experiments, it may likewise still be affirmed that the principle was correct. The only objection that can be raised from our present standpoint is that the same result can be attained in a more ready manner. In conducting such experiments on the microorganisms of the air, I should now employ a similar method to that described in the following section on the analysis of water.

As stated above, these investigations belong to the series of my writings which treat of practical problems relating to the fermentation industry. Those readers who wish to study them more in detail, are referred to my treatises (in Danish and French) in the Carlsberg communications mentioned above.

In recent years, several zymotechnologists have made similar experiments, especially P.

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Lindner in Berlin, Will in Munich, Grönlund and Alfred Jörgensen in Copenhagen.

Now that scientific aid is so readily obtainable, the occasional analysis of the air will, in the future, scarcely be omitted in the larger well-appointed breweries; by this means it will be possible to obtain a better insight into the working of the brewery, and it will also be sometimes possible to guard against mishaps. The condition of the atmosphere in the fermenting room is of particular importance in this respect.

Such analyses are of especial interest when as has been the case for several years in the Old Carlsberg and New Carlsberg breweries an arrangement has been adopted for the purification of the air. Recently, Linde's ice-machines and cooling-pipes have been employed for this purpose in many breweries. It is evidently necessary to investigate what has been thereby attained, unless we are satisfied to work in the dark. As far as I am aware, however, no analyses have been described which throw light on the effect of the Linde cooling-pipes in this direction; such analyses are very desirable, and I have repeatedly called attention to this fact in my lectures, and I take the opportunity of doing so again here.

2. All agree that the same principle should prevail in the biological examination of the water of a brewery as in the case of the air. It appears also to follow as a matter of course, that in the cultivation experiments which such an investigation involves, the very same liquids should be made use of which are employed in the brewery. Opinions, however, are not yet quite unanimous on this last point, and, especially towards the end of the last decade, a contrary view was expressed by several prominent bacteriologists. At that time a large number of investigations on the microorganisms contained in water were carried out on all sides by means of Koch's gelatin method, and not only in hygienic, but also in zymotechnic laboratories. In an extensive work on such analyses which was published in 1887 by Hueppe, the latter emphasizes his opinion that this method is the most important for the solution of practical questions which relate both to technology and to hygiene. He make no distinction in this respect; the gelatin should be employed in all cases.

I could not help at once regarding this as a great mistake; and after carrying out the necessary experiments in this direction, I replied in a short communication which appeared early in 1888 in the "**Zeitschrift für das gesammte Brauwesen.**" I thereby attained my object, in that Hueppe and some of the other advocates of the gelatin method changed their opinion on this point, but in the meantime it had become so customary in most zymotechnic laboratories to employ exclusively Koch's hygienic method, that it was very difficult to effect a change in this respect. My paper was read with attention, but, as was to be expected, was not received with favor. Even Hueppe could not suppress his dissatisfaction, although in the main he recognized that I was right. Curiously enough, my work was regarded by most of the German bacteriologists as an attack on Koch's method, which, however, it was not in the least; its object was merely to warn against the misuse of the latter.

When, during my visit to London in 1889, I read a paper on this subject before "The Laboratory Club," I was likewise opposed on the same grounds from several sides. In

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England it had, in the course of years, also become customary to employ the hygienic method, without examining whether questions relating to brewing could be solved in this manner. I do not intend to again enter into these disputes, as they are no longer of interest. Moreover, during the last few years my method has been adopted in several laboratories in various countries. My object now is to give an account of my treatises mentioned above, and with this I hope to bring this work to a conclusion.

According to Koch's method for the bacteriological analysis of potable water, 1 cc. of the latter is mixed with 10 cc. of melted (at 30° C.) nutritive gelatin (meat decoction peptone gelatin). This mixture is poured on to a plate, and is protected against infection by means of a moist bell jar. Under certain circumstances only 0.5 cc. or even only one drop of the water is employed. The plates are kept at the ordinary room-temperature, and are examined after 3-4 days. The number of vegetative specks developed is calculated, for practical reasons, for 1 cc. of the water under examination.

When a brewer desires to have a bacteriological analysis made of the water which he proposes to employ in his brewery, the point to be determined is not which and how many micro-organisms are present in the water, nor which growths develop in gelatin or other solid substrata, with or without meat extract peptone; all this is of no interest in the case under discussion, for neither the one nor the other of these substances is employed in the brewery. The simple question with which we have to deal is this: ***How does the water behave with the wort and with the beer; in what degree are such micro-organisms present which are able to develop in the above liquids, and are there amongst them such species: which are capable of producing ill effects?*** Briefly stated, our analysis must be carried out as far as possible under the conditions obtaining in the brewery; we must therefore, above all, work with the two liquids mentioned. It would also be desirable for the hygienist to be able to make direct experiments, but he cannot make use of the human body for this purpose, and in the place of this he has, therefore, to be satisfied with artificial nutritive substances. What I have just stated is so obvious that it really surprises me that it is necessary to call attention to it.

It is evident that the results of this zymotechnic method are not comparable with those obtained by means of the hygienic method alluded to above; this will be readily understood from what follows. There are special problems relating to the physiology and technology of fermentation which are different from those relating to hygienic bacteriology, and in each case, therefore, special methods must be elaborated.*

* The word "method" is used here in the same sense in which it is generally employed in modern physiological and chemical literature it does not signify new principles for investigation, but working methods, technical appliances, and modes of manipulation.

Starting from this point of view, I made the following experiments. Small flasks were charged with the nutritive liquids, beer and wort, and closed with cotton-wool plugs. The Chamberland

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flasks, or better still, the cylindrical Freudenreich flasks, are very suitable. I employed flasks of this description having a capacity of 22 cc., and into each were introduced about 10 cc. of the liquid. A considerable number were simultaneously sterilized in steam under pressure. This method is especially suitable for beer which, as is known, it is difficult to sterilize without its undergoing a great change; as has been emphasized, it is desirable to approximate as closely as possible to the conditions obtaining in practice. Possibly still better results could be obtained by sterilizing the beer by filtration (e.g. by means of a Chamberland filter), and under such conditions that neither the alcohol nor the carbonic acid could escape; it would, however, be considerably more troublesome to work in this manner. The wort can, of course, also be sterilized by merely boiling it.

In the case of the ordinary cold water used at the Old Carlsberg brewery in September, 1887, 5 cc. were mixed with 5 cc. of the nutritive liquid (in one series beer, in the other wort). One drop ($0^{\circ}04$ cc.) of each of these mixtures was added to each of a series of 15 flasks containing beer in one case, and another series of 15 flasks containing wort. The drops can be added from a pipette, the upper end of which is connected with a piece of rubber tubing, which is plugged with cotton-wool so as to completely free the air which passes through from germs. The whole is sterilized, fixed on a stand, and the flow is regulated by means of a pinch-cock. In all operations of this kind it is advisable to employ a pure air chamber. It is evident that all apparatus and nutritive media must be sterilized, and that care must be taken to work always with average samples. The quantity of water that is to be used for sowing must be accurately measured so that the result obtained can be calculated on 1 cc.

Simultaneously with the above, 0.5 cc. of the same water was examined by means of a plate culture according to Koch's method, and also 0.5 cc. by means of a similar plate culture, but employing wort-gelatin (wort with the addition of about 5 per cent. of gelatin) in the place of meat extract peptone gelatin. In addition to this, a considerable number of drops of the above mixtures of the water and wort, and of the water and beer, were sown on solidified gelatin plates containing no nutritive liquid. All the gelatin cultures were kept moist and covered over with bell jars, and, like the cultivations in the flasks, were placed in an incubator at $24-25^{\circ}$ C.

The object of this experiment was, in the first place, to obtain accurate information concerning the behavior of the beer and wort cultures, in comparison with the gelatin cultures, and from the results obtained to ascertain which method was most suitable for analyses in the brewery. The object of the lat-mentioned gelatin cultures was to determine whether it were possible in one way or the other to employ gelatin for such analyses. It is, for instance, often easier, especially for the less practiced, to operate with cultivations in gelatin than with cultures in fluids.

The following was the result of the Series of Experiments described: After about three days, the contents of both flasks containing respectively the mixtures of 5 cc. water with 5 cc. wort, and 5 cc. water with 5 cc. beer had become cloudy; they exhibited a very vigorous-growth of bacteria, and some yeast-like cells (Pasteur's so-called Torula) were also present, though in much smaller proportion. After three to four days, several of the drops on the pure gelatin

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showed growths visible to the naked eye. and similar growths were also found in Koch's gelatin and in the wort gelatin. After four to five days all the drops of the beer and wort mixtures which had been sown on the pure gelatin showed distinct growths; only in the case of two drops were the above-mentioned yeast-like cells found, and in three drops mold fungi (*Penicillium glaucum* and *Cladosporium*), and in all these five drops bacteria were also present. All the remaining drops contained bacteria only. In most cases these growths had liquefied the gelatin. The experiment was discontinued after fourteen days, when it was found that none of the flasks of beer and wort containing the small addition of water showed any signs of a growth.

In Koch's gelatin there were 16 colonies, which is equal to 222 calculated on 1 cc. of water; all of these contained bacteria, but only a few of the growths had produced liquefaction of the gelatin. The wort gelatin contained 15 growths, or 30 calculated on 1 cc. of water.

In a second similar Series of Experiments, the sample of water was taken three days later than in the above. The result was of the same character, but one of the flasks containing wort showed a growth of bacteria after four days, and another a growth of *Penicillium glaucum* after five days. The wort in the remaining 13 flasks, and the beer in all the 15 flasks, were still bright after 15 days, and in none of these was there a sign of growth. The number of growths calculated for 1 cc. of the water was, therefore, 6.6 in the case of the wort flasks, whilst it was 1000 for Koch's gelatin, and 34 for wort gelatin. As in the first series, all the drops sown on pure gelatin developed growths. The micro-organisms found were the same as in the first series.

Some analyses which were made in September, October, and November, 1887, and which were carried out under my supervision by Messrs. Karneef, Kukla, Terry, and Wichmann, also yielded, in the main, the same result.

It will be seen from all these experiments that the hygienic method always gives too high a result, and that the employment of wort gelatin is equally unsatisfactory. Whilst no growths were obtained in the beer, and in the case of wort the numbers obtained in different experiments were 0, 0, 6.6, 3, and 9, calculated on 1 cc. of the water, those representing the growths in Koch's nutrient gelatin obtained under otherwise similar conditions and for the same samples of water were 100, 222, 1000, 750, and in one case even 1500.

It is unnecessary to point out that the lat numbers must be regarded as valueless for brewery purposes. The analysis is somewhat more satisfactory when wort gelatin is employed instead of meat extract peptone gelatin, but even in this case the numbers obtained are too high, and give no serviceable information. ***The majority of the bacteria which developed in the gelatin were unable to grow either in the beer or in the wort, and are, consequently, of no importance for our purpose.***

For the sake of comparison, all the cultures in the above Series of Experiments were exposed to the same temperature namely, 24-25° C. It is customary, however, in the hygienic method

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to expose the cultures to the ordinary room temperature, and to examine them after three or four days.

Even when this method of experimenting was adopted, the results obtained were always too high in comparison with those obtained by means of the two nutrient liquids. Koch's method cannot, therefore, be employed even in this form.

If the hygienic method enabled us to determine with any degree of certainty whether pathogenic bacteria were present in a brewing water, we should, of course, always employ it in addition to the other method; as is known, however, this is, unfortunately, not the case, and its value for our purpose is at most that it affords a means for controlling the filters. Even in this test it is highly important to carry out the analysis in such a manner that the results obtained during a lengthened period and at different places are strictly comparable. In the text-books the directions given with regard to Koch's method are to leave the plate cultures for three to four days at the ordinary room-temperature. To this it may be remarked that, in the first place, *during this period, as a rule, only a small proportion of the bacteria present will show a distinct development, and, when regarded as a percentage, this will not, in all cases, be an expression of the total number of bacteria.* If accurate information is required with regard to the bacterial contents of a water, the cultivation must be continued for at least a fortnight. In one analysis, for instance, the number of growths found after four days was only one-tenth of those which developed after ten days, and only one-fifteenth of those found after sixteen days. Likewise, the room temperature is very uncertain and variable, and it will make no slight difference whether the cultures are exposed to a temperature of 20° C. or 10-5° C. *An analysis may give a very different result during the summer from that obtained during the winter.* If the work is carried out during the summer, and under fairly favorable conditions as regards night temperature, *the same water sample will give, under the same conditions of culture, an appreciably different result, according to whether the colonies which develop are examined after three or four days.* If a comparison is to be made, and the method is, in fact, entirely one of comparison, more account must be taken of these factors than has generally been the case hitherto. A further difficulty with the gelatin cultures is that growths of mold frequently spread to such an extent at the beginning of the experiment that it becomes impossible to complete the analysis. It is only when the above conditions are taken into account that the gelatin methods can be advantageously employed for testing the effectiveness of water and air filters.

If the above experiments are considered more closely, it will be seen that they not only indicate the means to be adopted for the rational analysis of brewing waters, but that they also throw light on more generally interesting biological conditions. Thus, we learn that *of the numerous bacteria present in the sample: of water examined, only extremely few were able to attack the wort, and none of them the beer.* It is evident that samples of other waters may give a different result, but the above appear to furnish a general rule.

Since each drop (half water and half nutrient) sowed on the pure gelatin gave a vigorous growth, it can be safely concluded that each of the flasks containing wort or beer, and to

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which similar drops had been added, also received living germs. In the case of the above two more fully described Series of Experiments it was proved that there were in several flasks as many as 60 bacteria, in many 20, and in none less than 4 bacteria. Most of these germs were therefore unable to develop in the two liquids. As some of the flasks" of wort showed a growth of bacteria, I assume that a few of the very numerous bacteria present in the water belong to species which are able to attack wort. This was distinctly seen in cases where a growth in wort consisted only of a single species of bacterium. When traces of such a pure culture were introduced into other flasks of wort, they rapidly produced bacterial turbidity in the latter, as was to be expected, but in no case was beer attacked by them. Bacteria which are able to attack beer would appear to be but very rarely present in water.

When, however, these liquids (wort and beer) were much diluted, the micro-organisms with which they were infected developed, as a rule, very freely. This was shown both in the case of the mixtures in the flasks and of the drop cultures on the gelatin plates, and still more distinctly by means of some special experiments which were made in this direction. Not only wort, but also beer, lost its original resistive power under these conditions. In this state of dilution, however, they are no longer what is understood in the brewery as wort and beer. In examining the flasks the fact must not be overlooked that there are bacteria which can grow in wort, and especially in beer, without producing cloudiness; amongst these are, e.g. the acetic acid bacteria described by myself a few years ago.

In order to gain further information with regard to the antiseptic power of the above-mentioned liquids, some special experiments were undertaken. Some of the wort and beer mixtures teeming with bacteria were introduced into a flask containing sterilized distilled water in sufficient quantity to render the latter cloudy. Single drops of this new mixture were then introduced into a number of flasks containing beer and wort. These were vigorously agitated, and consequently became aerated, and they were then set aside at a temperature of 24-25° C. In a short time the liquid in all the flasks became as bright as before infection; but after two days almost all those containing wort became cloudy, owing to very vigorous growths of bacteria. Those containing beer, on the other hand, were still bright after sixteen days, and did not show a trace of any growth, although they had been infected with a great number at least, hundreds of living bacteria, exactly as in the case of the wort flasks.

Identical results were obtained from similar experiments made with the drop-growths on gelatin. ***The highly interesting fact is time brought out that the bacteria present in tile water examined were not able to develop in beer, even when they had been introduced in considerable quantity.*** With regard to the wort, in view of the above results I am inclined to the opinion that the infection was caused, not through the abundance of the water bacteria, but rather by the presence of certain species which have the special power of attacking wort even when undiluted such species may be termed wort bacteria.

Based upon these observations, I have elaborated the following method for the analysis of the water of Old Carlsberg:

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One drop (0.04 cc.) of the water was added in the manner described above to each of a series of 15 flasks containing wort, and to each of a similar series containing beer, also 0.25 cc. of the water was added to each of a series of 10 flasks of either kind; these were then shaken and set aside for a fortnight at a temperature of 24-25° C. The beer contained in these flasks was low-fermentation lager beer, and the wort (about 14 per cent. Ball.) was that employed for the production of such beer.

An analysis made in November by means of this method showed that 1 cc. of the water gave only 1.3 growths of bacteria and 1.3 of mold i.e. 2.6 in all. No other growths developed, and the beer was not attacked at all. Applying these results to practice, we shall be able to add 2.5 liters of water to a hectoliter of beer without causing any bacterial growth. In December, 1 cc. of water gave 38 bacteria colonies on a wort-gelatin plate culture, whilst 2.25 cc. of the same water which was distributed between 9 flasks of wort did not give a single growth of bacteria, but gave rise to a growth of mold. The experiment was carried out in the same manner as the previous ones, and, like these, it also showed that the two methods failed to give concordant results.

In those cases where a trial was made, it was found that when 1 cc. of water was added to 10 cc. of beer, no growth occurred other than mold and Pasteur's so-called *Torula*, and not infrequently no growth of any kind developed. In other words, this shows, then, that the addition of 1 cc. of water to 10 cc. of beer was not sufficient to destroy the antiseptic power of the beer 3 regards resistance to the action of the bacteria present in the water, for none of the latter developed in the beer which was thus diluted.

It is self-evident that a microscopic examination should be made of each growth in the flasks, and in many cases it will also be advisable to make special cultivation experiments in order to ascertain to what extent the different species are dangerous.

All the experiments have shown that the gelatin method gives much higher numbers than the technical brewery method, and this applies to both wort-gelatin and meat decoction peptone gelatin. We have also seen that there is no fixed ratio between the numbers obtained by means of the two methods, and therefore any conclusions drawn from the one cannot be applied to the other. And with regard to these numerous colonies which develop in the gelatin, we stand face to face with the sad fact that even when we submit each of them to a thorough microscopic examination, we are still unable to decide whether they contain growths which are able to attack wort and beer. In order to arrive at an answer to this question and this is really the whole point it is necessary to make cultivation experiments in these liquids. A further important objection to the use if gelatin is that some of the micro-organisms, which are of the greatest practical importance as regard: the analysis, often do not develop in this medium. This applies, for instance, to acetic acid bacteria, *Saccharomyces* and other alcoholic fermentations. I have found by means of direct experiments with several such species that in the enfeebled condition in which they are present in atmospheric dust, in the soil and in water, they are either unable to develop in the above nutrient gelatins, or they give at most only a feeble growth, whilst similar germs present in the same samples give a vigorous growth when

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sown in the flasks containing wort. This will also, in all probability, hold good for several other species besides those investigated.

It was shown above that 1 cc. of a sample of water yielded 1500 growths by Koch's plate culture, whilst scarcely ten growths were obtained when the flasks of wort were employed instead of the gelatin. Although the technical brewery method gives comparatively very low results, the latter are also still too high. I will explain this more in detail. Thus the bacteria which develop in the flasks are cultivated under especially favorable conditions, and are removed from the retarding influence of competing organisms; if they had been introduced into some fermenting wort taken from a fermenting vessel in the brewery, a large number would have been suppressed. In my experiments with *Torula* and other species of alcoholic ferments, I have often found that species which when present alone in the wort produced beer of a very disagreeable flavor, were quite harmless under the practical conditions of a brewery, owing to the fact that when they have to compete with a good brewery yeast they become completely suppressed. Nevertheless, since the numbers obtained are low, the error which is introduced through counting these latter organisms as injurious is of but little moment. At all events, this holds good for the analysis" mentioned above. We approximate somewhat more nearly to the conditions obtaining in practice when we adopt the following grouping of the flasks. Those in which molds alone have developed are put side from the rest, for these are of importance only for malting. I know at least of no cases in which these organisms have produced diseases in beer. The remaining infected flasks are again separated into two groups namely, those in which growth soon occurred, and those in which the micro-organisms took several days (e.g. five or more days) to develop. Since the germs developed so slowly in these last flasks, there is reason to believe that they would not have developed at all if they had been introduced into the fermenting vessels or into storage casks. It is therefore only in that group of cultures in which the development had been rapid that we have to look for the bacteria and wild species of yeast which are likely to prove dangerous to the beer. The numbers found thus become further reduced, and, as stated, we approximate still more closely to the practical conditions which actually obtain in the brewery. To imitate these exactly is, of course, not possible, and we must be content with having approximated the desired end as closely as is really the case. The question of the effect of competition will vary not only according to the different methods adopted in different breweries, but also according to the varying composition of the wort in one and the same brewery in the course of the year.

In spite of all these restrictions, however, the described analysis gives us valuable information, and, as we have seen, of a kind which we can only gain in this way. It is important here to point out that ***by cultivation in our flasks of wort we are able to obtain growths of all micro-organisms which we know with certainty are able to produce diseases in beer.*** As has been already stated, several of these disease-organisms, on the other hand, do not develop at all when introduced into the beer. If they are to make themselves felt and produce sickness, they must be introduced into the fermenting wort. For this reason ***the cultivation in beer becomes of less moment for the analysis, and the cultivation in wort of by far the greatest importance.*** If we employ the method described with care, we shall thus be in a position to discover the enemies that are present amongst the

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microscopic germs which gain admittance to the brewery with the air and with the water. The fault which we are in danger of committing is to attribute somewhat too bad a character to the samples of air and water examined.

There are two points at which cold water is employed in the brewery in large quantities, but without its coming into direct contact with the wort or with the beer. I refer to the yeast vessels in the fermenting cellar and to the steep-water in the malt-house.

A practice frequently adopted is to keep the yeast covered with cold water. In some breweries pieces of ice are put directly into the yeast; in others greater care is employed, and the ice is placed in the cover of the yeast tub; in all cases care is taken to maintain a low temperature. If this were not done, most species of the water bacteria would in all probability find a favorable medium in the yeast-mass. As a rule, the yeast remains under the conditions named for 12-24 hours only, rarely 48 hours. When introduced into the tub with cold water, the temperature of the mass is about 10° C., but when the cover with the ice has been placed over it, the temperature sinks in a few hours to about 6° C.

I have examined yeast thus treated frequently and at different seasons, but when the cooling with ice was sufficiently ensured I never found that the bacteria multiplied to an appreciable extent; and as soon as the yeast is introduced into the wort the activity of most of the bacteria in the water becomes completely suspended, as has just been shown. It will, therefore, be of no importance if such species multiply even to a fairly considerable extent in the viscous mass occurring between the yeast cells. This is, in fact, a favorable medium for the development of by far the larger number of bacteria. That the multiplication of injurious bacteria at this point is also to be feared, is self-evident. In the above we have spoken of such species under the general name of wort bacteria. When these are present in the water which comes in contact with the yeast, they will, as a rule, multiply like the water bacteria, especially if the yeast mass has been exposed to too high a temperature. But since the wort bacteria do not appear often to occur in water (at least not in the samples examined by myself), the danger in this respect will not be very great under ordinary brewery conditions. As a rule, some wort bacteria from the previous fermentation will be present with the yeast itself. These are unable to develop during the vigorous fermentation in the fermenting vessel, but they will multiply in the yeast vessels as soon as the cooling is neglected. For this reason I am inclined to the opinion that at this point there is, as a rule, greater danger incidental to the yeast itself than to the water. ***At all events, it is certain that too much care cannot be taken that the yeast is kept at a low temperature during the intervals when it is not active in the fermenting vessels.*** This is the main point. That sterilized water may advantageously be employed for treating the yeast need scarcely be mentioned, although I am of opinion that, as a rule, this is not necessary.

I have likewise paid no special regard to the malting, for the reason that I am of opinion that this would be superfluous. The barley-corns have on their surface a multitude of bacteria and other micro-organisms before they come into contact with the steep-water; the few more or less due to ordinary water will, therefore, in this case be of very little importance. It is, indeed,

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mainly the molds which are feared in the malt house, and if these are present in the water they will also develop in the flasks of beer and wort employed in the analysis.

Bacteriological analysis applied to water is still in a phase of development, and has, indeed, as yet given no practical results of any great importance; still it certainly ought not to be neglected. We must not, however, forget that the result of an analysis of a single sample has only a very limited value, as it will only give us information with regard to the water at the time when the sample was collected. The condition of one and the same water is, however, subject to great fluctuations at different times of the year, and even at different hours of the day; and where, as in most breweries, it is pumped into large tanks, its condition will also depend largely upon whether the sample has been taken shortly before or after the tank has been cleaned. Consequently, if we desire any more accurate knowledge of the condition of a water, it is necessary to carry out a large number of analyses which must extend over a considerable period of time.

My zymotechnic investigations on the micro-organisms of air and water were carried out with special reference to the conditions obtaining in low-fermentation breweries, but in the main the results will also apply to high fermentation breweries.

Since my investigations on brewing water were published in 1887-89, several treatises on the same subject, and based on my method, have appeared in various journals; amongst the latter may be mentioned Holm's "*Analyses biologiques et zymotechniques de l'eau destinée aux brasseries,*" in the "*Compte-rendu des travaux du laboratoire de Carlsberg.*" 3 vol. 2 livr., 1892. There is, indeed, now scarcely a zymotechnologist who would recommend the employment of meat extract and peptone gelatin for the analysis of water when the question to be decided is whether it is suitable or not for brewery purposes. My work referred to has thus far, therefore, served its purpose.*

* After the above had been for some considerable time in the hands of the printer, Dr. Wichmann's "*Biologische Untersuchungen des Wassers für Brauereizwecke*" appeared in the "*Mitteilungen der cesterr. Versuehs~Station, V. Heft,*" Vienna, 1892. Wichmann points out the importance of noting the time when the decomposition of the test liquid commences. In my first publication in 1888 this factor was not introduced in the analysis, this was, however, done subsequently. Statements to this effect *occur not only in my treatise mentioned above, but likewise in Jørgensen "Micro-organisms and fermentation,"* also in Holm's treatise mentioned above. Thus the same idea occurred to Dr. Wichmann as to myself, and he likewise worked it out independently. I gladly take this opportunity to draw attention to his meritorious investigations.

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CHAPTER V. What is the pure yeast of Pasteur *

1892.

* Although this memoir is mainly of a theoretical nature, I have yet felt that it ought to be introduced into my collected writings relating to the fermentation industry. I decided upon this for two reasons: in the first place, the main question treated of is one of great practical interest, namely, what is to be understood by a pure brewery yeast? and, in the second place, the results of my investigations on the effect of treating brewery yeast with tartaric acid are of direct practical importance.

1. In the physiological experiments which I carried out with *Saccharomyces apiculatus* in 1879 and 1880, I prepared the necessary pure cultures in accordance with the principles laid down in Pasteur's "*Études sur la bière.*" The following is the account which I gave of the method.† "Into a large number of flasks containing sterilized wort as nutritive medium, fruits were introduced which were assumed to have *Saccharomyces apiculatus* on their surface; one piece of fruit only was introduced into each flask, and only such were chosen which were fairly clean and free from mold, and with a little practice this can readily be discovered with the naked eye. After a few days, one or several of the flasks, as a rule, contain an abundant and fairly pure growth of the desired yeast fungus. Whether bacteria are present or not is of little importance, for the latter can generally be easily suppressed by cultivation in acid liquids. Greater trouble is encountered when other yeasts or mold fungi are present together with *Saccharomyces apiculatus*. In such cases it is best to abandon the experiment at once and to start again from the beginning. If a satisfactory culture has been obtained in the manner described it is made use of to inoculate a two-necked Pasteur flask containing sterilized wort to which a little tartaric acid has been added. After a few days, when fermentation is in progress, the nutrient liquid is decanted from the yeast which has settled on the bottom of the flask, and fresh liquid of the same composition is added, the operation being performed with proper precaution so that external organisms do not enter. When this process has been repeated a few times a perfectly pure culture can, at last, be obtained." Notwithstanding the insufficiency of the method, I was able to express myself thus definitely, because the species in question possesses, in several respects, very marked characteristics. It is, indeed, one of the few species of yeast which, owing to the peculiar form of its cells, we are able to recognize by a simple microscopic examination, and, in addition to this, in its physiological aspect it also presents several peculiarities. In working with this species it is, indeed, possible at every stage of the experiment to prove whether we are dealing with a pure culture or not. This does not hold good for yeast cells with endogenous spore formation, i.e. the true *Saccharomyces*, * and it was just these which I wished to submit to a thorough investigation on account of their great theoretical and practical importance. In this field it was not possible to proceed further along the paths marked out by my predecessors. In order that I might be in a position to attack the problems I had set myself, it was, therefore, necessary in the first place to elaborate an exact method for the preparation of the necessary pure cultures. I was

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thus compelled against my will to devote some years to the study of this branch of bacteriological technique, and it was only after I had succeeded in this that I was able to really attack those questions which, for me, formed the main problem.‡

† Emil Chr. Hansen: "*Sur le Saccharomyces apiculatus et sa circulation dans la nature*" ("Compte-rendu des travaux du laboratoire de Carlsberg.") I vol. 3 livr. 1881).

* A few recently discovered species form the only exceptions.

‡ For my methods for the preparation of pure cultures, see p. 5.

In my first memoirs relating to the alcoholic ferments I confined myself to giving an account of the new results which I had obtained, and it did not then occur to me that it might be necessary to call attention to the errors of previous experimenters and to bring into relief the advance brought about by my researches. I have gradually learned from my opponents that the manner in which I stated my results is not sufficient when it is a question of promoting advance in a new direction. In recent years I have again been reminded of this by the attacks directed against me, and more especially by Duclaux and Velten.

Duclaux † attempts to prove the sufficiency of the methods described by Pasteur more than sixteen years ago for the preparation of pure cultures of yeast, and he particularly dwells upon the method of procedure, which consists in cultivating the yeast in a 10 per cent. solution of cane sugar to which a little tartaric acid has been added. In order to furnish proof of the correctness of his statement, he examined some of Pasteur's old flasks which contained yeast growths, partly in beer wort, and partly also in the tartaric acid and sugar solution; they had remained in the laboratory ever since Pasteur gave up these studies in 1876; some of the cultures were seventeen years old at the time they were examined. Duclaux found that out of 19 flasks examined, 14 contained pure cultures; in the case of three flasks he was not quite certain, but he assumed that each of these likewise contained one species only; in two flasks, containing brewery yeast, he states that mixtures of two species were present. He considers, therefore, that by means of these old flasks the method under discussion is shown to be an exact one for the preparation of pure cultures. If this were so, I should have to acknowledge having committed a great error, and having wasted my time with useless investigations.

† E. Duclaux: "*Sur la conservation des levures.*" ("Annales de l'Inst. Pasteur," 1889, p. 375.)

Against Duclaux's reasoning, Miquel ("Annales de Micrographie," 1889, p. 140), Alfred Jørgensen ("Botan. Centralbl.," xl. Bd., 1889, p. 316), and Denamur ("La Gazette du Brasseur," 1889, p. 887) immediately raised the same weighty objection, namely, that it is absolutely impossible, after so long a period, to ascertain with certainty what growths had been introduced into the flasks by Pasteur. Flasks which contained only one species when

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examined may very well have contained several species originally; in short, we are not able to discover those species which may have perished in the course of years. If Duclaux wished above all to revive and defend the old methods of Pasteur, it would have been more correct to have shown by means of theoretical investigations and practical tests what these methods are really able to effect. **As stated, the examination of the old flask: really throws no light on me subject.** In the following pages I intend, in the first place, to discuss the scope of Pasteur's method from a theoretical point of view, and then to show by experiments what can be attained by its means.

Duclaux's investigations, mentioned above, teach us that several species can live together in one and the same flask for even fifteen to seventeen years. It is evident that under these circumstances there can be no question of a pure culture, and thus we here have cases before us in which the method does not stand the test. I am ready to acknowledge that there are species of yeast which, under the conditions mentioned, possess different degrees of vital activity, and when a mixture of such species is present in one flask, the time will naturally come when all the weaker species will be dead and only the strongest will have survived; the question now is: how are we to know when this point has arrived and how to determine with certainty whether the flask contains one or several species? If the method is to be called an exact one it must satisfy these demands, but with regard to this neither Pasteur nor Duclaux gives any information. It must, therefore, be candidly stated that for this reason the method is an extremely uncertain one, and that in making use of it we are working in the dark and are really dependent more or less upon chance. On examining Pasteur's work more closely (p. 224-228), we find that on the whole he recognized the limits, and that he himself perceived that only a conditional certainty is attainable by the methods which he proposed. In order to obtain a pure culture, he therefore employed not one single method but several, and he states that in different cases it is necessary to employ sometimes one and sometimes another method, in fact, to set to work experimentally; there is no fixed rule. After describing the different methods, he expresses himself as follows: "By means of these different modes of manipulation, separately or combined, it is possible as a rule to obtain the yeast which it is desired to purify, in a very pure condition." It is thus not a question of pure cultures at all, in the sense in which we now employ the term. In describing his experiments with alcoholic ferments he states in several places (e.g. p. 179, in the note on p. 205), that it was not possible to determine whether he had one or several species in each of his flasks. Pasteur tries to attain his object by preparing a series of cultures under conditions as favorable as possible to the particular organism which he wishes to isolate, and at the same time attempts to check the development of such organisms as he desires to remove. By cultivating in this manner, it is naturally only possible to suppress those species which, under the given conditions of nutrition, are unable to withstand competition with the species the development of which has been favored. There may, however, be a considerable number of other species present, together with the latter, which require approximately the same conditions of nourishment. The principles of such a cultivation method are of a purely physiological nature, and, indeed, imply the assumption that the characteristics of the species which are being dealt with are known beforehand; but since we are dealing with unknown factors in by far the greater number of cases where we wish to prepare pure cultures, it is clear that the methods

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under discussion can, as a rule, give no certain results. They can, indeed, only be employed in such rare cases of species which possess sufficiently definite characters that they cannot easily be mistaken for others, and where there is the possibility of a control. A case of this kind is described at the beginning of this memoir in the account of my earlier studies of *Saccharomyces apiculatus*.

After the publication of the memoir mentioned in the "*Annales de l'Institut Pasteur*," Duclaux again attacked the question, namely, at the French Brewers' Congress at Paris in 1889 ("Le génie civil," p. 110), and at the Congress at Lille in 1890 ("La Gazette du Brasseur," p. 447, No. 141, 1890). He acknowledges that my method implies a real advance, and that my researches have brought about a reform in fermentation in the brewery. He only concedes this, however, with reference to low fermentation, and for high fermentation he still advocates the adoption of the old methods of Pasteur. These lectures appeared in journals which have only a limited circulation, and consequently his views of the question of pure culture are known to most readers only through the "*Annales de l'Institut Pasteur*." If my famous colleague had also published his new views in the last-named journal, I might, perhaps, have refrained from again entering into the discussion of this old question.

The question as to whether my method is applicable or not to high fermentation has been answered in the affirmative, in Denmark by the experiments of Alfred Jørgensen, in Australia by De Bavay, and in North France and Belgium by Kokosinski, Van Laer and Vuylsteke ("*Station Scientifique de Brasserie*," "*Comptes-rendus*," Gand, 1890, p. 13-21; "*La Gazette du Brasseur*," Bruxelles, 1890). Even in France my method has been successfully introduced into fifteen high fermentation breweries, and its employment is therefore not confined, as Duclaux believes, to low-fermentation breweries. The reader may also be referred to the account in a subsequent chapter of the extent of the application which my system of pure yeast culture has now acquired. *

* Addition 1895. As shown in a subsequent chapter the pure yeast cultivation in top fermentation has been much more widely propagated since I wrote the above. Up to recent times Van Laer employed my system in its simple form, but now in a more complicated and uncertain manner, in that he recommends a composite yeast. The name is also the case with Vuylsteke.

Velten commenced his attacks against myself in the lectures which he delivered at the French Brewers' Exhibition in Paris in 1887 ("*Revue Universelle de la Brasserie et Malterie*," 1888, No. 742 and 743), and he repeated them at the Antwerp Congress in 1889 (see Report, p. 82). He maintained that I made a great mistake in introducing yeast consisting of a single species or race into the brewery; according to his view, brewery yeast should consist of several species, and in reference to this he expresses himself as follows: "It is to the mixture of these pure species of yeast, different in their race and in their nature, that beer acquires the desired taste and bouquet." This result, he states, is obtained by the employment of Pasteur's method, namely, by cultivating the yeast in a solution of cane sugar to which a little tartaric acid has been added, or in wort containing carbolic acid and alcohol. He gives no

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further particulars of the method in the lectures mentioned, but these are to be found on referring back to the lectures which he delivered at the Paris Exhibition in 1878, and which were afterwards published under the following title: "*De la fabrication de la biere par le procédé Pasteur. Conference faite par Eugene Velten au Congrès Internationale des Brasseurs de Paris en 1878*" ("*Revue Universelle de la Brasserie*," Paris, 1881, No. 372). They also appeared in the report published by the Congress. In these lectures he says: "If an acid be added to a saccharine solution favorable to the growth of alcoholic ferments, the development of disease ferments is thereby prevented. Acetic bacteria can indeed live in an acid liquid, but they require a high temperature for their development; the other disease ferments, on the other hand, cannot live in an acid liquid. For the purification of brewery yeast, 4-5 per cent. of acid (for instance, tartaric) may be employed. After four or five cultivations in this liquid, one can be sure that the yeast is pure; the alcoholic ferments alone survive, being the most vigorous and the most numerous." Pasteur, says Velten, makes use of four such cultivations and each occupies 48 hours. It follows from the above that the sole object of this purification of the yeast is the removal of the bacteria, and it is stated that yeast thus purified consists of several species. Duclaux also advocates the employment of this method for purifying brewery yeast ("*Chimie Biologique*," 1883, p. 301), but he recommends only a small addition of tartaric acid.

Shortly after the publication in 1876 of Pasteur's famous "*Études sur la bière*," some experiments were made by the late Captain J. C. Jacobsen at Old Carlsberg, and by Carl Jacobsen at New Carlsberg, on the methods described in that work for the purification of brewery yeast, and both tartaric acid and carbolic acid were tried; they gave no satisfactory result, however, and were consequently completely relinquished. This was also the case in breweries abroad in which similar trials were made; and Pasteur's methods did not even find favor in France. At the present time Velten is the only brewer who recommends them, and his own writings ("*Wochenschrift für Brauerei*," Berlin, 1886, p. 5) show that he does not invariably employ them. Velten's name is of high standing as the old associate of Pasteur in the domain of brewing science, and this has lent a certain weight to his attacks, ill-founded as they are. Neither he nor my other French opponents have been able to induce brewers to adopt Pasteur's methods; they have, however, aroused mistrust towards myself and the reform for which I am contending; and thus it is that its advance has been much slower in France than would otherwise have been the case.

2. When in the year 1882 I published my first papers on the preparation of absolutely pure cultures, I had already made numerous experiments on Pasteur's methods, but I confined myself to a brief theoretical discussion, and considered that the matter was then closed. That I was mistaken in this, has been proved by these attacks. Being thus compelled to take up these questions again, it was at once clear that, if I wished to make an end of the matter, I could not confine myself to a theoretical discussion, but that it would be necessary to make further experiments. In these I was assisted by my assistants Mr. Holm and Mr. Nielsen, and especially a large proportion of the analyses were carried out by Mr. Holm. I attempted, as is my wont, to investigate the question exhaustively, to study it from different points of view. It was a pleasure to me that the great pains bestowed upon this work by me and my assistants

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not only led to the important result that it enabled me to rectify the incorrect and arrogant assertions of my opponents, but it also added new information of both practical and theoretical interest. The experiments form two groups; the first, which includes the first four experiments, has regard to the theoretical side of the question; the second group includes the remaining experiments, the object of which was to test Velten's statements; the two groups of experiments help reciprocally to elucidate the matter.

Experiment I.

A 10 per cent. aqueous solution of cane sugar, to which one-twentieth per cent. of tartaric acid had been added, was introduced into two-necked Pasteur flasks and sterilized. After cooling, these flasks were inoculated with absolutely pure cultures of the yeasts mentioned below.* The cultures employed were obtained from a ten days' growth in wort cultivated at the ordinary room-temperature. A fairly large quantity of yeast was introduced into each flask, and, as far as possible, an equal amount of each species. .

In A, *Saccharomyces cerevisiae I.*, *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*, *Saccharomyces ellipsoideus II.*

In B, *Carlsberg bottom yeast No. 1.*, *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*, *Saccharomyces ellipsoideus II.*

In C, *Carlsberg bottom yeast No. 1*, *Carlsberg bottom yeast No. 2*, *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*, *Saccharomyces ellipsoideus II.*

* These and the species mentioned later are described in different places in my "*Recherches sur la physiologie et la morphologie des ferment alcooliques.*" They will also be found in Jörgensen's "*Micro-organisms and Fermentation,*" New edition, London, 1893; and in Zopf's Handbuch "*Die IPilz.*" Breslau, 1890.

The flasks were allowed to stand for a month at the ordinary room temperature; they were then shaken up and small average samples from each were introduced into fresh flasks containing the same liquid; a month later a third series of flasks were similarly inoculated from the second series, and these were again allowed to stand for a month. By the inoculation of such average samples, the species which preponderate are brought under still more favorable conditions, and thus the development of a pure culture was favored in this way.

After this process of cultivation had been carried on during three months, the problem was to bring about a new growth of any cells which were still alive, and thus to determine which species were still living in each flask. For this purpose average samples from each flask were introduced into two other flasks, one of which contained beer wort, and the other a 10 per cent. solution of dextrose in yeast-water. These flasks were set aside at a temperature of 25° C.,

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and as soon as a growth had developed, average samples were taken for fractional cultivation in gelatin to which, in the one case wort and in the other the solution of dextrose in yeast water had been added. The flasks from which these samples had been withdrawn were then left at the ordinary room-temperature until the primary fermentation was finished, and average samples were then again withdrawn for fractional cultivation as above. Similar cultivation: were likewise prepared from average samples taken direct from the growths which had been cultivated for three months in the sugar solution in the manner described. The experiments were thus made with the respective yeast growths in three phases of development. The gelatin plates in which the yeast cells had been sown were exposed to a temperature of 25° C. until numerous colonies had developed, and from these a large number of flasks containing beer wort were inoculated: in addition to this the colonies themselves were submitted to a microscopic examination. The growths which developed in the last-mentioned flasks of wort were also examined microscopically, and in all cases for the properties which I knew beforehand characterized the respective species. It is self-evident that in all cases sterilized liquids and gelatin were employed, and that the work was carried out with the care necessary to guard against infection from without.

The result arrived at was that out of the six species of yeast which were sown in the three flasks containing the solution of cane sugar and tartaric acid at the commencement of the experiment, only two species had survived, namely, ***Saccharomyces ellipsoideus II***, and ***Saccharomyces Pastorianus***: L; the former of these survived in all the flasks, the latter only in one, or perhaps in two of them. ***Saccharomyces Pastorianus I***. could only be detected with certainty in one of the flasks, and then only after cultivation in the solution of dextrose in yeast-water. ***Saccharomyces ellipsoideus II***. proved, therefore, to be the strongest species under the condition: of culturization described; but not even in the case of this species was there any certainty that the method had yielded a pure culture.

Experiment II.

This was carried out in the same manner as the first experiment; whilst, however, in the latter the cultivation: employed were ten days old, in this case the growths made use of were quite young and were obtained by cultivation in wort for twenty-four hours at 25° C. Otherwise the method was the same, and ***the end result of the experiment was likewise the same***.

Experiment III.

This was conducted in essentially the same manner as the two previous experiments, but with only one flask as the starting point. The yeast sown consisted of ***Carlsberg bottom yeast No. 1***, ***Saccharomyces cerevisiae I***. and ***Saccharomyces Pastorianus III***.

The mode of experimenting differed from that adopted in the two previous experiments in that the cultivation in the solution of cane sugar and tartaric acid was carried out in the course of only four weeks, and during this period four consecutive growths were obtained at about

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equal intervals, and in the manner described. On then examining as to which species were still living, it was found that ***Saccharomyces cerevisiae I.*** and ***Saccharomyces Pastorianus III.*** had survived. The former species was especially noticeable on cultivation in beer wort, whilst the latter was only found after cultivation in a solution of dextrose in yeast-water. ***In this case also a pure cultivation was, therefore, not obtained.***

Experiment IV.

The cultivation in the solution of cane sugar and tartaric acid was in this case carried on for a month, and during this time the culture was only transferred once to fresh solution, namely, after a fortnight. The experiment was commenced with two flasks, and into each of these were introduced the following species, namely, ***Saccharomyces Pastorianus II.***, ***Saccharomyces Pastorianus III.***, and ***Saccharomyces ellipsoideus II.***. They were all obtained from vigorous growths which, however, had been cultivated for three months on a nutrient gelatin containing fish decoction and cane sugar. ***At the conclusion of the experiment, *Saccharomyces ellipsoideus II.* was found in both flasks and this alone.***

The methods of cultivation which were employed in order to bring about the development of the species which had survived the described treatment in the sugar solution, and thus render their recognition possible, were such as, after several years' experience, had been found to be favorable to the growth of the species mentioned. If I had conducted a still larger number of cultivations and had varied the conditions in several other ways, I should probably have had a chance of getting at least some of the species which, under the circumstances, appeared to have perished. Where the limit would be, cannot well be determined. Some of the colonies in the gelatin cultures may also have contained more than one species. In short, it is probable that more species may have survived than were found. Those which were detected must, therefore, in the first place be regarded as the preponderating species. But even if we assume that those flasks in which we found only ***one*** living species, only contained this one, ***we have still as the main result the fact that the method described gives us no certainty of obtaining a pure culture. Out of nine flasks, three contained two species each at the end of the experiments;*** in two experiments, however, the treatment was carried on for three months. On the other hand, it is not improbable that, if the experiment had been carried further, all the species employed in one experiment would have perished with the exception of one; this was the case with ***Saccharomyces ellipsoideus II.*** in the first two experiments. But there is nothing here to guide us in reference to this point; as was pointed out above, we have no indication by means of which we can decide whether this result has been attained or not, and if we carry the treatment further there is the danger that all life will perish. In short, this method of working is and remains one dependent upon chance, and it can never become an exact method. The chief difficulty in the employment of the physiological method for the object in view is, as already stated, that we do not know beforehand how the species with which we are working will behave in respect to it; and even if we make preliminary trials, the same result would by no means be attained under all conditions. In this case, the individual characters of the cells of the species will play a part,

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and it is highly probable that when a species is exposed to the treatment mentioned for a number of generations, it becomes influenced in such a way that it gradually becomes better able to struggle on under the unfavorable conditions of nourishment. This constitutes a further objection to the employment of the physiological methods for the purpose mentioned.

The only certain way under all circumstances by which we can obtain a pure culture of a micro-organism, whatever physiological and morphological properties it may possess, is to saw a single cell in a sterilized nutrient medium.

Experiment V.

This and the following experiment were specially undertaken in order to test the method described by Velten in the lecture mentioned above, and which he employs for the purification of brewers' yeast in accordance with Pasteur's directions. The liquid in this case was an aqueous solution, containing ten per cent. of cane sugar and four per cent. of tartaric acid. The experiments were made with cultures consisting of young, vigorous cells, which were obtained by twenty four hours' cultivation in wort at 26° C., and each species was introduced in equal amount into the flasks.

In A, *Saccharomyces cerevisiae I.* *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*

In B, *Carlsberg bottom yeast No. 1*, *Carlsberg bottom yeast No. 2*, *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*

In C, *Carlsberg bottom yeast No. 1*, *Carlsberg bottom yeast No. 2*, *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*, *Saccharomyces Pastorianus II.*

In D, *Saccharomyces cerevisiae I.*, *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.*, *Saccharomyces ellipsoideus II.*

After the flasks had received the above mixtures of yeasts, they were set aside at the ordinary room-temperature and after two days they were well shaken and average samples from each were introduced into fresh flasks containing some of the same sugar solution. Five cultures were prepared in the manner described in the first experiment, and these were left undisturbed for two days. The cultures contained in the fourth and fifth flasks were tested, the fourth after eight, and the fifth after ten days, calculated from the commencement of the experiment. This was done in both cases by well shaking the flasks so as to mix the yeast with the sugar solution, and then introducing average samples into a series of flasks containing beer wort. The flasks from which the samples had been withdrawn were then left at rest for a short time until the remainder of the yeast had settled to the bottom; the liquid was then poured off as completely as possible, and in its place a suitable quantity of wort was introduced. In this way it was not only possible to work with average samples, but all the yeast

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present in the sugar solution of the corresponding culture was, so to speak, collected in the two series of flasks, and the last series containing the wort received scarcely any of the strong acid liquid. The importance of this may here be emphasized, where it is a question of bringing about the multiplication of yeast cells which have become enfeebled. In all cases sterilized liquids were employed and the introduction of foreign organisms from without was carefully guarded against.

If the described treatment with the solution of cane sugar and tartaric acid had really effected a purification, these flasks with the cultures in wort must have contained the purified brewery yeast freed from the disease germs originally present. Accordingly, we must, therefore, expect to find in the cultures from **A** a pure growth of the brewery yeast ***Saccharomyces cerevisiae I.***, in the cultures from **B** a pure growth of the ***Carlsberg bottom yeast No. 1 and No. 2***, in the cultures from **C**, also the ***Carlsberg bottom yeast No. 1 and No. 2***, and in the cultures from **D** a pure growth of ***Saccharomyces cerevisiae I.***. The result, however, was a very different one.

The cultures were placed in an incubator at a temperature of 26° C., but only those flasks which contained the yeasts from **A** and **B**, and which had been submitted to the described treatment in the sugar solution during eight days, showed signs of development; all the other cultures must be regarded as having perished; even after they had stood for several weeks there was no sign of life. The flasks in which growth had occurred showed distinct low-fermentation phenomena, and the beer had the disagreeable bitter taste and unpleasant odor which are produced by the disease yeast ***Saccharomyces Pastorianus I.*** Even from this it might be concluded that it did not contain a pure culture of the species of brewery yeast originally introduced. The problem now was to more closely investigate what was the composition of the yeast; for this purpose a dilution and examination of the cells was carried out in accordance with the method described in the first experiment.

The result arrived at was that only a single species was present, namely, the disease producing ***Saccharomyces Pastorianus I.***, this species alone had survived the treatment in the sugar solution.

Experiment VI.

Whilst in the previous experiments equal quantities of the different yeasts were taken, in this case the "disease" producing yeasts were mixed with the culture yeasts in the proportion of one to five. The brewery yeast present in each flask was thus in positive excess at the very beginning. The experiment was conducted with the following five flasks :

In A, ***Saccharomyces cerevisiae I, Saccharomyces Pastorianus I.***

In B, a ***brewery bottom yeast, Saccharomyces Pastorianus I.***

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In C, *Carlsberg bottom yeast No. 2.*, *Saccharomyces Pastorianus I.*

In D, *Saccharomyces cerevisiae I.*, *Saccharomyces ellipsoideus II.*

In E, a *brewery bottom yeast*, *Saccharomyces ellipsoideus II.*

The sugar solution in this case contained only 3-8 per cent. of tartaric acid. In other respects the conditions were the same as in the fifth experiment.

It was found that the yeasts contained in the flask *D*, namely, *Saccharomyces cerevisiae I.* and *Saccharomyces ellipsoideus II.*, had perished after they had been subjected for ten days to the described treatment in the sugar solution; on the other hand, after eight days' treatment, they were still alive. In all the other flasks some, at least, of the species withstood the treatment, not only for eight but for ten days. The yeasts were diluted, and in the case of each flask a very considerable number (in some cases as many as eighty) of cultures were made from the cells in accordance with the methods described above; the following results were obtained :

A. Both species sown were alive, but whilst at the commencement of the experiment the cells of *Saccharomyces cerevisiae I.* were five times as numerous as those of *Saccharomyces Pastorianus I.*, the ratio was now completely changed. The disease yeast predominated, whilst the brewery top-yeast had become suppressed to such an extent that it could only be detected by special cultivation in wort at 37-38° C., a temperature which, under the given conditions, is still favorable to *Saccharomyces cerevisiae I.*, but not to *Saccharomyces Pastorianus I.*.

B. *Saccharomyces Pastorianus I.* only was found, and not a trace of the *brewery bottom yeast* was detected.

C. The cells of *Saccharomyces Pastorianus I.* were present in large preponderance; only a doubtful trace of *Carlsberg bottom yeast No. 2* was found.

D. *Saccharomyces ellipsoideus II.* was in superabundance; *Saccharomyces cerevisiae I.* could in this case also only be detected by cultivation in wort at 37-38° C.

E. Both species sown, the *brewery bottom yeast*, and *Saccharomyces Pastorianus II.* were found, but the experiment showed that the latter now formed half of the mixture, whilst at the commencement, as will be remembered, it only constituted one-sixth; the disease yeast had, therefore, multiplied also in this case at the expense of the brewery yeast.

As the main result of this experiment it is found that the two disease yeasts, *Saccharomyces Pastorianus I.* and *Saccharomyces ellipsoideus II.*, had suppressed the brewery yeasts. The first of them, as has been already mentioned, imparts to beer a disagreeable taste and odor, and the second produces in low fermentation beers the disease known as yeast

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turbidity. The presence of an excess of the brewery yeasts at the commencement of the experiment was therefore of no avail.

It will be remembered that in the lectures quoted above, Velten bases his arguments entirely upon Pasteur's standpoint, as enunciated in his "*Etudes sur la bière*." With him the question was merely the removal of the bacteria. My doctrine of the alcoholic ferments he disregards, and he considers a brewery yeast to be pure when he has freed it from bacteria. My investigations have, however, definitely proved that the three species of *Saccharomyces Pastorianus I.*, *Saccharomyces Pastorianus III.* and *Saccharomyces ellipsoideus II.* produce diseases in low-fermentation beers (see the account in subsequent pages of this book). The correctness of my results has been confirmed by Alfred Jörgensen, Grönlund, Will, Lasche, Kokosinski, and others. It has been further proved in recent years that in addition to those mentioned there are several other *Saccharomycetes* which are able to produce diseases in beer. Indeed, many facts point to there being a large number of such species. The question whether a purification of the brewery yeast has or has not been effected by its cultivation in the sugar solution must therefore be considered not only with reference to the bacteria, but also with very special regard to the species of disease yeast mentioned. Regarded from this point of view, not only are the results of the fifth and sixth experiments opposed to Veltcn's statement, but this is really also the case with the first four experiments. *Pasteur's method for the purification of brewery yeast recommended by Velten and Dueaux effects therefore no purification whatever as regards the disease yeasts, but on the contrary it brings about a more vigorous development of the disease-producing ferments. This holds good for the experiments with both high and low fermentation yeasts. Pasteur's method is consequently absolutely useless in the brewery.* Where it is introduced it will lead to great loss and difficulties.

The sixth experiment likewise showed that in the manner employed the method does not with certainty lead to a pure culture.

The above experiments were published in 1891 in the "*Compte-rendu des travaux du laboratoire de Carlsberg*." It might have been expected that in view of such facts, opposition would have ceased, especially as my opponents were not able to detect any error in my experiments or in the conclusions which I drew from them. This, however, was not the case. Velten tacitly recognizes that my experiments are correct, and he therefore cannot oppose the results obtained under the given conditions; but then he introduces new objections ("*La Gazette du Brasseur*," 1891) *that the disease yeasts were present in my yeast mixtures, in too high a proportion in comparison with the brewery yeasts, and further, that the experiments should have been conducted at a lower temperature than 25° C, which I in part employed.* If I had experimented as he suggests, I should, he states, have obtained quite a different result, and he designates Pasteur's method as simpler and more rational than my method, which, as is known, starts with a single cell. In the detailed description of Pasteur's method which Velten gave in 1878 there is not a word to the effect that the cultivation should be conducted at a low temperature, and neither does any such statement occur in the works of Pasteur and Duclaux. Velten has only words to bring forward;

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he has no proof and no experiments. My colleague, Mr. Jörgensen, has published a sharp rejoinder to Velten's assertions in "*La Gazette du Brasseur*" (1891, No. 215), and in the "*Allgemeine Brauerand Hopfenzeitung*," Nuremberg (1891, No. 142). In this Jörgensen gives the following account of his own experience:

"Eleven years ago, when the Tuborg brewery at Copenhagen was suffering from a very pronounced yeast turbidity of the beer, and which was produced by wild yeasts, I made use of Pasteur's method for purifying the yeast on a large scale, the yeast being treated with the tartaric acid solutions in vats of 5 to 6 hectoliters capacity in the fermenting cellar, and at the temperature employed in low fermentation. No improvement whatever was "obtained, the disease always occurred again, and only disappeared when a true pure yeast was introduced in accordance with Hansen's method in the place of the yeast purified by tartaric acid. It may further be stated that during the last half year a large number of experiments have been made in my laboratory with mixtures of culture yeasts with widely differing amounts of the various wild yeasts, and amongst the latter disease yeasts, these mixtures being cultivated in wort containing about 0.5 per cent. of tartaric acid. It has always been found that the wild yeasts gradually developed at the expense of the culture yeast, and that the latter became completely suppressed. It is thus shown that the addition of tartaric acid to the wort also fails to effect any purification, but rather that it favors the development of disease yeasts."

In order to, if possible, silence my opponents, and to thoroughly investigate the question, I carried out the following experiments, in which low temperatures were employed, I as demanded by the last objections raised by Velten, and a yeast mixture was made use of in which the proportion of disease yeasts was extremely small. My assistant, Mr. Nielsen, assisted me in these experiments.

Experiments VII and VIII.

In these new experiments I employed the ordinary pitching yeast of a low-fermentation brewery in which the fermentations were perfectly normal and the beer of which was in every respect satisfactory. This brewery was provided with yeast from a pure yeast propagating apparatus, containing an absolutely pure culture of one of the species of bottom yeasts which yield spores with great difficulty. In the ordinary gypsum block cultures of this yeast, the cells developed either no spores at all, or, at most, very few after six to seven days at 25° C. The spores found had further the characteristic appearance of the spores of a culture yeast, and could be distinguished microscopically from the spores of a wild yeast. My method for the analysis of brewery yeast could, therefore, be employed with safety. In the samples which I examined in this way, I was unable to discover traces of wild yeast, and the microscopic examination likewise indicated that the whole of the yeast consisted only of the low-fermentation species of the brewery. Holm and Poulsen have submitted my method to a

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thorough examination, and have found that by its means it is possible to detect as little as 0°5 per cent. of wild yeast when admixed with a brewery yeast ("Compte-rendu des travaux du laboratoire de Carlsberg," 2 vol. 4 and 5 livr. 1886-88). The examination of the pitching yeast under discussion showed then that it contained either no wild yeast at all, or, at any rate, only extremely minute traces. As I took this yeast as the starting point for my analysis, it is evident that the objection raised by Velten that my yeast mixture had an abnormal composition such as never occurs in practice, does not apply in this case. The cultivation of the yeast in a solution of cane sugar and tartaric acid was carried out in the manner described in the fifth and sixth experiments. Two Series of Experiments were conducted with this brewery yeast, one at the ordinary room-temperature (as a rule 17° C. during the day, and 10° C. at night), and the other at a constant temperature of about 9° C.

In the first series the composition of the yeast had not only changed after four and five cultivations in the sugar solution, but even in the third cultivation, and to such an extent that after cultivation in wort a growth was obtained which consisted in the main of wild yeast. The ordinary gypsum block cultures showed, after three to four days at 25° C., a very abundant development of wild yeast cells with spores of a highly typical appearance, and the microscopical examination of the newly formed yeast likewise showed that most of the cells had the same appearance as those of the *ellipsoideus* and *Pastorianus* groups. In the second cultivation in the sugar solution the yeast had not yet experienced so marked a change, and the brewery yeast still preponderated.

The second series of these experiments was carried out at a temperature of about 9° C., as stated above. When the third culture in the sugar solution had stood two days, an average sample was introduced into a fourth flask. The yeast from the third cultivation was then introduced into wort also at 9° C. In the same manner, the yeast from the fourth and fifth cultivations in sugar solution, after standing the usual time, was likewise cultivated in wort. In consequence of the low temperature employed, it was only after about twelve days that a perceptible development of yeast in the last-named wort culture was detected, and only after fifteen days in the two others. If Pasteur's method were correct, the yeast growths in these three flasks should consist, therefore, of the pure brewery yeast. A contrary result was, however, also obtained in this case. *The brewery yeast had, namely, as in all my other experiments, been completely suppressed by the wild yeasts*, and yet at the beginning of the experiment these were, as stated above, only present in an extremely minute proportion. The new objections brought forward by Velten are, therefore, completely refuted.

The fact that Pasteur and his collaborators recommended a method which even brought about diseases in beer, clearly shows that they had no knowledge of the part played by certain wild yeasts in producing disease, and that the nucleus of the problem of the pure cultivation of yeast had altogether escaped their notice. I have, therefore, been compelled to conduct these researches on new lines, and my opponents have not been able to pardon me for this. In the attacks, whether small or great, which have been directed against me, a wrath is manifest that the new reform came from Denmark and not from France.

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3. The above experiments have shown that the **brewery bottom yeasts** which were investigated are unable to resist the treatment with the tartaric acid and sugar solution; the top-fermentation yeast ***Saccharomyces cerevisiae I.***, appears to possess a somewhat higher resistive power, but this species was also suppressed by the wild yeasts. The disease yeasts ***Saccharomyces Pastorianus I.*** and ***Saccharomyces ellipsoideus II.***, exhibited the greatest resistive power. It is possible that somewhat different results might be obtained under other conditions.

The objections which have been brought forward against the employment of tartaric acid for the preparation of pure yeast, also apply in principle to other similar substances and methods. It would, therefore, be an error to assume that by ***substituting another antiseptic for tartaric acid, as, for example, carbolic acid, salicylic acid, hydrofluoric acid, &c., it would be possible to destroy not only all bacteria present, but likewise all the species of wild yeasts, so that only the desired species or race of culture yeast would remain, freed from all competing organisms.*** Such a universal remedy does not exist. It must also be remembered that even if we have got rid of the bacteria and wild yeasts, we are still far from having attained the desired pure culture. There are, namely, a large number of species of "good beer yeasts, both high and low, and many of these, although widely different in other respects, exhibit the same behavior towards antiseptics. The conditions are further complicated owing to the fact that cells belonging to one and the same species may behave differently under the same treatment according to their varying conditions, according to whether they are young or old, well or ill nourished, &c. It is also by no means a matter of indifference whether the pitching yeast consists only of the desired species, or whether this is mixed with other brewery yeasts; this is shown by the fact that species of culture yeasts, each of which when used alone gives a good beer, may under certain conditions produce disease when mixed (see following chapter). In short, certainty cannot be attained in the manner mentioned above; it is, in fact, necessary to start from the single cell and from this to prepare the absolutely pure culture.

It is a known fact that an addition of tartaric acid retards the development of most species of bacteria which occur in pitching yeast and in wort; from what has been stated above, it will be seen how dangerous this remedy may become. When it is proposed to employ it in breweries, it is, therefore, advisable to add it in only small quantities, and, indeed, to use it with caution. Good breweries are conducted even at the present day without resort to antiseptics, and the way to do so is to maintain strict cleanliness and order, and to employ a good pure culture yeast in the fermenting vessels.

When it is a question as to whether wild yeasts are present or not in a certain pitching yeast, the method which I indicated some years ago is employed, that is, culture tests for spore formation are carried out at 25° and 15° C. Under these conditions, the wild yeasts form spores earlier than the brewery yeasts, and there is a further difference in the appearance of the spores. As was mentioned above, it is possible by means of this method to detect as little as 0.5 per cent. of wild yeast in a brewery yeast. Nevertheless, the detection of such a small admixture is no easy matter, and requires a good deal of practice. It has been shown,

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however, from the above that cultivation in a 10 per cent. solution of cane sugar containing 4 per cent. of tartaric acid, affords an excellent means of ascertaining the presence or absence of wild yeasts in a pitching yeast. In all the cases examined, the brewery yeast was suppressed by the wild yeasts when the latter were present at the commencement, and this occurred both with high and low brewery yeasts. My experiments in this direction were so numerous that the rule which they enabled me to establish is certainly one of wide application; it is, however, highly probable that exceptions may be found by further research. Every method is known to have its limits, and this is especially the case with biological methods. For the practical analysis of the yeast in the fermenting vessels, this test is, however, too sensitive, for, as we have seen, my experiments proved that a good brewery yeast showed, by this test, a high degree of contamination with wild yeast. Its employment might, therefore, lead to a pitching yeast being condemned, which for practical purposes is really a good yeast. Whether the method could be so modified as to render it applicable to the above purpose, has not been investigated. The question, however, it deserves further investigation, and may be recommended to the attention of zymotechnologists.

In many of the breweries where my pure yeast system has been introduced, the closed propagating apparatus mentioned above is now employed in which the yeast is developed in a state of absolute purity. It is evident that there must be no contamination whatever in the fermenting cylinder, and, therefore, of course, no trace of wild yeast. If the apparatus is handled with strict care, the yeast in it will remain pure for an indefinite period. I know breweries in which the apparatus has been -in uninterrupted use for years without it being necessary to introduce a fresh pure culture. But if the apparatus is not properly handled, contamination may readily occur. There is, however, no necessity to further discuss this matter, as full information, both with regard to the construction of the apparatus and its employment in the brewery, will be found in the earlier part of this book.

From what has been stated above, it is evident that the yeast in the apparatus should, from time to time, be submitted to a rigorous test. For this purpose, samples of the fermenting wort should be carefully drawn from the fermenting cylinder; this is best done towards the end of the primary fermentation, for at this stage it is easier to detect both bacteria and wild yeasts if they are present. From these samples small portions are introduced into flasks containing yeast extract, and these are exposed to a moderately high temperature e.g. 25° C., in order to ascertain whether bacteria are present. The remainder is allowed to stand until the yeast has settled to the bottom; the beer is then poured off and average samples of the yeast introduced into the tartaric acid and sugar solution. The further treatment is carried out in the manner described in the fifth and sixth experiments at the ordinary room-temperature or at 2 5° C.; it is sufficient to carry out three or at most four cultivations in the sugar solution. After this treatment, the yeast is cultivated a few times in wort and then it is examined under the microscope, and by means of my method of analysis by spore formation. When wild yeast is present in such small traces that could not be detected by previously known methods, the treatment with tartaric acid will cause them to develop sufficiently strongly to render their detection easy. The same also holds good for some of the species described under the name **Mycoderma cerevisiae**. For this analysis, therefore, I recommend the occasional

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employment of the described method of treatment with tartaric acid.

4. As formerly, I should still prefer to avoid all criticism of Pasteur's work, but my opponents have not permitted this. When we examine more closely as to what Pasteur really meant when he spoke of the preparation of a pure yeast for brewery purposes, we do not find any such definite statement in his work as we could wish, but there is much to indicate that he himself recognized the limit of his methods (*"Études sur la bière,"* p. 227), and that his object was merely to free the yeast from bacteria. Where he gives an account (loc. cit, pp. 4-7) of the disease micro-organisms, which, in his opinion, are able to attack beer, it is, therefore, only bacteria which he describes, and there is no mention of alcoholic ferments. (This view is, as was previously stated, also held by Velten, and has likewise been expressed by Duclaux in both of his works, namely, *"Chimie biologique,"* 1883, p. 618, and *"Le microbe et la maladie,"* 1886, pp. 91-95). After mentioning the different methods which he employed in 1876 for the purification of yeast, Pasteur says (p. 227): "The best way to determine whether a yeast is pure or not, is to use some of it for making some beer in a two-necked flask; when the fermentation is at an end, the flask is placed in an incubator at 20 - 25° C. If, after some weeks, the beer does not become cloudy nor covered with a film, if the sedimentary yeast appears to be pure when examined under the microscope, and finally, if the taste of the beer does not become impaired in any other way than through flatness, there is every reason to presume that the yeast was pure. "The test affords a good illustration of the standpoint of research at that time.

If it is merely desired to ascertain whether bacteria and film-forming species of *Mycoderma* are present, the method is very useful. On the other hand, it is quite useless when it is also a question as to whether the yeast cells present belong to one or several species. The sedimentary yeast may, in fact, consist of a mixture of a good beer yeast with some of the worst species of disease yeasts, without the possibility of these being discovered under the conditions described. The microscopic examination does not suffice in this case, and the same applies to the other characters. This is seen even from theoretical considerations, and tangible proof is obtained by direct experiment. Pasteur's test can thus only be of service where it is exclusively a question of bacteria and *Mycoderma*.*

* In some zymotechnic journals, the attention of brewers has recently been called to Pasteur's test described above as a means for ascertaining how the beer will turn out in practice. This is, however, a great mistake. The beer produced in the flask is of a very different character from that produced in the brewery, even when the yeast and the wort are the same. The fermentation and the struggle between the organisms present take place under such different conditions in the two cases that no comparison can be made.

The most definite statement of Pasteur's, with reference to this question is in the *"Bulletin de la Société d'encouragement pour l'Industrie nationale"* (Janvier 1887, p. 45), in which he says "Hansen was the first to perceive that brewery yeast must be pure not only as regards bacteria, the true disease ferments, but that it has also to be freed from the wild species of

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yeast."

Pasteur's work and mine start from two different standpoints. With Pasteur it was the bacteria which produce the diseases of beer, and accordingly the problem with him was to free the yeast from those minute organisms, and this he attained by means of the method described. His object was to purify the brewery yeast (*purification des levûres*), not, however, to prepare a true pure culture from it.

From my standpoint, on the other hand, the alcoholic ferments play a chief part. Since I showed, in 1883, that some of the commonest and most dangerous diseases of low-fermentation beer are not caused by bacteria, but by certain species of the **Saccharomycetes**, it followed that a purification of the yeast, such as that proposed by Pasteur, would not lead to the end in view, but that the true pure culture was necessary. And since a careful study of the **Saccharomycetes** led me to the conclusion that the views of my predecessors in this field were incorrect, and that, for instance, the systematic name **Saccharomyces cerevisiae** included a whole series of species and races of high and low yeasts differing widely in their action, it followed further that it did not merely suffice to prepare a pure culture, but that, in order to satisfy the different conditions required in the manufacture of the various kinds of beer and wine, and also of spirit and bakers' yeast, a systematic selection of the most suitable species or race must be made. ***In this way I was led to introduce into the fermentation industries the same principles which I have long been adopted in horticulture and agriculture for the cultivation of the higher plants.***

Starting as I did from a different point of view and with different methods from those of my famous predecessor, the results were of necessity also different. In my writings, I have often emphasized the great importance of Pasteur's "**Études Sur La Bière**," in relation to my researches, and I again gratefully acknowledge it. On the other hand, I cannot help protesting against the attempts made in France to check development, and to bring everything back to the standpoint of 1876, for this is antagonistic to all progress. If the famous teacher of my opponents had continued his studies in this field, he would himself have carried them much further.

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CHAPTER VI. Investigations on the "diseases" of beer, produced by alcoholic ferment.

1892.

1. Introduction.

My first memoir on this subject was published in the "*Transactions of the Carlsberg Laboratory*," vol. ii., No. 2, 1883. The division of my researches on the organisms of fermentation into two series had not then been introduced, and thus it is that this memoir appears amongst my "*Recherches sur la physiologie et la morphologie des ferment alcooliques*," although, from the matter which it contains, it belongs rather to the new series which I commenced publishing in 1888 under the title "*Untersuchungen aus der Praxis der Gärungsindustrie*." In 1884 I published in the "*Zeitschrift für das ges. Brauwesen*," a short account of some new studies in the direction indicated. This paper, however, contained only the main results of my experiments carried out at that time, and I promised to give a more detailed account later on, and at the same time to discuss the different sides of the question. I have since continued these investigations at intervals, and have carried out more than fifty Series of Experiments. I believe they are now completed, _at least for the present, and in publishing the results I have attempted to fulfill the promises given in the two preliminary communications mentioned.

Those of my researches which have a direct bearing on the fermentation industry may be grouped around the three main questions, namely: *the question of the diseases of beer, the question of the pure cultivation of yeast, and the question of the employment of systematically selected species or races of yeast.*

It was the result obtained on attacking the first of these questions which caused me to include in these practical studies also the other two questions. If, for instance, it had been found that the alcoholic ferment do not cause disease, there would have been no cogent reason for introducing true pure cultures into practice, and in consequence also none for the selection of a certain single species or race. Thus the question of the diseases of beer and other fermented liquors is one of great importance. Its solution was not arrived at all at once, many investigators having worked at this problem for a long time past. The investigations in this field were closely connected with those relating to spontaneous generation, which, as is well known, led to the development of a new experimental science, namely, the science of micro-organisms. Amongst these, the doctrine relating to the diseases of fermented liquids occupies a conspicuous place. An account of the manner in which this doctrine gradually developed will therefore be of interest both to the biologist and to the practical zymotechnologist. This, however, will naturally only be the case when the description is based upon a thorough study from the original sources, and when it presents to us in a clear manner the variable

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standpoints, not detached, but in their consecutive order. In the following part of this chapter I have attempted this. It is the first time that the history of this question has been written.

2. How the doctrine of "diseases" in fermented liquids was gradually developed.

It may here be pointed out that in speaking of "diseases," this word is intended to apply to those objectionable changes which fermented liquids, especially beer and wine, are liable to undergo in consequence of the attack of micro-organisms. Closely connected with the investigations on the diseases of fermented liquids is, as mentioned above, the important question of spontaneous generation (*generatio æquivoca*). **By spontaneous generation is understood the development of living organisms from dead matter, especially from shapeless organic matter without eggs, seed or germs.**

There have been naturalists at all times who embraced this view. In the years 1745-1756 it was revived by the writings of Needham. One of Needham's experiments consisted in strongly heating meat extract in closed flasks, and since organisms developed in these flasks, he considered that they must have been produced by spontaneous generation. Buffon and a large number of other **savants** adopted his doctrine.

There were, however, also opponents to this view, the most famous of whom was Spallanzani. In 1765 he commenced the publication of a Series of Experiments against the view upheld by Needham. The flasks with which he made his experiments were closed hermetically and placed in a vessel of boiling water, and the high temperature maintained for about an hour. No micro-organisms appeared in the flasks after this treatment, not even after they had been cooled; this occurred, however, at once when air was allowed to enter. Spallanzani concluded from his experiments that spontaneous generation did not take place, and that the germs, or, as he called them, the eggs for the development of microorganisms were present in the air. When these gained access to the dococtions with which he and Needham experimented, their further development took place.

It would lead us too far from our subject to discuss the history of this remarkable doctrine, and we will therefore only mention those points which are of special importance in relation to the investigations on the diseases of fermented liquids. Consequently, only that portion of the literature will be quoted which has a direct bearing on this question.

As long ago as 1782, the famous Swedish chemist Scheele made practical use of Spallanzani's experiments; namely, he published a method for the preservation of vinegar.* According to this method, the vinegar is put into bottles, which are then well closed and placed in a vessel containing water. The latter is then heated, and after it has boiled for some time the bottles are taken out. Vinegar which has been treated in this manner will, as Scheele states, keep indefinitely without becoming turbid or spoiled. It is the same method which is employed to this day.

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* Carl Wilh. Scheele, Anmärkningar om sättet att conserven Ättika (Kongl. Vetenskaps Academiens nya Handlingar, tom. iii. Stockholm, 1782, p. 120. Also in French (according to Pasteur) in Scheele's "**Mémoires de Chimie,**" Dijon, 1735. After Scheele": death, his works were published also in other languages.

The first indication of the relation of micro-organisms to the diseases of fermented liquids, is found in Chaptal's work, "**L'art de faire le vin,**" which appeared in 1807.† "There is," he says, "a phenomenon which has not only attracted the attention of numerous authors who have interested themselves in the diseases of wine, but has also perplexed them. I refer here to the films (*les fleurs du vin*) which develop in the casks, and especially in the bottles, on the surface of the wine. This film-formation always occurs prior to the wine becoming sour, and foretells that this will take place. In my opinion, it is a vegetation a byssus growth which belongs to the ferment-substance." We find here the conception of disease connected with that of growth, but we can clearly place no weight upon this; it must be regarded more or less as an isolated idea, a vague conception. At any rate, Chaptal's statement had no appreciable effect on the progress of research, and I have only mentioned it because it appears to contain the first germ towards the assumption that the relation between the diseases and micro-organisms is one of cause and effect.

† My source in this case is Pasteur's "**Études sur le vin,**" as there is not a copy of Chaptal's work in any of the public libraries at Copenhagen.

A practical application of Spallanzani's experiments, similar to that of Scheele, was made by Appert at the beginning of the century. The latter, in 1810, published in Paris a noteworthy book, in which he gave a detailed description of a method of preserving various foodstuffs by means of heat. In this book he states that he had spent a great part of his life in kitchens, breweries, wine cellars, in confectioneries, distilleries, and in grocers' storehouses. In short, he was a thoroughly practical man; at the same time, his book shows that as an experimenter he was highly talented, and that he had studied at least a great part of the literature which was of importance for his experiments. However, he does not mention Scheele's discovery, and he probably did not know of it. If he had heard of it, it may have been through Gay-Lussac. In 1810, the latter chemist read before the Paris Academy a treatise on Appert's method, and it is therefore not improbable that they were personally acquainted. Appert states that the details of his method are in the main as follows: (1) the substances to be preserved are enclosed in bottles or other glass vessels; (2) these various glass vessels are corked with the greatest care, for success depends upon the manner in which the vessel is closed; (3) the substances thus enclosed are submitted to the action of boiling water, heated in a water bath for a longer or shorter time "according to their nature, and in the manner described for each substance; (4) the vessels are taken out of the water at the appointed time. All the apparatus and the details of manipulation are fully described, and accurate directions are given for the treatment of various fruits, vegetables, soups, milk, fruit juices, &c. In a comparatively short time, several editions of his work appeared both in France and in other countries, and Appert became a rich and a famous man.

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In the fourth French edition,* he gives detailed instructions for the use of the autoclave, a modification of Papin's digester. In this edition there are, however, two chapters which are for us of still greater interest, one treating of wine, p. 131, and the other of beer, p. 167. Appert states that in his time the finest wines of France would not bear even a short sea voyage; some, indeed, were so easily spoiled that they could not be exported at all, but had to be consumed at the place where they were made. Appert treated these wines as follows: The wine was drawn off into bottles which were filled to the neck; these were then hermetically closed and the stoppers secured with iron wire; a small air space was left between the surface of the wine and the stopper. The bottles were placed in a water bath, the temperature of which was cautiously raised to 70°. Some were then shipped to St. Domingo, and on their return, after a lapse of two years, they were examined. For comparison he had set apart some bottles of the same wine, but which had not been heated. The latter had a disagreeable taste, whilst the wine which had been heated proved highly satisfactory in every respect. His experiments thus showed that a wine, which under ordinary circumstances would not stand a journey, had in this case borne the voyage without any ill-effects whatever. He was, therefore, justified in pointing out the great benefit which his method would bring to France in that it would render possible the exportation of the fine wines of the country to the most distant regions of the earth. He also submitted beer to the same treatment, and obtained a similar favorable result.

* *"Le linceul de tour: is ménnges, ou l'art de conserver, pendant pluaieurs années, toutes les substances animales et végétales"*; par M. Appert. Quatrième édition, Paris, 1831.

Appert was not able to give an explanation of what actually took place on heating, and he did not get further than to perceive that it was the "principle of fermentation" which was destroyed. He saw, that neither fermentation nor putrefaction took place in the substances which he submitted to the action of heat. It was only after Cagniard Latour and Schwann had shown that fermentation was caused by the activity of microscopic organisms that the explanation was forthcoming.

Thus, long before anything was known as to the causes which bring about the diseases of fermented liquids, a method had been found for their prevention, which, in fact, is the best that we have at the present day. What has been added later by various technologists, consists only of small improvements; in all essentials we employ the method of heating elaborated by Scheele and Appert. There is, however, no rule of universal application. If a satisfactory result is to be attained, the heating process must be arranged to suit the character of the different liquids under treatment. What will suit one kind of beer or wine will not always prove satisfactory with another.

Appert's method of preserving wine and beer does not appear to have found any general application until Pasteur took the matter in hand. Pasteur made great efforts to bring about the general application of this method of treatment to wine. His associate, Velten, carried out some experiments with beer. At the present time, the method is known all the world over

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under the name Pasteurization.

Scheele's name has been completely forgotten in connection with this, and there are not many who know ***That we are indebted to a Scandinavian for this beautiful and practical discovery.***

Spallanzani's experiments and results were received favorably only by the few, and in particular it was argued against them that the air enclosed in his hermetically closed flasks was changed by the boiling, and also that it was present in too small a quantity to enable spontaneous generation to take place.

In 1836-1837 a number of experiments were carried out by Franz Schulze and Theodor Schwann separately, which proved that various readily fermentable and decomposable substances could be preserved unchanged if they were boiled and if care were taken that the air with which they subsequently came into contact, was freed from its germs. Both these experimenters, therefore, fitted their flasks with doubly bored stoppers through which bent glass tubes passed. The tubes served for the passage of the air into the flasks. In Schulze's experiments, the air which was drawn into the flask after boiling was purified by passing through sulfuric acid. Schwann, on the other hand, effected this purification by heat. The experiments of both showed that any quantity of air, however large, could be passed through such decoctions without putrefaction, fermentation or the development of micro-organisms taking place; these experiments prove, therefore, exactly the opposite of Needham's statement, and they confirm the correctness of Spallanzani's results.

The years 1836-1839 are notable in the history of microbiology for the epoch-making researches of Cagniard Latour and Theodor Schwann, who, for the first time, proved that yeast consists of living cells, and that it is these which bring about alcoholic fermentation. What had been previously stated in connection with this, consisted only of conjecture.

Kützing simultaneously arrived at a similar result, and in his treatise he not only gives descriptions and figures representing yeast cells, but also the acetic ferment and various mold fungi. * He distinctly noted the difference between yeast cells and the cells of the acetic ferment; and faulty as his figures of the latter are, they still show that even at that time he had really discovered one of the species of bacteria which bring about the production of acetic acid. This was described by him under the systematic name *Ulvina ateti* on account of its giving the acid fermentation. When wine and beer become sour from the formation of acetic acid, the effect is spoken of as a disease, and is indeed regarded as one of the very worst. In Kützing's writings we find the first indications as to how such a disease can occur. He did not, however, make use of the term disease, and neither did it occur to him to make any practical application of his investigations.

* Kützing, "***Mikroskopische Untersuchungen über die Hefe und Essigmutter,***" "Journal f. praktische Chem.," 1837, 2 Bd. p. 385.

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The same applies also to Turpin. The latter published in 1838, a memoir * in which he described just as Kützing did not only alcoholic and acetic ferments, but also several other micro-organisms. The main result at which he arrived is given (p. 134) in the following proposition: "No decomposition of sugar, no fermentation without the physiological activity of vegetation." Thus even at that time it was known that there are different micro-organisms which bring about different fermentations; but a clearer understanding of these relationships was wanting, and both Kützing and Turpin held quite wrong views regarding the origin of the microscopic organisms.

Against the experiments of Schulze and Schwann mentioned above, the objection could still be raised that the air which was allowed to enter their flasks, had been previously submitted to such a violent treatment in Schulze's experiments by washing with sulfuric acid, and in Schwann's by heat- that it was possibly altered in such a way that, for this reason alone, the dead matter was unable to enter into life. This argument was answered by the beautiful experiments which Schröder and Dusch conducted in 1854. The method of experimenting adopted by these investigators was the same as in the preceding cases, except that a cotton-wool filter was attached to one of the tubes passing through the stopper. The air entering the flask after its contents had been boiled, was made to pass through this filter; in this case, the purification of the air was, therefore, effected by filtration. They chose cotton-wool as filtering medium "because" as they stated, "as is known, it is able to retain on its surface the miasmata of infectious diseases." The boiled organic substance in the flask came into contact in this experiment not only with a large volume of air, but also with air the composition of which had in no way been altered; the small particles suspended in it had alone been removed. In their experiments with meat extract and wort they obtained the same results as Schwann; they did not always succeed, however, in their experiments with milk and some other substances. There was thus still some uncertainty in several points.

* Turpin, "*Mémoire sur la cause et les effets de la fermentation alcoolique et acétouse. Paris, Lu à l'Académie. 1838*"

These old experiments relating to spontaneous generation are still of especial interest for the fermentation industry in that they established not only the principle of sterilization, but they furnished also a model for the necessary apparatus. They also showed that beer wort is sterile after boiling, and that it can be kept in this condition even when air is passed through it, provided that care be taken to free the air from germs. In practice, the purification of the air has been effected partly by Schwann's and partly by Schröder's and Dusch's methods, and the latter especially has received a very wide application. The flasks with bent tubes employed by Schulze, Schwann, Schröder, and Dusch have become models for the different culture flasks which are now employed in bacteriological and zymotechnic laboratories. In most cases these flasks are plugged with Schröder and Dusch's cotton-wool filters. The high degree of perfection which has been attained in the technique of this subject is due, however, more especially to Pasteur and his pupils. The culture flasks of the laboratory have likewise served as models for the larger forms of apparatus which are now employed in many breweries for the pure cultivation of yeast.

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The first indication that some of the alcoholic ferments are to be regarded as the cause of disease, is found in Bail's writings. * Thus in 1857 he expressed the opinion that different species of yeast often bring about different kinds of fermentation; and he further suggests that it might possibly be of practical importance to carry out the systematic cultivation of any one species. As is known, it is easier to give expression to an idea than to prove _ by experiment how far one's views are correct; but it is only by means of such proof that true success is attained, whether in science or in practice. Bail conducted no experiments, and confined himself to the expression of the above views. He likewise advocated the doctrine, both in the treatise referred to and still more so in his later writings, that ***Hormiscium cerevisiae*** (beer yeast) and also other yeasts are merely a stage in the development of higher forms e.g. ***Mucor*** and other mold fungi. That his researches were quite incorrect, has been proved by the investigations of De Bary and Reess.

* Th. Bail, "***Ueber Hefe,***" Flora, 1857, Nos. 27 and 28, p. 438.

In the zymotechnic journals of the years following, the opinion was expressed here and there that in the fermentation industry, different species or races of both top and bottom yeast occur. Observations in practice would naturally readily lead to such a view, and the researches mentioned above no doubt also helped.

We have now reached the point where Pasteur took the subject in hand. He published in 1857 a treatise on the lactic acid fermentation, and in this he showed that the fermentation was brought about by an organized body which, according to the opinion he then held, was closely related to beer yeast. That he was not at that time able to decide as to what kind of a micro-organism it was, is evident.

In 1860 Pasteur published the chief results of his numerous and very comprehensive investigations relating to spontaneous generation. He continued this work during the years following, and opposed with great energy and ability the experiments which were made again and again in support of Needham's doctrine of spontaneous generation. Pasteur was able to prove that in such experiments, where the method was correct in principle, some source of error had always occurred; for instance, that impure air had not been absolutely excluded, or the heating had not been sufficient. Details which appear trifling may here acquire great importance. Thus the views which were defended by Spallanzani and his supporters finally prevailed, owing to the ability and perseverance with which Pasteur conducted his researches.

It was mentioned above that in 1837 Kützing published some observations relating to an acetic acid bacterium which he had discovered. This subject was again taken up by Pasteur, and subjected to a thorough experimental examination. * He gave a good description and figure of the bacterial growths found in French vinegar factories, and which he named "***Mycoderma aceti.***" His experiments definitely proved that it was this growth which gave rise to the formation of acetic acid. He also dealt with the chemical side of the question. This is,

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indeed, one of his most important researches. In France, wine is employed for the manufacture of vinegar. The fermentation takes place in casks which are only partially filled, and which have a capacity of two to four hectoliters, and are of the usual form. At suitable intervals a certain amount of the vinegar is drawn off, and a corresponding quantity of wine is added to take its place. The same casks are thus kept in use for several years, without being completely emptied, and therefore, also without being cleaned. Under these conditions, the so-called vinegar eels develop in enormous numbers. Pasteur proved that these small worms are able to check the development of the acetic acid bacteria, and so prevent the fermentation from proceeding properly in the casks thus infected; the vinegar is then said to be "sick" (**malade an tourné**). He now devised a new method, founded upon his theoretical investigations mentioned above. In the place of the casks with the comparatively deep layers of liquid, he employed shallow vats, in which the liquid presented a large surface, and on this he sowed a small portion of a film-growth from a previous fermentation. Under these conditions the whole of the alcohol contained in the wine will, as a rule, be converted into vinegar within a few days. The vat is then cleaned, and a new fermentation started in the same manner. With this method the process is quicker than with that described above, and the eels are avoided; nevertheless, it has acquired no application. In France the old slow method, especially the form known as the Orleans process, is still always employed, whilst that in vogue in other countries is almost exclusively the new German quick process (Schnellessigfabrikation). It would, however, be out of place here to further discuss the advantages and defects of the various methods of manufacture.

* Pasteur, "*Mémoire sur la fermentation acétique*," "*Annales scientifiques de l'École Normal supérieure*," t. I, 1864. Also, "*Etudes sur Le Vinaigre*," Paris, 1868.

Pasteur regarded the bacterial growth mentioned as consisting of a single species. In 18791 showed that it contained two very distinctly different species, and one of these I named ***Mycoderma Pasteurianum***, after the famous French savant. Zopf and other bacteriologists subsequently changed the specific name to *Bacterium*. The number of species has lately been further increased by recent investigations, conducted partly by myself and partly by others. Amongst these acetic acid bacteria, there are several, the activity of which is distinctly different, and it is, therefore, probable that a similar reform will some day be introduced in the manufacture of vinegar, such as I succeeded in effecting in the brewing industry namely, the employment of a pure culture of a systematically selected species. As yet, however, nothing has been done in this direction, and the process is still carried on hap-hazard.

The investigations of Pasteur, and of those before him, thus established the fact that there are different micro-organisms which induce different kinds of fermentation, alcoholic, lactic, acetic, butyric, &c. Therefore, if it is desired to bring about a pure alcoholic fermentation, free from the formation of objectionable acids it is evidently necessary to keep out those micro-organisms which produce these acid fermentations. Pasteur drew this conclusion, and first made use of its application to wine.*

* Pasteur. "*Études sur le vin*," Paris, 1866.

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It was known long ago that wine is subject to different changes, which may seriously affect both its taste and its appearance; for instance, it may become sour, bitter, viscous, cloudy, &c. It was Pasteur, however, who first discovered the causes of these disagreeable changes (diseases); he showed, namely, that they were produced by different bacteria. As a remedy for such diseases, he recommended the employment of heat in accordance with the method of Scheele and Appert already described. This must, of course, be carried out at an early stage of the disease, before the bacteria have been able to develop to any considerable extent; when the wine is once spoiled, it is of no avail to kill the disease germs.

As regards the alcoholic fermentation of wine, Pasteur considered that, on account of the favorable composition of the grape juice, it can, without danger, be left to the yeast fungi which happen to be present on the surface of the grapes and in the air. Subsequently he also gave expression to this view in his work, "*Études Sur La Bière*," p. 4.

In his book on the alcoholic ferments,† Reess gives a systematic description of various yeast forms, and he starts with the view that the form and size of the cells, taken alone, constitute specific characters. The large oval yeast cells are, accordingly, classed as *Saccharomyces cerevisiae*, the smaller oval cells as *Saccharomyces ellipsoideus*, the sausage-shaped cells as *Saccharomyces Pastorianus*, and so forth. As will be recollectcd from my earlier treatises, my investigations have shown that this view is quite incorrect. One and the same species may occur with cells which might be taken as belonging to all the forms classed by Reess as distinct species. Associated with the conception of different species, there is naturally also the conception of a difference in the activity; both these views were expressed by Reess. On p. 21 he suggests that the reason for the custom, then prevalent in all breweries, of getting a change of yeast, is, possibly, that the yeast becomes contaminated by the various fungi occurring in the brewery premises, and that during their growth these have an injurious effect on the activity of the yeast. On p. 40 he also expresses the opinion that besides *Saccharomyces cerevisiae*, other alcoholic ferments can also occur, which are capable of exciting injurious fermentations.

† Reess, "*Botanische Untersuchungen über die Alkoholgärungspilze*," 1870.

In the following year two communications appeared on diseases in beer produced by alcoholic ferments, one by Holzner, the other by Lintner, sen.* As both these investigations led to the same result, they may here be discussed together. A dangerous disease is described in them, which was at that time prevalent in low-fermentation breweries. This disease manifested itself in such a manner that the beer fined in the lager casks only with very great difficulty, and when this finally occurred it became thick again when tapped, the turbidity being caused by the presence of numerous small yeast cells. A microscopic examination led to the belief that this yeast was the species described by Reess under the name *Saccharomyces exiguum*. No experiments were made, and at that time it was assumed that the species described by Reess were true species. Since this, as we have

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seen, is not the case, these investigations were unable to throw any certain light on the question. However, they are at any rate deserving of credit, since they, for the first time, called the attention of zymotechnologists to the small light yeast cells and to the dangers which these may be able to bring about.

* "Der Bayerische Bierbrauer," Munich, 1871, pp. 14 and 64.

Since then, brief accounts by different authors having reference to *Saccharomyces exiguum* [bakers yeast] have frequently appeared in the brewing journals; these, however, contained nothing new, and may here be passed over. With regard to my own investigations in connection with this species, I refer the reader to the account on subsequent pages.

It was imagined that this small yeast fungus was to be found in all cases where anything was wrong with the beer, and as a starting-point it was always erroneously assumed that this species could be detected with certainty with the help of the microscope. Engel * also took this view of the question. The well-known brewer, Gruber, of Strasbourg, had noticed that his beer became attacked by a characteristic disease after it had been six to nine months in the lager casks. In this disease a new fermentation set in which rendered the beer opalescent and gave it a greenish hue. When this fermentation ceased, the beer became bright, but in the place of its original good fresh taste, it had acquired a vinous flavor. On examining this beer under the microscope, Engel found numerous small yeast cells, which, in accordance with the custom of the day, he, without hesitation, stated to be *Saccharomyces exiguum*, and he likewise expressed the opinion that the after fermentation in question must have been produced by these cells. At that time investigations were carried out rapidly, and the results were in accordance with the methods.

* Engel, "La ferment: alcooliques," Paris, 1872, p. 30.

A view, which was also advanced at that time was that each of the various kinds of wine and beer had its own species of yeast. Cohn, for instance, expresses this view in his "Beitrage zur Biologic der Pflanzen," 1872, vol. i. heft 2, p. 136.

Engel adopted Reess's view without criticism; this, was, however, not the case with Cienkowski. * The latter considered that Reess's species of the *Saccharomycete*: were only forms of development of *Mycoderma vini*. Several other investigators expressed an almost identical view, as, for instance, Harz in his "*Grundzüge der alcoholischen, Gärerungslehre*," 1877. This standpoint was perfectly justifiable since the basis of Reess's description of species was, as we have seen, quite untenable. If, with this altered view, there could be any question at all of the yeast cells themselves producing diseases, this would, at any rate, have to be regarded from quite a different point of view from formerly. It was no longer a question of foreign species gaining admission from without to the wort and beer, and here competing with the brewery yeast, but the whole question had to be attacked from quite a different direction. It could then only be a question of a deterioration of the brewery yeast itself under different conditions of nourishment. Regarded from a practical point of view the

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problem was therefore restricted to determine these chemical conditions.

* Cienkowski, "**Die Pilze der Kahmhautl**" "Bulletins der Petesb.Akadmie," t. xvii. 1873.

These important questions were discussed again and again without any decisive experiments having been made.

In 1876 appeared Pasteur's famous work on beer and its diseases. † It will be recollect that in his studies on wine, Pasteur showed that a number of its diseases are caused by bacteria. In his new work (pp. 4-7) he proved that with beer the case is similar. The fact must also be emphasized that by means of exact experiments, he furnished a complete proof of the correctness of this doctrine (pp. 20 and 26). He came to the conclusion, so important in practice, that any bacterial growth which can attack wort or beer, must be regarded as dangerous, and that every precaution must be taken to exclude bacteria as far as possible. As these small organisms are distinguishable by their form from yeast cells, he recommended that a microscopic examination should be made in the brewery, both of the pitching yeast and of the beer. He described several methods for the purification of yeast, but for this purpose he especially recommended cultivation in a solution of cane sugar containing a little tartaric acid (p. 224). At all events it was this method in particular which was recommended by his pupils.

† Pasteur, "**Etudes sur Le Vin,**" Paris, 1876.

With regard to the yeast cells Pasteur repeated in several parts of his work (especially on pp. 218-220) the views of previous investigators, Reess, Engel, Holzner and Lintner. In other places (c. g. p. 193), he appears, however, to have adopted the opposite view of Cienkowski and Harz, namely, that the cells of yeast are subject to an endless and rapid variation, and that there are no fixed species of **Saccharomyces** (and consequently also no species of disease-yeasts). In agreement with this is also the opinion which he expresses on p. 333 that, under conditions obtaining in the brewery, low beer-yeast can become changed into high-yeast. He investigated (p. 199) a special form of yeast (caseous yeast) which he found in an English brewery-yeast, and he suggests as possible that this might be a form of development of the culture yeast. Where he discusses the yeast fungi, everything is in a state of uncertainty; nowhere does he indicate any certain boundary lines.

With regard to the question of pleomorphism, Pasteur's view was similar to that held by Bail, since he assumed that the **Saccharomycetes** were forms of development of certain brown mold fungi (*Dematiuum* and *Alternaria*) which are found on the surface of different fruits (pp. 154-155, 164-165, 177). It is easy to understand how Pasteur could acquire such an erroneous conception when we recollect that he never made a distinction between the **Saccharomycetes** (yeast cells with endospore formation) and the non-**Saccharomycetes** (yeast cells giving no endospore formation). His statements in connection with the whole of this subject are full of contradictory views, and must be characterized as an exposition of different possibilities. A scientific conclusion was not arrived at at any point. As a rule he only

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alludes to previous investigators when he wishes to correct some error in their works; a historical account of the previous development of the science is not found in his book. Pasteur, however, never undertook to give any such account, and it is, therefore, a great mistake which is often committed to look for such in his work. If we wish to acquaint ourselves with the advance made by investigators prior to Pasteur, we must seek this information elsewhere.

It is on p. 218 that he expresses himself most clearly with regard to beer diseases caused by alcoholic ferments. He mentions here that in some breweries a lager beer is brewed during the winter months, which is intended for consumption in August or September of the following year, and that much anxiety is felt lest the beer acquire a vinous flavor during this long storage period. He writes, "From my observations, the cause of this vinous flavor appears to be due in the main to the brewery yeast being mixed with **Saccharomyces Pastorianus** or varieties of this species." Pasteur is thus very cautious in expressing his opinion; he states nothing definite. Against the supposition of Engel that this disease is caused by **Saccharomyces exiguum**, he simply brings forward a new supposition, as we have seen, namely, that it may, perhaps, be another species. Neither Engel nor Pasteur attempted to separate this or other micro-organisms which cause disease from the good brewery yeast in order to prepare beer fermented in the one case with the latter species alone, and in the other case with a mixture of this with the supposed disease yeast. This is, however, the only way in which we can determine on the one hand what disease yeast, and on the other hand, what good brewery yeast is. The reason why neither Pasteur nor any of the previous investigators carried out such decisive experiments is that the methods which were then at their command, did not enable them to do so.

The confused ideas which prevailed even in 1876 with regard to the two great questions of pure cultivated brewery yeast and the diseases of beer produced by alcoholic ferments, are also seen from the fact that Pasteur recommended the method described above (cultivation in a solution of cane sugar containing a little tartaric acid) for the purification of yeast. So far as it is a question of suppressing the bacteria, his method is irreproachable; but since brewery yeast, as a rule, contains also wild yeast in greater or less amount, the treatment with tartaric acid will, as my experiments have shown, in most cases cause the suppression of the good brewery yeast, whilst the development of the wild yeasts will be promoted (see "What is the pure yeast of Pasteur?" Chapter II., p. 130). In order to properly judge Pasteur's standpoint, it must, however, be regarded in the light of his time. It can then scarcely be conceived that it was even possible at that time to clearly appreciate these fundamental questions. As we shall see, no further progress had been made even six years later.

A method such as that proposed by Pasteur must naturally soon be found to be quite unsuitable in the brewery, and in fact it was quickly given up wherever it was tried.

Pasteur constructed a special form of apparatus (pp. 326-340) for cultivating yeast, and it was also his intention to do away with the open coolers in breweries, and to substitute closed vessels in which the boiling wort from the hop-back could be cooled and aerated without

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becoming infected. He also constructed a suitable form of apparatus for this purpose (pp. 371-378). These forms of apparatus were shortly afterwards adapted by Pasteur's associate, Velten, to the practical requirements of the brewery. If on this account Velten has for some years appeared as the discoverer of something quite new, he forgets that the principle and also the apparatus necessary to effect sterilization were made known by Pasteur's predecessors; and further, it was Pasteur and not Velten who first made use of its technical application in the brewery. The form of construction adopted by Velten is, moreover, in several respects not to be recommended. It is evident that these forms of apparatus acquire all their importance through the yeast. If the yeast is not really a pure culture free from all disease germs, they will be worthless. Since Pasteur's yeast does not at all satisfy this requirement, it also follows that the apparatus could not find any application in the breweries.

Nine years later their application for practical brewing purposes was fully appreciated. As will be remembered, I succeeded in 1883 in introducing the pure cultivation of a systematically selected race, and when in the year following this important reform was adopted in the Old Carlsberg brewery and elsewhere, the impetus was given in the direction of the abolition of the open coolers. The apparatus which was accordingly constructed in Carlsberg differed in several respects from Velten's, and especially in the manner in which the air is sterilized. Velten employed heat for this purpose in accordance with Schwan's method; in the Carlsberg apparatus, on the other hand, the air is purified by means of the cotton-wool filters of Schröder and Dusch, and this method has proved to be much more practical. (Directions for the purification of the air by means of cotton-wool filters, and also a description of the pure yeast propagating apparatus constructed by Captain Kühle and myself, are given in the earlier part of this book.) The employment of this apparatus with my pure cultivated yeast has spread in recent years over a large portion of the globe. As was to be expected, the pure cultivation of systematically selected races had to be first introduced.

I have now discussed that portion of Pasteur's work which bears directly upon the questions to be dealt with in this treatise. I have not only emphasized the important advances brought about by the discoveries of the famous French savant, but have also given the reasons why they were unable to bring about the solution of the two important problems of the diseases of beer and pure cultivated yeast. By means of the method recommended by Pasteur, the good brewery yeast became suppressed and the growth of the disease yeast promoted. It was, indeed, not possible to achieve success by the means which he adopted. I

In the same year in which Pasteur's "*Études Sur La Bière*," appeared, Lintner, sen., published the results of some zymotechnic experiments.* He described various irregularities in the fermentation and diseases in the beer, which were a source of great trouble and loss to the breweries in which they occurred. The microscopic examination gave no information, and Lintner, indeed, states that a yeast which, judged by this test, would be regarded as satisfactory, nevertheless gave a bad result in the brewery. On the other hand, he obtained a good result with another yeast which a microscopic examination indicated to be unsatisfactory in that it contained a number of small and irregular cells (light yeast). This objectionable-looking yeast, nevertheless, gave perfectly normal fermentations, and was employed with

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decided success in different breweries. In fact, this forms an excellent illustration, showing how little information is gained in this field by a simple microscopic examination alone. A more rigorous criticism of the methods of examination then in vogue can scarcely be imagined.

* C. Lintner, sen., "**Ueber einige Resultate zymotechn. Untersuchungen,**" "Zeitschrift f. das ges. Branwesen," Munich, 1876, p. 221.

† Such inadequate methods are unfortunately still made use of in several brewery-laboratories, and, what is still worse, theories are built up upon them and are boldly expounded with the conceit which characterizes the *half-scientific literature*.

Most of the brewing journals are only too ready to open their columns to such article. This half-scientific literature has continued up to the most recent times, one communication after another appearing on "light yeast" and "degenerated" yeast (*verwilderte Hefe*). The authors describe no scientific experiments, and believe that they are able to settle the matter by a microscopic examination; they cause great confusion, but they add no information. They might with profit study the above-mentioned treatise of the famous old symtechnolog-ist.

The terms "light yeast" and "degenerated" yes.st.as employed in this literature, imply two very different things Sometimes, asl.intner's observations show, they are only taken to imply cells which have an unsatisfactory appearance under the microscope, but nevertheless give a good result in the brewery. Sometimes, on the other hand, cells are meant which have suffered some change which makes them less suited to carry out the work desired by the brewer. In my various tratises, from **1883** to the present time, oburvations occur here and there which have reference to these two cases, and I hope later to be able to publish a more extensive experimental investigation in this direction. The question relating to the laws affecting the variation of species of yeast is a very complicated one. If the authors, to whom I here refer, had studied my invstig-ations with some degree of thoroughness, and especially those which I have published during the last years, they would have perceived that especially in this field it requires great labour to achieve only modest raults, and that a great deal more has to be accomplished before we can really thinlr of establishing theories of general appliubility. They would then also know that it is not possible by means of a microscopic examination of a yeast cell to judge how it will behave in the brewery. And if they had carried out experiments such as those conducted by myself and my pupils, they would have learned that the cells, which from their standpoint they describe as large and satisfactory, will in many cases produce a yeast which the brewer has to reject. whilst inversely their so-called "light" and "degenerated" yeast cells are often good vigorous cells which in the brewery develop a good yeast. If the authors to whom my advice is directed could be converted, they would take up a more modal position with regard to the difficult

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problems mentioned, and they would in the future write less but make more experiments. I heartily wish that this might happen, for the half-scientific literature has always been, and is still, a source of mischief for zymotechnic science.

Few works have on their first appearance attracted as much attention as Nägeli's book on the lower fungi.* The influence which it exerted did not, however, correspond with the expectations which it raised, at many of the statements which it contained were wanting in proof. With regard to the *Saccharomycetes* and bacteria, Nägeli expresses on pp. 20-22 the view that the species are able to undergo rapid and abundant variation, both in their morphological and physiological properties, and that many of the forms can be readily converted one into the other. Also, with regard to the special fermentative activity which one form may possess, he considers that this may quickly disappear on cultivation, or that it may become changed into quite a different function. According to Nägeli neither the morphological nor the physiological forms are constant, but readily merge into one another. The chemical composition of the substratum and the external conditions form important active factors which bring about transformations in various directions. Nägeli's standpoint with regard to the idea of species is difficult to understand, and, as already stated, he gives no experimental proof for the different cases. In his "Theorie der G'zihrungr," 1879, p. :20, he also expresses a similar view.

* Nigeli, "*Die niederen Pilze in ihren Beziehungen zu den Infektionskrankheiten und der Gesundheitspflege,*" Munich. 1877.

In his zymotechnic retrospect of the year 1877,* Holzner pointed out that the investigations hitherto conducted gave brewers no reliable information with regard to the irregularities which so often occur in the fermentation, and he refers especially to the confusion which prevails with regard to the species of yeast. Some regard each form as a special species. According to others, the different species readily change one into another. We are here confronted with enigmas and problems which still remain unexplained. With regret he points out that "hitherto the number of hypotheses regarding fermentation and the morphology, biology and physiology of the fermentation fungi (and ferments) has not diminished but has continually increased." And he refers to his famous countryman, Nägeli, as one likely to help in throwing light on the subject.

* Holznet, "*Zyrnotechn. Ruckhlicke auf den Jahr 1877,*" "Zeiuchr. f. das ges. Braunwesen," Munich, 1878, p. 142.

On looking through the various brewing journals of the period to which we are now referring, it will be found that they contained frequent complaints of disturbances and irregularities in the fermentation, and of difficulties and losses caused by diseases of the beer. A similar complaint occurs in a paper by C. Lintner, sen.,† in which he points to the prevalence of the disease known a yeast turbidity. He writes: "The finished lager beer is drawn off from the store "zslr in the cool cellar, apparently perfectly bright, but it soon b....-nes cloudy in the bottles or casks

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when placed in a warmer situation, or during transport. The examination of such beers, then, reveals small yeast cells which rapidly develop and increase and finally completely settle. Brewers call this yeast light yeast (Flughefe)." Lintner considers that the cause of this is to be attributed to the influence which an inferior malt may have on the nourishment of the yeast, partly also to an insufficient quantity of yeast having been added to the wort, and to the fermentation having been conducted at too low a temperature. Lintner assumes that under such conditions the normal brewery yeast suffers some undesirable transformation, which causes it to develop these small light cells, and that the resulting disease is thus explained. He regards the theoretical speculation of Nägeli as supporting the correctness of this view. In the year following he repeated the same opinion.*

† C. Lintner, sen., "Ueber Mal: und dessen Einfluss auf die Innernkeil und Gule des Bierel," "Zeitschr. f. das ges. Br:mwesen," Munich, 1880, p. 384.

* C. Lintner, sen., "**Altes und Neues über Bierbrauerei,**" "Zeitsehr. f. das ges. Br:uwesen," Munich, 1881, p. 153.

As will be remembered, it was formerly held that the disease mentioned was caused by **saccharomyces exiguum**. This view had been completely discarded in 1881, and the cause of this and similar discuss is now no longer sought in foreign yeasts, and in contamination occurring from without, but in the conditions as regards nourishment under which the good brewery yeast is placed. After the doctrine of Reess and Pasteur failed to help brewers, both Lintner and Holzner appealed to the physiological theories of Nägeli in the hope of obtaining light on the subject. Most of the zymotechnologists of that time adopted similar views, as also did Delbrijck and Hayduck in Berlin. Discussions now arose concerning the degeneration and transformation of brewery yeast, and it was thought that the yeast question would be solved by chemical-physiological methods.*

* Those who desire to study the details of this subject may be referred to the oft quoted "**Zeitschr. f. des ges. Braunwesen.**" In the twenty-six yearly volumes of this journal will, in fact, be found the records relating to the history of brewing matters during that period.

previously. In the attacks which Duclaux and his associates have directed against me, they thus always start with the assumption that Pasteur had found the true solution.

The chemical-physiological researches which Nägeli promoted, however, threw no light whatever on the yeast question, nor concerning the degeneration and transformation of the brewery yeast. My botanical investigations had, in fact, to be undertaken before it was possible to attack these problems in a scientific manner. In that way inquiry will again be directed to these important problems, and it is not improbable that Nägeli will then receive credit for his views.

After Pasteur had discontinued (1876) his studies on beer and its diseases, some

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investigations in the same direction were published by his pupils in the years following, but none of these have any direct bearing on the questions to be treated here. The standpoint at which the French school had arrived in the year 1883 is described in Duclaux's handbook.[†] Thus, on p. 300, Duclaux discusses the purification of brewery yeast, and recommends Pasteur's method, which has been already mentioned. That this method is quite unsuitable for the purpose named for, as has been shown, its employment favors the development of the most dangerous disease germs was, therefore, not then known. With reference to the examination of brewery yeast, Duclaux states, on p. 471, that, with the help of the microscope, it can be ascertained whether the yeast is pure or not. In accordance with this it is seen, on careful perusal, that when speaking of disease germs, he refers only to bacteria, and not to the *Saccharomyces*. This view is likewise repeated on p. 618, where he treats of the diseases of beer in a separate chapter. It is exactly the same standpoint at which Pasteur had arrived seven years

† Duclaux, "*Chimie biologique*," Paris, 1883.

About this time a new impulse was given to bacteriology in Germany by the investigations of Robert Koch, and numerous pupils quickly gathered around this famous investigator. The problems attacked by this school were, in the main, such as have a direct importance in medicine, and it was only exceptionally that investigations were published relating to the physiology of fermentation; prominent amongst the latter are Hueppe's studies on the lactic acid bacteria. Neither Koch nor his pupils devoted any attention to the alcoholic ferments, and, where they make any reference to these, it is only in a very cursory manner. The cause of this is easy to understand, as these fungi have little or no interest for the pathologist and hygienist.

Some years earlier Fitz had commenced his valuable investigation on various species of bacteria, and their fermentation-products. These researches have as little to do with the diseases of fermented liquors as Hueppe's, yet, indirectly, they throw some light on this question.

In the year 1884, Thausing * expressed himself as follows: "Science has furnished valuable results in connection with the organisms of fermentation and fermentation itself, but for the brewer it has yielded practically no result of direct application, and now, as ever, the process of fermentation is shrouded in a deep mystery. Hansen's investigations on the cultivation of pure yeast certainly justify great hopes; if they do not deceive, we are on the threshold of an achievement, the importance of which cannot be over-estimated. For the present we have still to deal with the conditions which now prevail."

* Julius Thausing, "*Einfluss der Hefegabe auf Hauptgärung, Hefe und Bier.*" In the "14. Jahresberichte der ersten österr. Brauerschule in Mödling." Also in the "*Allgem. Zeitschr. f. Bierbrauerei*," Vienna, 1884, p. 872.

This was the universal standpoint in 1884. The researches which I published in the course of

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the following year, and the results thereby achieved in the fermentation industry, caused Thausing's doubts to disappear, and in the third edition of his famous handbook on the manufacture of beer, he gave me full credit for my system of pure yeast culture.

We have now reached the end of this historical review. The following part of this chapter will deal with the investigations concerning the diseases of beer which I have carried out since 1881. Several investigations which have had an indirect bearing on the development of the doctrine of the diseases of fermented liquors are only briefly touched on, whilst others are not mentioned at all. Amongst these are especially such as treat of the diseases of plants which are brought about by fungi, and contagious diseases in man and animals produced by bacteria. Experimental investigations in the former direction were undertaken as early as the beginning of this century, and the Dane, Scholer, may be named as a pioneer of that time. Advance was likewise also slow in this field, but it continually brought greater clearness. In more recent times the Danish botanists, A. S. Ørsted and E. Rostrup, have been conspicuous, whilst the names of Tulasne and De Bary are especially famous in this field.

Even in the writings of Linné and several of his contemporaries we find the idea expressed that fermentation, putrefaction and contagious diseases are caused by microscopic organisms. In the year 1840, Henle showed, with great acuteness, that known facts and observations indicate that contagious diseases must be attributed to the attack of microscopic organisms. It was, however, only in more recent times that experimental proofs were forthcoming, and it was then that Pasteur's investigations brought him his greatest fame.

The young science of micro organisms has developed in all essential particulars from the much older science relating to the higher forms of plants and animals. Just as biology in general, so also micro-biology has received a very powerful impetus through the epoch-making theories of Darwin concerning the transformations of species. This has been brought about, especially, by Nägeli's work, which was mentioned above.

Finally, stress must be laid upon the reciprocal action which there has always been between micro-biology and chemistry.

All these different lines of investigation have mutually helped and have reacted on one another in many ways. If in the above description we had been able to further dwell upon the subject, more life and fullness could have been given to it, but this would have carried us beyond the limits of this treatise.

3. My investigations.

PROBLEM AND METHOD.

In the foregoing historical portion of this treatise we have seen how, in the course of time,

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guesses were thrown out in all directions, and the most varied possibilities were considered in order to explain the relationship which the alcoholic fungi bear to the diseases of beer, and that as often as the right road was on the point of being struck, it was again soon abandoned on account of some unskillful turn, just as if the wrong direction had been aimed at.

After the appearance of Nägeli's work, the idea of the degeneration and transformation of brewery yeast was, as we have seen, brought to the front. It was in such occurrences that the cause was sought of those diseases in the beer, and those troubles in working, which it was assumed were brought about by the yeast. Thus, the other possibility was more and more lost sight of namely, that the cause of these troubles might also be attributed to foreign species which had gained admission, and which, in their competition with the culture species, had given rise to the symptoms of disease. As already stated, the view which was the most widely held, was that deep-seated transformations in a good brewery yeast can readily occur in practice, a view which gave rise to much discussion about the degeneration of yeast; no experiments, however, were undertaken in support of this view.

In my treatise, which was published in 1879, I took up essentially the same standpoint; after carefully considering the question, however, I soon perceived that it would be useless to continue the discussions of my predecessors, but that decisive experiments were necessary, and that, until the latter had been made, it would be best to remain silent. Several years were spent on the preliminary work. First of all it was necessary to elaborate a method of pure cultivation, in order that I might know with perfect certainty whether I was working with one or with several species. I therefore took as my starting-point the individual, the single cell. My next problem was to discover characters which would enable me to solve the intricate questions of species, race, and variety. I have, in the course of some years, treated these problems from different points of view. The first characters which I found related to the development of spores, with especial reference to the cardinal points of temperature. It is now generally acknowledged that this method of spore analysis is of importance, and on this basis I have further devised the above-mentioned method for the practical examination of brewery yeast. I therefore still lay great weight on these characters; but I have never held the opinion so often stated by superficial readers of my writings that they are, in themselves, sufficient for the determination of all species; on the contrary, I have always endeavored to discover new distinctive characters, and I have also already given a whole series.

In 1882 and 1883, two exceptionally favorable opportunities occurred for testing the applicability of my new weapons in practice. I refer to the maladies with which the Tuborg and Old Carlsberg breweries were at that time afflicted. The beer at the first-named brewery was attacked by the disease known as yeast turbidity, whilst in the case of the latter brewery, the beer acquired a disagreeable odor and an objectionable bitter taste. Since it was not possible to discover any fault either in the wort or in the fermentation, I had to assume that these objectionable effects were caused by micro-organisms. Tests made with Pasteur's method for the purification of yeast, proved of no avail. By comparing all my observations I arrived at the opinion that the cause of the disease must, without doubt, be sought in the pitching yeast. Although a microscopic examination revealed no foreign organisms in the yeast except a few

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bacteria, I nevertheless started with the idea that yeast cells which were to all appearance similar, might yet belong to several species, and that some of these might have caused the diseases. The method, therefore, to be adopted was to split up the brewery yeast into its constituents, to prepare a large number of pure cultures of them, and, finally, to carry out fermentation experiments partly with each species separately and partly with mixtures, and in such a manner that the conditions would correspond with those obtaining in the brewery. If the opinion with which I started was correct, I should in this way find out which of my pure cultures contained good brewery yeast, and which of them contained disease yeast. An investigation of this kind can, at the present time, be carried out with comparative ease in any zymotechnic laboratory; at that time, however, there were difficulties to be overcome. Since then the technique in this field especially has been developed in a high degree.

The experiments showed that the diseases mentioned were caused by species of yeast which were quite different from the preponderating culture yeasts present in the pitching yeast of the two breweries, and that each of the latter when employed alone gave a good beer. As will be shown in the following pages, the number of species capable of producing similar diseases is by no means small. All these disease yeasts exhibit several characteristics, by means of which they can be distinguished from brewery yeasts. Accurate experiments have further shown that the disease yeasts gain admission into the brewery from without, and that they are not forms of development of the brewery yeast.

Since the introduction of my pure yeast system into breweries, favorable opportunities have occurred in practice of making important observations in different directions. In the two breweries, Old and New Carlsberg, I have thus for many years studied the cultivation of pure yeast on the large scale, and never have the brewery yeasts shown any sign of developing forms like those of the disease yeasts mentioned; on the contrary, under the conditions obtaining in the brewery, they always retained their specific characters. ***The theories of the degeneration and transformation of yeast have thus, in this respect, proved to be quite untenable.***

Just as all organisms are subject to variations, so this is also evidently the case with the species of brewing yeasts. As long as they are subject to the conditions obtaining in the brewery, however, they only exhibit slight modifications, and these are only of a temporary nature. When we regard them from a biological point of view, we are inclined to look upon them as quite insignificant; for the practical brewer, however, the matter is quite different. These changes can, indeed, occur in a very disagreeable manner, and sometimes cause an appreciable irregularity. In the course of a year they pass like a wave through the brewery, and in most cases we have no idea of their cause.

The question of the variation of species of yeasts is thus not only of great theoretical, but of equally great practical interest. A review of the experimental investigations which I carried out in this direction will be found on pp. 92-102. These studies are being continued without break in my laboratory. Any further discussion of them is, however, out of place in this chapter. Irregularities caused by the brewery yeast itself, cannot be regarded as diseases, at any rate

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not in the sense in which the word is here made use of, and has hitherto been employed in the literature of this subject.

In the following pages are described my experiments on the diseases caused by alcoholic ferments, as well as some investigations which are related to this subject. In the numerous experiments which this work involved I have been assisted by Mr. Gram and Mr. Nielsen.

YEAST TURBIDITY IN BEER.

When I commenced the study of this disease in 1882-83, it was regarded as one of the most dreaded maladies, not only in Denmark, but to a still greater extent in Germany; and it not infrequently caused great losses in low-fermentation breweries. As we have already seen, it was for a time assumed that this disease was caused by a foreign yeast fungus, *Saccharomyces exiguum* later, the cause was attributed to the degeneration of ordinary brewery yeast, *Saccharomyces cerevisiae*, in that the latter developed only small light cells instead of large heavy cells.

In the Tuborg brewery at Copenhagen, this disease manifested itself as follows: When, at the end of the storage period, the beer was drawn off in the cold lager-cellars, it was bright and faultless in appearance; but after the casks or bottles into which it had been drawn off had been exposed for a few days to a warmer temperature than that of the lager cellar, a more or less abundant yeast deposit had formed, and, on slight agitation, rendered the beer cloudy. When the malady was strongly developed, the beer became so cloudy, only a few days after being drawn off?" from the lager casks, that it was quite undrinkable. The experiments which I made in connection with this were published in 1883, in "*Compte-rendu des travaux du laboratoire de Carlsberg.*" An account of the most important of the results is given below, and then follow the later experiments made in more recent years.

I. Series of Experiments

From the sick beer of the brewery mentioned I separated the alcoholic ferments present, and obtained three species namely, one belonging to the group *Saccharomyces cerevisiae* (the main constituent of the bottom yeast of the brewery), and two wild yeasts, to which I gave the names *Saccharomyces Pastorianus III.* and *Saccharomyces ellipsoideus II.** My problem was to determine whether one of the last two species was the cause of the disease. With this object in view, I first of all made a Series of Experiments with six two-necked flasks, each of which contained 700 cc. of the same sterilized wort. Two of these flasks, marked A, were inoculated with 1} cc. of the species *Saccharomyces cerevisiae*, two others, marked B, were inoculated with 1 cc. of the same *Saccharomyces cerevisiae*, and further with i cc. of Sac:r. *ellipsoideus II*, whilst the remaining two flasks, C, were likewise inoculated with 1 cc. of the same *Saccharomyces cerevisiae* and with § cc. of *Saccharomyces Pastorianus III.* In each case the yeasts employed were thick, and of about the same consistency; they were pure cultures prepared under the same conditions, and they consisted of young vigorous

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cells. The primary fermentation was carried on at the ordinary room-temperature, the secondary fermentation during storage at about 7° C. Two-necked flasks were also made use of for the storage, and for this purpose were well filled with the respective beers. After about three months' storage the beer was drawn off* into other sterilized flasks, and these were then set aside in a cupboard at the ordinary room-temperature. In less than eight days it was found that the beers from B and C were quite cloudy from suspended yeast, whilst after fourteen days the beer from A was still faultless.

* With reference to these and the species mentioned later on, I would refer the reader to the description given in my "*Recherches sur la physiologie et In morphologie des fermentes alcooliques,*" and also in the text books of Jörgensen and Zopf

It was thus shown that one of the three yeasts found in the sick beer, namely, *Saccharomyces cerevisiae*, gave a stable product when present alone in the fermenting liquid, but that the malady manifested itself when, in addition to the above, either of the other two species, no matter which, was also present under the conditions named.

In this, as in all the experiments which were undertaken with the view to ascertain not merely the nature of the diseases here in question, but also of other diseases, care was naturally taken that the fermentations of each series were conducted in all respects under the same conditions, except as regards the composition of the pitching yeast, upon the action of which the whole question turned. Absolutely pure cultures were also, of course, always employed.

A considerable number of experiments, modified in various ways, were made in the laboratory in connection with the question of yeast turbidity, and especially with other yeasts in addition to that mentioned from the Tuborg brewery. The result was the same. On extending the investigation, I obtained the interesting result that the *two disease yeast: did not produce the malady when they were only added to the beer at the end of the primary fermentation, that is, at the stage when storage commences.*

These experiments were now repeated on a larger scale. The problem was the same as before, but I also wished to determine what proportion of disease yeast must be present in the pitching yeast in order to produce the disease, and finally, what will be the effect of a lower or higher attenuation during the primary fermentation, and also of a shorter or longer period of storage. The following will serve as examples of the experiments which I undertook with a view to the solution of these questions.

II. Series of Experiments

Two Pasteur fermenting vessels, A and B, were charged, each with 165 liters of aerated wort (I 3-5 per cent. Ball) such as is employed in the brewery for the production of ordinary lager beer. A was pitched with 660 grams of thick beer yeast, the species used being the one which I subsequently described under the name ***Carlsberg bottom yeast No. 1***; the second vessel,

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B, was pitched with 644 grams of the same yeast, with the addition of 16 grams of ***Saccharomyces ellipsoideus II.*** of the same thick consistency. The growths of both yeasts were young and vigorous, and were produced under the same conditions. The temperature of the wort when the yeast was added was 7° C., and the temperature of the room was 710° C. during the primary fermentation. After eight days the extract in A was 7-6, and in B 7.5 per cent. Ball. From each fermenting vessel a cask of 66 liters capacity was then filled, and these were placed in the lager cellar, the temperature of which was 2° C. The remaining portion of the beer was left to ferment further, and, after ten days from the commencement, the extract in both A and B was 6-7 per cent. Ball. The beer was then drawn off into casks, and these were placed in the same lager cellar with the first portions.

After the two first casks had been in the lager cellar for 2; months, the beer, which previous to storage had a gravity corresponding with 7.5 per cent. Ball, was drawn off into clean bottles of clear colorless glass, and these were then placed in a dark cupboard in the laboratory. At the time of bottling both kinds of her were, as in the previous experiments, free from all trace of yeast turbidity; but even after one day's standing, the development of yeast in the beer B was noticeable; after five days B was distinctly cloudy, whilst A was still bright. The contents of the other casks, the gravity of which, previous to storage, corresponded with 6.7 per cent. Ball, were treated in the same manner after three months' storage. The beer from both B and A proved to be perfectly stable; its extract amounted to 5-9 per cent. Ball.

These experiments thus show that ***the disease can still develop when Saccharomyces ellipsoideus II. constitutes only 1/41 part of the pitching yeast, but only when the beer had an extract of at least 7.5 per cent. Ball. at commencement of storage, and when under these conditions the storage was discontinued after 2 1/3 months. On the other hand, when the fermentation was carried further in the fermenting vessel, so that the extract had diminished to 6.7 per cent., and the beer then stored for at least three months, the disease did not manifest itself.***

This experiment was repeated, but with the modification that the yeast used for pitching B contained 15 part ***Saccharomyces Pastorianus III.*** in the place of ***Saccharomyces ellipsoideus II.*** The main result was the same as before; it was found, however, in this and in some other experiments, that the latter species was the more objectionable one.

Finally, experiments were made on a large scale, in order to determine what is the effect when infection occurs at the end of the primary fermentation.

III. Series of Experiments

The lager beer and export beer used in these experiments were taken from the fermenting cellar of the Old Carlsberg brewery at the stage when ready for removal to the lager cellar. Three casks, A, B, and C, each of 16% liters capacity, were filled with each kind of beer. B was then inoculated with to cc. of the yeast ***Saccharomyces ellipsoideus II.*** and C with an equal amount of ***Saccharomyces Pastorianus III.***; A was not inoculated, but was kept as a

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control The yeast was thick, and, as in all the previous experiments, consisted of young vigorous growths which had been cultivated in wort. The experiments having been started in this manner, the casks were placed in the lager cellar of the brewery, and were stored there in the ordinary manner for nearly two and a half months, a comparatively very short storage period for the export beer. The temperature of the cellar was 2° C.

At the end of the experiment it was found that ***the strongly infected beer was excellent in every respect, and that its stability was equal to that of the beer which had not been infected.*** The result in this case was thus the same as in the laboratory experiments with small quantities.

The above experiments are described in my treatise of 1883, which was mentioned above. I will now give some account of the experiments which I have made since then in connection with this disease.

The experiments which were carried out in the large Pasteur fermenting vessels agree so closely with the conditions obtaining in the brewery that I did not hesitate to apply the results obtained to practical conditions. The only objection which can be raised against this is, that these fermenting vessels differ from those ordinarily employed in the brewery in that the carbonic acid gas cannot as readily escape as under the normal conditions of the brewery. Further, the temperature of the room in which my fermenting vessels stood was slightly higher than is customary in the fermenting cellars of a brewery. It was, therefore, of great advantage to me that the director, Captain Kühle, gave me a portion of the fermenting cellar at Old Carlsberg for my new experiments. From this time all my practical investigations were tested in the brewery before their completion. The laboratory experiments conducted on a small scale can, in fact, only serve a a preliminary guide, and from these alone conclusions cannot be drawn as to what will occur under practical conditions on the large scale. When such experiments are carried out in the brewery it is evident that the greatest care and precautions must be taken; only then can they be undertaken without incurring danger.

IV. Series of Experiments

Three fermenting vessels, A, B and C, were fitted up in the fermenting cellar mentioned above; they were made of wood, and their form was that of the ordinary fermenting vessels. Into each of these 1} hectoliters of wort (14 per cent. Balling) were introduced.

A was pitched with 400 grams of ***Carlsberg bottom yeast No. 2.***

B was pitched with 350 grams of ***Saccharomyces Pastorianus III.***

C was pitched with 350 grams of ***Carlsberg bottom yeast No. 2,*** and 50 grams of ***Saccharomyces ellipsoideus II.***

The temperature of the wort was 7.5° C at pitching. After eight days the extract was 8.13 per

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cent. in A, 8.21 per cent. in B, and 8.29 per cent. Ball. in C. As regards brightness, A was good, and B and C only moderately good. The beer from each vessel was drawn off into two similar casks, which were then placed in a lager cellar at a temperature of 0.5 – 2.5° C.

After the beer had remained in the cellar for about a month, it was in each case bright, and had the appearance and taste of a good normal beer such as occurs in commerce. A considerable number of bottles were filled from each cask, and these were set aside in a dark cupboard at the ordinary room-temperature. After eight days the beer from A was still bright and without appreciable sediment, whilst the beers from B and C had developed a fairly pronounced sediment, which on agitation rendered the beer cloudy. The species of brewery yeast employed in this experiment is one which gives a beer which is bright after short storage, and has a full taste, but only a moderate degree of stability. After it had been twelve days in bottle, the beer from A also began to show a distinct sediment; in B and C, however, the sediment was much more strongly developed.

The same result was in the main obtained from a similar experiment with **Carlsberg bottom yeast No. 1** and the two disease yeasts, but in this case the storage was carried on for a month longer. As in some earlier experiments, it was found that the beer which had been fermented with this yeast possessed much greater stability than that fermented with the **Carlsberg bottom yeast No. 2**.

As was to be expected, the two species of wild yeast likewise produced the disease when the fermentation was conducted in the brewery and under the ordinary conditions: **Saccharomyces ellipsoideus II.** proved to be the more dangerous of the two species.

It still remains to be ascertained what will occur when the beer becomes infected with the two wild yeasts after the completion of the storage period, that is to say, in the small casks and bottles from which the beer passes direct to the consumer. For these experiments I employed bottles of clear, colorless glass, and of the customary size and form as ordinarily employed in the trade for the bottling of beer; the capacity of each bottle is about 350 cc. After they had been cleaned, they were sterilized, together with the corks. The greatest care was taken in filling the bottles, after which the two disease yeasts were introduced, and the bottles carefully corked. They were then well shaken, and finally put away in a dark place, and at the ordinary room temperature. The infection was in all cases abundant, but the amount of yeast introduced was such that the beer remained bright directly after shaking. The flasks which were not infected, and which were placed with the others as a control, were of course also agitated and were treated in every way like the latter, the only difference being that they were not infected. Moreover, in these, 3 in the earlier experiments, importance was attached to the imitation of the conditions obtaining in practice. The beer ordinary lager beer was from the Old Carlsberg brewery, and it was only in a few cases that I employed beer from some of my own fermentations with pure cultures of low brewery yeast. The chemical composition of the latter beer approximated to that of the ordinary lager beer, the alcohol of which amounted to 4.3 per cent., and the extract 5.6 per cent. The three following examples will serve as illustrations of the experiments which were carried out with reference to the above questions.

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V. Series of Experiments

Young vigorous growths of the two disease yeasts, which had been developed in ordinary wort, were introduced into twelve bottles of lager beer. Three of the bottles were inoculated each with one drop of ***Saccharomyces Pastorianus III.***, and three drops of the same species were introduced into each of three other bottles; six bottles were similarly infected with ***Saccharomyces ellipsoideus II.***; three bottles which had not been infected were kept as a control.

After ten days all were still bright, and showed no appreciable sediment. Four days later this was still the case with the uninfected beer. The bottles infected with ***Saccharomyces Pastorianus III.*** showed a slight sediment, which rendered the beer slightly cloudy when shaken. The bottles into which the other disease yeast had been introduced contained at this time a more pronounced sediment. The three bottles to which one drop had been added became slightly cloudy when shaken, whilst those to which three drops were added exhibited a marked yeast turbidity.

VI. Series of Experiments

Twenty-four bottles were inoculated, twelve with one and twelve with the other species. The yeast had been grown in bottles of lager beer, which had stood for about ten days at the ordinary room-temperature, and which were frequently shaken in order to hasten the growth. It was in a vigorous condition, and was employed in moderately thin consistency. Twelve bottles were inoculated with ***Saccharomyces Pastorianus III.***, four of which received one drop each, four others two drops each, two more four drops, and two eight drops. In the same way twelve bottles were likewise inoculated with ***Saccharomyces ellipsoideus II.***; three bottles were kept as a control.

After seven days an appreciable sediment was found only in the two bottles to which eight drops of ***Saccharomyces ellipsoideus II.*** had been added; the beer in these became slightly cloudy on shaking, whilst in all the others it was bright and showed no sediment. After fourteen days the bottles which had not been infected, and also ten of those infected with ***Saccharomyces Pastorianus III.***, were still perfectly bright, and showed no appreciable development of yeast; the two bottles which had received eight drops of this yeast were also bright, but on closer examination a slight sediment could be detected, and on agitation the beer became slightly cloudy. The four bottles which had been inoculated with one drop of ***Saccharomyces ellipsoideus II.*** were bright, whilst the remainder showed signs of yeast turbidity, varying according to the amount of infection. On agitation, however, only those which had been most strongly infected (with four and eight drops) became distinctly cloudy.

Some experiments which were carried out in the same manner as those of the last two series, but in which cask sediment was used, gave essentially the same result. This sediment was

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obtained from some of the lager casks mentioned in the description of the experiments, in which the two disease yeasts were added with the pitching yeast at the beginning of the primary fermentation. The cells which were introduced into the bottles in these experiments had therefore been produced under brewery conditions in the fermenting and lager cellars. They were less vigorous than in the previous experiments, and to this I attribute their more feeble action.

Thus, in the experiments which were undertaken in order to ascertain the effect when the infection occurs at the time of bottling, it was also found that ***Saccharomyces ellipsoideus II.*** was the more vigorous of the two disease yeasts. It was also ascertained that the effect of the infection was more pronounced when the yeast consisted of young vigorous cells which had been grown in wort in the course of a few days, than when produced by a protracted fermentation. In order that ***Saccharomyces Pastorianus III.*** may assert its influence it must be introduced into the bottles in quantities which in my opinion are much greater than ever occur in practice. As already mentioned, the other species behaves somewhat differently. When the yeast of a thin consistency, which was employed for the infection, consisted of young vigorous cells, the introduction of one drop into each bottle was sufficient to cause the beer to become cloudy after fourteen days, whilst the uninfected beer could be kept for about three weeks. A greater degree of infection produced yeast-turbidity more quickly. The species in question is therefore able to cause trouble in practice also at this stage. The development of wild yeasts is promoted by vigorous aeration of the beer whilst it is being drawn off, and also through the bottles being badly corked. Beer which has undergone a feeble fermentation, and which has a high extract, is also more subject to contamination than another beer. This holds good for beer both at the commencement and at the end of the storage period. The slight infection due to atmospheric dust can scarcely acquire any importance in this respect. When beer which has remained sound in the lager casks is attacked by this disease after it has been drawn off, this must accordingly be attributed to the bottles and carriage casks not having been properly cleaned. ***A slight infection of the bottled lager beer is without effect; even in the case of Saccharomyces ellipsoideus II. the beer must be comparatively strongly infected before any effect is produced.*** What has been said with regard to the infection of lager beer in bottles will, in the main, also apply to the same beer in the small carriage casks.

Faultless beer, which has been fermented with a pure culture of a good brewery yeast, will also form a yeast sediment after it has remained a sufficient length of time in the bottles and casks. As has already been shown, however, such a sediment is much slower in forming than a disease yeast sediment. As a rule, I also found that there was a distinct difference in the character of the sediment. When the good beer was shaken it did not become cloudy; the yeast collected in small conglomerations, and these rapidly settled again to the bottom. The yeast in the sick beer, on the other hand, was loose, and rose as a cloud of cells when the beer was slightly agitated, and when vigorously shaken the beer became muddy.

In none of the numerous experiments with the two yeasts did I notice that they imparted any disagreeable taste or odor to the beer, not even in cases where they had produced

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pronounced yeast turbidity. A difference was detected by experienced tasters, but was not recognizable as a disease.

Before concluding this chapter, some observations relating to one of the disease yeasts, namely, ***Saccharomyces Pastorianus III.***, may here be mentioned. In one of my earlier researches I briefly alluded to some observations I had made, with regard to the importance of aeration of the wort. By cultivating the Carlsberg bottom yeast: No 1 and No 2 separately in aerated wort, I obtained pitching yeast which, under brewery conditions, was normal in its clarifying properties. On the other hand, when the same yeasts were grown in perfectly similar wort, but which had not been aerated, I obtained yeast which only behaved normally after it had gone through several fermentations in the brewery. The No. 2 yeast, however, re-acquired its normal properties more quickly than No. 1; both underwent temporary modification, the one to a greater degree than the other.

The beer produced by the fermentation of the non-aerated wort was highly opalescent; and this opalescence was, as a rule, only slightly diminished by protracted storage; the beer also remained cloudy after it had been exposed for several days to the ordinary room-temperature. This applies especially to the beer produced from ***Carlsberg bottom yeast No. 1.*** The aerated wort gave bright beer, the non-aerated gave cloudy, opalescent beer.

On the other hand, the result was quite different when the non-aerated wort was pitched with a yeast consisting not only of one of the bottom yeasts mentioned, but which contained also a small quantity of the disease yeast ***Saccharomyces Pastorianus III.*** In this case, the beer produced from the non-aerated wort was also bright; the disease yeast thus played the part as it were of a curative.

On repeating these experiments some years later, a different result was obtained. As a rule, it was found that the beer obtained from non-aerated wort was also bright; yet these new experiments, like the previous ones, were made with ordinary lager-beer wort from the Old Carlsberg brewery, and with the same species of yeasts. Some of the experiments were made in the laboratory with vessels of ten liters capacity and containing seven liters of wort, others in a fermenting cellar under the conditions obtaining in the brewery. In all cases the beers were stored at a temperature of 1 - 2° C., and, in fact, care was taken to imitate as far as possible the conditions obtaining in practice. It was only in one of these new experiments that the beer from non-aerated wort was opalescent. This experiment was conducted in four of the fermenting vessels in the fermenting cellar of Old Carlsberg, and which have been previously mentioned; in the first of these the fermentation was carried out with ***Carlsberg bottom yeast No. 1.*** in the second, with a mixture of this with ***Saccharomyces Pastorianus III.***; in the third, with ***Carlsberg bottom yeast No. 2.*** and in the fourth, with a mixture of the latter species with ***Saccharomyces Pastorianus III.*** The main result obtained was that the beer produced with the help of ***Carlsberg bottom yeast No. 2.*** was only faintly opalescent, whilst the beer from the No. 1 yeast showed a marked opalescence. The beer which had been fermented with the mixture of ***Carlsberg bottom yeast No. 2*** and ***Saccharomyces Pastorianus III.*** was bright at racking, whilst that fermented with the mixture of Carlsberg

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bottom yeast No 1 and ***Saccharomyces Pastorianus III.*** was rather strongly opalescent, though in a much less degree than the beer from the first vessel. ***Saccharomyces Pastorianus III.*** had thus produced the effect on the opalescent beer which was previously mentioned.

The most probable explanation of these varying results is, that the worts with which I experimented at different times varied in their composition. The experiments have, at any rate, shown that ***Saccharomyces Pastorianus III., which under certain conditions can play the part of a dangerous disease yeast, can under others act as a curative. We have further seen that wort may be so constituted that it does not require the customary aeration which has hitherto been regarded as perfectly necessary for the attainment of a good fermentation and of a bright beer.*** With regard to the importance of aeration, our knowledge is still very slight, and a thorough investigation of this subject would, therefore, be of great value. I have given the above results in this place, as I believe that there will not be another opportunity of returning to the subject.

Whilst speaking of variations in the activity of yeasts, I may here also mention that ***the addition of the same amount of a disease yeast produced very marked ill effects in some of my experiments, and only very feeble effects in others;*** and yet the experiments appeared to have been carried out essentially in the same manner. The variation occurring in the composition of the wort in the same brewery during the year may in part be the cause; but it is also conceivable that a temporary modification of the condition of the cells has something to do with it. We are here dealing with phenomena similar to those which we often find described in connection with pathogenic bacteria. In the competition which occurs between the brewery yeast and the disease yeasts, the power which the cells possess of accommodating themselves to external circumstances also gradually comes more and more into play. In my theoretical studies I hope to be able to give some information in connection with this question.

Main Result. We have now followed the beer through all the stages of fermentation, in its course from the fermenting vessel to the lager cask, and finally from this to the consumer. The investigations have shown that there are two species of yeast, ***Saccharomyces Pastorianus III.*** and ***Saccharomyces ellipsoideus II.***, which produce the disease when they are present in the pitching yeast, and are, therefore, introduced at the commencement of the primary fermentation. One Series of Experiments showed that the disease can occur when the disease yeast amounts to 1/41 part of the pitching yeast, but, on the other hand, that it can be checked by a strong attenuation and a sufficiently long storage. When the disease yeast is present in larger amount, it is more difficult and sometimes quite impossible to ward off the disease.

When the infection occurs at the end of the primary fermentation, when the beer is removed to the lager cellar, it is without effect. Beer which leaves the fermenting cellar without having become infected, is as a rule not attacked by the disease, even when it comes in contact with the two disease yeasts in the lager casks or in the pipes through which it passes. It must,

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however, be borne in mind that there are a large number of other micro-organisms besides those mentioned, and which can also produce dangerous effects. A careful cleansing of the pipes leading into the lager cellar, and a frequent pitching of the lager casks, is therefore, and always remains, of the greatest importance.

When the infection was not great, it had no influence on good beer which was bottled in the ordinary way. Indeed, comparatively very large quantities of ***Saccharomyces Pastorianus III.*** could be added to such beer without producing any disease whatever. The addition of one drop of the other species of disease yeast, of a thin consistency, to 350 cc. of lager beer produced faint yeast turbidity, but this via the case only when the yeast consisted of young vigorous cells.

The main rule is, that both species are dangerous when present at the commencement of the primary fermentation, and, in fact, only at this stage. In all cases, ***Saccharomyces ellipsoideus II.*** was found to be the stronger of the two species. The varying results which the same infection may produce have already been mentioned.

In recent years the above-mentioned wild yeasts have been observed by Lasche in Chicago, and by Kokosinski in Lille. These investigators have proved that they produce similar ill-effects in the low-fermentation beers of North America and France, as in those of Denmark and Germany. In one of the following chapters we shall become acquainted with other species which also produce yeast turbidity in beer. The two species mentioned in this chapter I regard as especially dangerous in this respect, and this is particularly so in the case of ***Saccharomyces ellipsoideus II.***

SACCHAROMYCES EXIGUUS

From the above historical survey we perceive that, after the publication of Reess's investigations on the alcoholic ferments, there was a tendency to attribute to ***Saccharomyces exiguum*** the irregularities which can occur in the fermentation, when the beer refuses to clarify, when it becomes cloudy with suspended yeast after storage, or when it acquires a disagreeable taste. As stated, no experiments were undertaken, but a simple microscopic examination was made to suffice. The small yeast cells which could be detected in such bad beer were definitely stated to belong to Reess's ***Saccharomyces exiguum***, and this micro-organism was assumed to be the cause of a whole series of different maladies. At that time it was not known that all, and every one of the ***Saccharomycetes*** can develop cells which might be regarded as belonging to Reess's ***Saccharomyces exiguum***.

If, with the knowledge which we now possess, this systematic name is to be still retained, it must be applied to the species which I have described in my treatise "***Action des ferment alcooliques sur les diverses espèces de sucre***" ("***Compte-rendu des travaux du laboratoire de Carlsberg.***" tom. ii. livr. 5, 1888). With this species I carried out some experiments of which the following will serve as examples.

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I. Series of Experiments

Three vessels, A, B and C, in the fermenting cellar of the Old Carlsberg brewery were charged with 1 1/3 hectoliters of wort of 14.3 per cent. Balling. They were the wooden fermenting vessels mentioned in the last section.

A was pitched with 400 grams of ***Carlsberg bottom yeast No. 2.***

B was pitched with 350 of ***Carlsberg bottom yeast No. 2*** and 75 grams of ***Saccharomyces exiguum***

C was pitched with 400 grams of ***Carlsberg bottom yeast No. 2.***

In all cases the yeast was moderately thick and consisted of young vigorous cells which had been grown at about 10° C. The temperature of the wort at pitching was 7.5° C., that of the fermenting cellar during the whole experiment was 8-9° C. After seven days the extract in A was 7-37, in B 7.45, and in C 7.21 per cent. Ball The beer clarified well in all three vessels, the odor and taste were also faultless and alike in all the beers.

The beer from each fermenting vessel was run into two similar casks, and into one of those filled with beer from C was introduced 15 grams, and into the other 30 grams, of ***Saccharomyces exiguum*** of a thick consistency. All the casks were then placed in the lager cellar, the temperature of which was 0"§ z"5° C.

After three months' storage some of the beer from each cask was drawn off into bottles, and these were preserved in a dark cupboard at the ordinary room-temperature. The beer was in all cases perfectly bright when bottled, and it was also good as regards taste and odor. After standing for fifteen days, the beer from A, B and C was still alike and faultless in every respect.

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II. Series of Experiments

The method was the same as before, but six vessels, A, B, C, D, E and F, were made use of.

A was pitched with 400 grams of **Carlsberg bottom yeast No. 1.**

B was pitched with 400 grams of **Carlsberg bottom yeast No. 2.**

C was pitched with 350 grams of **Carlsberg bottom yeast No. 1,** and 50 grams of **Saccharomyces exiguum.**

D was pitched with 350 grams of **Carlsberg bottom yeast No. 2,** and 50 grams of **Saccharomyces exiguum.**

E was pitched with 400 grams of **Carlsberg bottom yeast No. 1.**

F was pitched with 400 grams of **Carlsberg bottom yeast No. 2.**

The extract of the wort was 13.9 per cent. Balling, and its temperature at pitching was 7° C. The primary fermentation was finished after ten days, and the extract was then 6.80 per cent. Balling in A, 7.78 per cent. in B, 7.13 per cent. in C, 7.70 per cent. in D, 6.72 per cent. in E, and 7.86 per cent. in F. The clarification was good in all cases, and was best in B and F. As regards odor and taste all the beers were alike. The beer from E and that from F were each infected with 75 grams of **Saccharomyces exiguum,** as in the first experiments.

After scarcely two months' storage the extract was 5.74 per cent. Balling in A, 6.72 per cent. in B, 5.90 per cent. in C, 6.56 in D, 5.74 per cent. in E, and 6.64 per cent. in F. The beer, which was now perfectly bright, was drawn off into bottles as described above, and after these had stood eleven days they showed only a very slight sediment. No difference could be detected in this respect between the beer which had been infected with Sarah. enguu: and the uninfected beer. All the samples had a good taste and odor; after fourteen days there were still no signs of disease. After three months' storage the extract was 5.74 per cent. Balling in A, 6.39 per cent. in B, 5.82 per cent. in C, 6.39 per cent. in D, 5.74 per cent. in E, and 6.31 per cent. in F. The beer had in each case preserved its brightness perfectly, and also its good taste and odor. After being fourteen days in bottle under the conditions mentioned, there was no sign of yeast turbidity or of any disease whatever.

In the experiments described, **Saccharomyces exiguum** was in some cases added to the wort with the normal pitching yeast at the commencement of the primary fermentation, and in others was introduced after the termination of the primary fermentation, at the commencement of the storage period. In addition to these experiments I made others in which the beer was infected with the yeast mentioned at the conclusion of the storage period. The method was the same as that adopted in the experiments with **Saccharomyces Pastorianus III.** and **Saccharomyces ellipsoideus II.**, which were described above. I made

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use partly of a young vigorous growth which had been cultivated in flasks of wort, partly of cask sediments which were obtained after storage of the beers produced in the experiments just described, and partly also of yeast sediment from bottles of ordinary lager beer which had been infected with ***Saccharomyces exiguum***, and subsequently kept for a time in the room; these bottles had been frequently shaken, in order to hasten the growth of the yeast. The yeast obtained in these different ways was employed in a thin condition; in some experiments two drops, and in others three drops, were added to the bottles under investigation. In spite of this considerable contamination, no effect could be detected, and, after fourteen days, none of the bottles showed any signs of yeast turbidity.

Main Result. The experiments just described show that a considerable addition of ***Saccharomyces exiguum at the beginning of the primary fermentation, or at its end, or after storage, does not produce any disease in lager beer.*** As the experiments were carried out entirely under the conditions prevailing in the brewery, the results obtained may, with perfect justice, be applied to practice.

It is, of course, not possible to determine of what kind were the yeast cells referred to at the time when ***Saccharomyces exiguum*** played such an important part in the zymotechnic literature. Since the problem of the diseases of beer caused by alcoholic ferments has been attacked experimentally, it has no longer been a question of this species. The possibility is, indeed, not excluded that a disease yeast consisting of small cells may some day be found, and assumed to be the old ***Saccharomyces exiguum*** of Reess; but, for the present, this dread has disappeared from the zymotechnic field.

In addition to the ***Saccharomyces exiguum*** which I have mentioned above, there are, as I have pointed out on different occasions, several other species of wild yeasts which can grow freely in wort, but which produce no disease in beer. The same holds good also for several bacteria.

My experiments described above were carried out with reference only to the practical points in question, and, regarded from this point of view, they show that the addition of ***Saccharomyces exiguum*** is without influence. If we were to follow out the question of competition from a theoretical standpoint, we should find, however, that ***Saccharomyces exiguum*** is not absolutely without effect. In several experiments, for instance, I noticed that the addition of this species in larger proportion had a retarding influence on the attenuation during the early stages of the fermentation, as compared with the result when the brewery yeasts are alone present.

DISAGREEABLE ODOR AND TASTE PRODUCED IN BEER BY SACCHAROMYCES PASTORIANUS I.

The main result indicated in the above heading was published very briefly in the "***Zeitschrift für das ges. Brauwesen,***" in 1884., and I then promised a more detailed account of my

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investigations. This will be given in the following pages, together with an account of some more recent experiments.

In the preliminary notice mentioned I stated that, in 1833, the beer of the Old Carlsberg brewery was attacked by a disease which communicated a disagreeable bitter taste and an unpleasant odor to it. Some beer experts designated the taste and odor as smoky; all agreed that the beer had suffered. By separating the yeast into its constituent species, I succeeded in isolating four. In the experiments which I made with these in flasks of wort, only one of them gave a beer of good taste and odor; this was the species to which I gave the name **Carlsberg bottom yeast No. 1**, and which since then has been employed on a large scale in Scandinavian breweries. Amongst the other yeasts present, I found the species which I have named **Saccharomyces Pastorianus I. It was only when this species was present in the pitching yeast that the disease occurred.** However convincing such laboratory experiments may be, they do not carry the same demonstrative force as trials made in the brewery itself, and under the conditions obtaining in practice; in the following. only experiments of this nature will, therefore, be described.

I. Series of Experiments

Three of the above-mentioned small fermenting vessels, C, D, B, were charged in the fermenting cellar of the Old Carlsberg brewery with 1.5 hectoliters of ordinary aerated wort of 13.3 per cent. Balling. The temperature of the wort at pitching was 7.8° C., that of the fermenting cellar 5-6° C.

C was pitched with 500 grams of **Saccharomyces Pastorianus I.**

D was pitched with 400 grams of **Carlsberg bottom yeast No. 1**, and 100 grams of **Saccharomyces Pastorianus I.**

E was pitched with 500 grams of **Carlsberg bottom yeast No. 1.**

The yeast was of a thick consistency in all cases, and was grown in similar wort at 8-10° C. After eleven days the extract was found to be 6.03 per cent. Balling in C. 5.54 per cent. in D, and 6.27 per cent. in E. The beer from each vessel was then drawn or!" into three small casks, and these were placed in the lager cellar at a temperature of 2-3° C. The beers from C and D had an objectionable odor and a bitter disagreeable taste, whilst that from E had a satisfactory taste and odor; the bitter taste was strongest in C. Although a vigorous fermentation had taken place, the beer from C and D clarified badly, that from E, on the other hand, was faultless.

After being stored for about a month, samples from one series of the casks were drawn off into colorless glass bottles. C was quite cloudy, D was nearly bright, and E was perfectly bright. After standing for five days, at the ordinary room-temperature, D showed a slight yeast turbidity, whilst E showed no signs of cloudiness after twelve days. In the case of the beer

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which had been fermented partly with ***Saccharomyces Pastorianus I.*** the disagreeable taste and odor were very marked, whilst the beer which had been fermented with Carl-berg bottom yeast No. 1 alone, possessed the same good taste and odor as the ordinary lager beer of Old Carlsberg. After the beer had been a little more than two months in the lager cellar, D and 1-2 were perfectly bright, while C was still cloudy. The extract was 5.54 per cent. Balling in C, 5.37 per cent. in D, and 5.29 per cent. in E. With regard to taste and odor, there was the same difference as before. The beer from D had now also become stable again, and, like that from E, it remained twenty-one days in bottle in the room without exhibiting signs of yeast turbidity.

After five months' storage the beer from D still possessed the disagreeable bitter taste in a high degree. The beer from C was at this time still cloudy, and it was only after six months' storage in the lager cellar that it became bright; the taste was still as nauseous as before.

The result is, in the main, the same when a mixture of ***Saccharomyces Pastorianus I.*** with another brewery yeast in the place of the ***Carlsberg bottom yeast No. 1*** is employed.

Thus the disease mentioned is very pronounced when one fifth of the pitching yeast consists of ***Saccharomyces Pastorianus I.*** In one experiment made with the large Pasteur vessels containing 18 hectoliters of aerated wort of the same character as that previously mentioned, it was found, however, that the disease was also produced when the same disease yeast was present to the extent of only 15 of the pitching yeast. This experiment, however, was not made exactly under brewery conditions, and I will, therefore, pass on to the description of the following experiments.

II. Series of Experiments

These were conducted in the same manner as the first experiments, and in the same fermenting cellar. The wort had an extract of 13.9 per cent. Balling; its temperature at pitching was 7° C.

A was pitched with 400 grams of ***Carlsberg bottom yeast No. 1.***

E was pitched with 380 grams of ***Carlsberg bottom yeast No. 1,*** and 18 grams of ***Saccharomyces Pastorianus I.***

F was pitched with 380 grams of ***Carlsberg bottom yeast No. 1,*** and 18 grams of a variety of ***Saccharomyces Pastorianus I.***

In E and F the disease yeast thus formed 1/22 part of the total pitching yeast. After ten days the extract was 6.80 per cent. Balling in A, 7.37 per cent. in E, and 7.86 per cent. in F. The beer clarified well in A, and only fairly well in E and F. As regards taste and odor, there was no marked difference in the beers from the three vessels.

When the beer had been in the lager cellar for about two months, A was found to have an

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extract of 5.74 per cent. Balling, E 6.15 per cent. and F 6.23 per cent.; the beer was bright in all three cases. A had the usual good taste and odor, E and F, on the other hand, possessed an objectionable odor and a bitter disagreeable taste, but only in a very slight degree. This test was made directly after the beer had been drawn off, and, as usual, by several persons: in fact, by her experts. After the beer had stood in bottles for a few days at the ordinary room temperature, it was again examined; the difference appeared to be still less pronounced. It was, indeed, only when a comparison was made with the infected beer that it was possible to detect that which had been fermented partly with *Saccharomyces Pastorianus I.* and its variety. **After standing six days, the bottles containing the beer from E and F showed a rather considerable sediment of yeast, and when shaken up it became cloudy. The beer from A, on the other hand, was stable.** Samples were again collected after three months' storage; the extract of A was 5.74 per cent. Balling, that of E and F 5.9 per cent. **The beers from all three were bright. With regard to taste and odor the same observations were made as before.** When the beer had been fourteen days in bottle in the dark, and at the ordinary room-temperature, A contained no appreciable sediment, and when shaken showed no sign of yeast turbidity. In the beer from E and F, on the contrary, there was a distinct yeast sediment, which on agitation rendered the beer slightly cloudy.

The disagreeable taste and odor were noticeable also in this case, although to a very slight extent. We have seen above that, on the other hand, these symptoms of disease manifest themselves in a marked degree when *Saccharomyces Pastorianus I* is present in considerable quantity in the pitching yeast. That this applies also to varieties of the above species, I have convinced myself by direct experiment.

In the experiments described, the disease yeast was added to the wort at the commencement of the primary fermentation; the following experiments, on the other hand, were undertaken in order to ascertain the effect when infection occurs at the end of the primary fermentation.

III. Series of Experiments

At the conclusion of the primary fermentation in the above first Series of Experiments, and after the beer had been run from the fermenting vessels into the small lager casks, 20 cc. of sedimentary yeast was taken from each of the vessels, C, D and E, and introduced into about 17 liters of fermenting lager-beer wort, the primary fermentation of which was just finished, and which was then removed to the lager cellar. The small lager cults were treated in the manner previously described, and were set aside in the lager cellar, together with a control cask containing uninfected beer; the temperature of the cellar was 3-6° C. When it is recollect that the weight of yeast of the consistency usually employed at least in the Copenhagen breweries amounts to 4 grams to the liter of wort, it will be seen that the infection mentioned was very considerable. As stated above, C contained exclusively *Saccharomyces Pastorianus I.*, D a mixture of this with *Carlsberg bottom yeast No. 1*, and E, the latter species alone; they had in all cases gone through a primary fermentation in the fermenting cellar. The conditions were, therefore, similar to those obtaining in practice.

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When this beer had been two and a quarter months in the lager cellar a considerable number of bottles were filled from each cask. The beer was bright in each case, and contained no sediment. Experts who tasted the samples were generally inclined to pronounce the beer from all the casks as faultless; only in the case of the bottles containing the beer from C, in which the infection consisted entirely of ***Saccharomyces Pastorianus I.***, was a faint disagreeable bitter taste noticed by most. ***The infection had, therefore, in this respect had little or no effect, and the same holds good for the stability of the beer.*** Alter the bottles had stood at the ordinary room-temperature for twenty-one hours, there were still no signs of yeast turbidity.

IV. Series of Experiments

The method adopted was the same as in the last Series of Experiments. The beer was of the same character, and was taken at the same stage, namely, at commencement of storage. In this case, however, to each quantity of seventeen liters was added 10 cc. of yeast of fairly thin consistency, and which consisted of a vigorous growth of ***Saccharomyces Pastorianus I.*** produced by twenty-four hours' cultivation in wort. The temperature of the lager cellar was 2.5° C.

After about three months' storage, and after the beer had been bottled, both the infected and the uninfected were bright and free from sediment; the taste and odor were good in both cases. and also at regard: stability no difference could be detected; after standing fourteen days under the conditions mentioned above, no signs of yeast turbidity were perceptible.

The following examples will serve to illustrate the experiments which were carried out with a view to ascertain the effect of infection occurring at the end of the storage period.

V. Series of Experiments

Nine bottles of lager beer were infected with ***Saccharomyces Pastorianus I.***, as follows: three of them with one drop each of the yeast, two with three drops, and four with 1 cc. each. The yeast consisted of a young vigorous growth cultivated in wort, and was of a very thin consistency. Three uninfected bottles served as a control. In other respects the method was the same as before.

The bottles which had been infected with one and with three drop: showed no .rr"g"n: o/ yeast turbidity after fourteen day, and the taste and odor of the beer was a: good at at the commencement of the experiment; the beer, in fact, was the same as that in the control bottles. The four bottles which had been ***very strongly infected, namely, with 1 cc of the yeast, were cloudy after only four days; the beer, however, had only acquired a faint indication of the bitter taste.***

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VI. Series of Experiments

After the beer of the second Series of Experiments was drawn off" from the casks, which had been about two months in the lager cellar, some of the cask sediment from E (**Carlsberg bottom yeast No. 1** and **Saccharomyces Pastorianus I.**), of fairly thin consistency. was introduced into three bottles of ordinary lager beer, one drop being added to each. In this case, therefore. the yeast consisted of a growth which had gone through both primary and secondary fermentation under the conditions obtaining in the brewery. Three bottles which were not infected were kept as a control. **After standing for sixteen days no effect had resulted from the infection, either as regards taste and odor or stability of the beer.**

VII. Series of Experiments

Sixteen bottles of lager her were infected, and three uninfected bottles were kept as a control. For the infection the sediment was used which had formed in bottles which had stood for a long time at the ordinary room-temperature. This beer had been fermented in one case with a mixture of **Carlsberg bottom yeast No. 1** and **Saccharomyces Pastorianus I.**, and in the other case with a mixture of the same brewery yeast with the above-mentioned variety of **Saccharomyces Pastorianus I.**. These yeasts had not only carried through the primary fermentation in the fermenting cellar and the normal secondary fermentation in the lager cellar, but had also given rise to a new after-fermentation and to a multiplication of the cells after the beer had been drawn off from the lager casks. Eight bottles were infected with each of the two yeast mixtures, three with one drop, three others with two drops, one with four drops, and one with eight drops of the fairly well diluted yeast.

The bottles to which eight drops had been added became cloudy from suspended yeast after seven days, those to which four drops had been added were affected in the same way after twelve days, whilst those infected with only one and two drops were still mostly faultless after fourteen days, or at most they showed a faint indication of yeast turbidity; in short, their stability was practically equal to that of the samples kept for control As regards odor and taste, no difference should be detected between the infected and the uninfected beer.

Main Result. The disagreeable odor and the unpleasant bitter taste, communicated to lager beer by the disease we have been discussing, exhibits itself not only in the finished stored beer, but also even in the fermenting wort at the end of the primary fermentation. The experiments have shown that this disease is caused by **Saccharomyces Pastorianus I.**, and by the varieties which I have obtained from the latter. Strictly speaking, the disease only occurred when the infection had taken place at the commencement of the primary fermentation. The disease, germs must be looked for in the pitching an yeast, and in the wort contained in the fermenting vessels. When **Saccharomyces Pastorianus I.** formed one-fifth of the pitching yeast, the disease was produced in a marked degree; on diminishing the proportion it was less pronounced, and when the wild yeast, or its varieties, formed 1/22 part

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of the total pitching yeast, the disease could only just be detected. Under the conditions described this appears, therefore, to be the limit. A smaller quantity still will, therefore, scarcely have any deleterious action in this direction. In an experiment made with a strongly infected pitching yeast, it was found that the beer still retained the disagreeable taste and odor produced after no less than five months' storage.

It was easy to foresee that beer which had been fermented exclusively with a pure culture of this species, or its varieties, must also acquire the same disagreeable taste and odor.

When the infection only occurs in the lager casks, or in the pipes leading to these, it is, under ordinary brewery conditions, without effect. This is shown by the experiments which were carried out partly with young vigorous growths obtained by one day's cultivation in wort, and partly with growths which, in conjunction with a brewery yeast, had carried through a primary fermentation in a fermenting vessel in the brewery. Only in the case of one experiment, in which an extremely large proportion of a pure culture of ***Saccharomyces Pastorianus I.*** was added at the commencement of storage, did the beer acquire a faint indication of the disagreeable bitter taste.

An equally insignificant effect was produced when the infection occurred at the end of the storage period.

It is, however, not merely as regards taste and odor, but also as regards the stability of the beer, that ***Saccharomyces Pastorianus I.*** can act injudiciously. In the experiments in which this species was present in the pitching yeast, it also interfered with the brightening of the beer at the end of the primary fermentation. Even when ***Saccharomyces Pastorianus I.*** formed only 1/22 part of the pitching yeast, the resulting beer proved, after normal storage, to be appreciably less stable than the corresponding beer which had been fermented with a pure culture of the brewery yeast.

As in the experiments described above with ***Saccharomyces ellipsoideus II.*** and Sac:/1. ***Saccharomyces Pastorianus III.***, the attenuation and storage of the beer also plays an important part in this case. The highly attenuated beer of the first Series of Experiments was perfectly stable after two months' storage in the lager cellar, in spite of the fact that one-fifth of the pitching yeast consisted of ***Saccharomyces Pastorianus I.***. When the beer attenuates well during the primary fermentation, and is then stored for not too short a period in a good cellar, it will not, as a rule, be subject to yeast turbidity after it is subsequently drawn off. When the diseased yeast is present in larger quantity, however, it will be able to attack the beer in another way, and there will be a deterioration as regards taste and odor.

With regard to the effect produced by this species of yeast, irregularities were also observed similar to those mentioned in the case of ***Saccharomyces Pastorianus III.*** and ***Saccharomyces ellipsoideus II.***

In those cases where the infection occurred only after the conclusion of the primary

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fermentation in the fermenting cellar, that is to say, either during or after storage, no effect was produced on the stability of the beer, except when a comparatively large proportion of the disease yeast had been present.

Likewise, when a small quantity of the disease yeast is present in the lager casks, in the pipes leading to these, or in the bottles and small casks from which the beer passes to the consumer, it will have no effect either in the one direction or the other. This result is thus in agreement with the main results of my experiments with *Saccharomyces ellipsoideus II.* and *Saccharomyces Pastorianus III.*

The treatises which I published in 1883 and 1884 on the disease yeasts again brought this question to the front, and, indeed, in quite another manner than previously. Similar experiments to those which I described above were now undertaken in most zymotechnic laboratories. The time for experimenting in this field was introduced through my researches.

In my first studies I confined myself to the investigation of the effect of infection occurring at the commencement of the primary fermentation, and for the time I left the other stages of fermentation out of consideration. The same method has been followed by those authors who have been subsequently engaged with the study of the relationship of the alcoholic ferments to the diseases of beer. A review of the results of these investigations will be of interest, and may, therefore, follow here.

In 1887 Grönlund published an elaborate investigation of similar disease phenomena to those mentioned above ("Zeitschr. für das ges. Brauwesen"). He stated that a Danish low-fermentation brewery had suffered in this respect, where the beer formerly had been stable and of good flavor. It had now not only become bitter, but it left an after-taste which was highly objectionable, sharp and astringent. In this sick beer he found a yeast which possessed all the characters of my *Saccharomyces* Pa.rtorianu.r I, and which he therefore identified as this species. He also proved, by direct experiments, that it wn the cause of the disease.

The more recent investigations of Kokosinski in Lille, and of Lasche in Chicago, likewise confirm the correctness of my experiments.

We thus not only learn that *Saccharomyces Pastorianus I.* is of very frequent occurrence in breweries, but also that this species give: rise to the universally dreaded disease in question, even under the varied condition: obtaining in the breweries of the different countries mentioned.

In the reports for 1885-1888 of the Scientific Station for Brewing at Munich, and in the "Zeitschrift fiir das ges. Brauwesen" for 1891, Will gives an account of a series of elaborate investigations which he carried out with two new *Saccharomyces*. The effect of one of these species was to communicate to low-fermentation beer a characteristic sweetish taste, and a harsh, bitter after-taste, whilst the clarification of the beer during the secondary fermentation took place more slowly than when the cells of this species were not present. The

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effect of the other species was, in the main, the same. Both species are dangerous in the manufacture of low fermentation beer.

Krieger has also published accounts from the Brewing Station at New York, in which he mentions wild yeasts which diminish the stability of beer, and at the same time communicate to it a bad flavor.

In the "***Wochenschrift für Brauerei***" for 1889, Windisch describes some fermentation experiments with different species of brewery yeasts, and with a species of the group of ***Saccharomyces Pastorianus***, which is not further defined. These experiments were carried out with flasks charged with sterilized wort. The beer obtained with the wild yeast mentioned did not become bright, and it had a disagreeable bitter taste, which was followed by a harsh after-taste.

In the same journal for 1891, P. Lindner stated that he had come across a yeast which closely resembled the low fermentation yeast of a brewery. Judging from the appearance of the fermentation, the clarification of the beer, and the character of the sedimentary yeast, the practical brewer would have regarded this as an excellent bottom yeast, but it, nevertheless, produced a beer having an abominable bitter and harsh taste. This dangerous yeast had gained access to a brewery in Berlin, and gradually increased to such an extent in the pitching yeast that the beer soon began to acquire the offensive flavor mentioned. ***This is not only a new example of the manifold nature and the danger of the disease yeasts, but also of the insufficiency and uncertainty of the characters which brewers judged - formerly exclusively, and unfortunately still to too great an extent the progress and condition of the fermentation.***

Lasche has also observed some new disease yeasts, a description of which may be expected in the journal of the Chicago Station.

The above investigations relate exclusively to low-fermentation beer. On reading the English brewing literature of recent years, it is seen, from a number of observations, that ***the wild yeast: can give rise to irregularities to an equally great extent in high fermentation as in low-fermentation breweries***. The experimental investigation of De Bevay, of Melbourne, shows that the beer-disease known in Australia as "summer-cloud" is caused by a ***Saccharomyces*** ("***The Brewers' Journal***," London, 1889, p. 490). high fermentation beer which has been attacked by this species of wild yeast becomes cloudy, and acquires a sour bitter taste. This malady is mentioned as one of the most serious occurring in Australian breweries.

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Whence Come The Disease Yeast

In investigations such as those under discussion, the problem is not merely to ascertain the causes of the diseases, but there at once present themselves the new questions: How can we recognize the disease germs? Where is their habitat? How do they gain admission into the brewery?

As to the characters by means of which the disease yeasts can be distinguished from the good brewery yeasts, these have been described in the above account of my theoretical investigations. The important question of habitat cannot unfortunately be completely answered, and I will here briefly state all that we now know.

In 1881, I published in the journal of the Carlsberg laboratory a treatise on ***Saccharomyces apiculatus*** and its circulation in nature. My investigations not only proved that this fungus occurs on ripe, sweet, succulent fruits, but, what is more important, they also showed that these fruits form its ***normal*** habitat. As the fruits increase in the garden, numerous generations of the cells of this fungus are produced, and they then become more and more abundant in the dust particles of the atmosphere. ***Saccharomyces apiculatus*** is regularly observed first on the sweet succulent fruits which ripen earliest, and afterwards on those which ripen later. In the Carlsberg garden it is found, at the commencement of the season, on the strawberries, gooseberries and cherries; and, at the end of the season, on the plums and grapes. It is carried to the soil by rain and falling fruit on dry days it is again borne into the air with the dust, and the cells, which settle upon the fruits named and gain access to their juice, are enabled to bud and produce new generations. All this may be repeated several times in the course of the summer, so that ***Saccharomyces apiculatus*** passes alternately from the fruit to the soil and ***vice versa***. It passes the winter in the soil in order to recommence the same migrations in the following summer. It cannot leave its winter habitat spontaneously but requires assistance: in dry seasons the wind carries it into the air with the dust; the rain may also splash its cells on to low plants, such as the strawberry plant; likewise insects and other animals may take part in their transference. When the cells are brought into contact with nourishment they commence budding; otherwise they soon dry and perish.

In a small communication, which I published in 1882 in the Danish journal, "***Tidsskrift for populære Fremstillinger af Naturvidenskaben***," I described the results of some experiments on the part played by bees, wasps and flies in disseminating the small yeast fungus. I pointed out that at the time when fruit is ripe ***Saccharomyces apiculatus*** is carried, especially by the agency of these insects, to places widely distant from those where it originally multiplied. When these insects come into contact with the juice in which ***Saccharomyces apiculatus*** has developed, considerable quantities of the yeast often stick to their hairy coverings, where it slowly dries. The cells, as my experiments have proved, can keep alive for a longer period in this manner than when they are dispersed in atmospheric dust. In the latter case the drying up will, as a rule, have a more destructive effect.

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These were the results of my studies mentioned above. ***The sweet succulent fruits of the garden proved to be the normal place of development of the small yeast fungus, and (lie soil its normal winter habitat.**** My experiments have thus shown that insects and other small animals are active in distributing the cells of this yeast, but that the wind also plays a highly important part. This latter means of transport especially demands the attention of brewers in connection with the question of micro-organisms.

* I published some recent investigations on ***Saccharomyces apiculatus*** in "Botanisches Centralblatt," Bd. 21, No. 6, 1835, in "***Annals des sciences naturelles. Botnnique,***" t. ii. No. 3, 1890, and in "***Annales de micrographie,***" 1890.

Saccharomyces apiculatus is as yet the only species of yeast whose migrations in nature are known. My experiments on the true ***Saccharomycetes*** (yeasts exhibiting endospore formation, a character not possessed by ***Saccharomyces apiculatus***) in connection with this question have not yet led to a successful issue. Our knowledge of the most important species relating to the fermentation industry is in this respect still very imperfect. The investigators who first studied yeast cells, observed that these occurred on sweet succulent fruits, especially on those which were damaged, and that they multiplied there. My numerous experiments have also confirmed the fact that this occurrence is very general; fallen fruits especially give rise to a luxuriant growth. With regard to the wine yeasts, according to Pasteur's view, these do not pass the winter in the soil. My experiments are, however, opposed to this view. I have, in fact, found these yeasts alive in the soil under vines in several parts of Germany both in the spring and in the summer months, that is, at a time when there were still no ripe grapes.

It is highly probable that the yeast cells which I found were carried into the soil in the previous autumn, when the grapes were ripe and the damaged fruit had given rise to endless generations of such cells. These investigations can, however, naturally not prove with certainty that such was the case. I have proved, however, by direct experiments, that cells of various ***Saccharomycetes***, which were placed in the soil in the month of September, were still living after the expiration of a year, that is, from one fruit-time to the next. My first experiments in this direction are described in the journal of the Carlsberg Laboratory for 1882 (French Résumée, p. 203); my later investigations are described in the recent communications mentioned above; amongst the ***Saccharomycetes*** there was also a typical wine yeast, which I described in 1883 under the name ***Saccharomyces ellipsoideus***, also the disease yeast ***Saccharomyces Pastorianus I.***

The fact is thus established that at least some of the true Saccharomycetes can pass the winter in the soil,* and, further, that sweet succulent fruits offer a favorable medium for their growth. Nevertheless we do not yet know whether their ***normal*** habitat is the soil during the winter and spring, and the fruits mentioned during the summer and autumn. The observations which have hitherto been made do not justify us in drawing this conclusion. Before this can be done, similar experimental proof is required to that furnished by my

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investigations on the migrations of *Saccharomyces apieulatus*; as stated, I have, however, not yet succeeded in furnishing such proof. From the investigations hitherto carried out we must still admit the possibility that there may be other places in nature where the true ***Saccharomycetes*** multiply, and other winter habitats than those mentioned, and which perhaps are of greater importance. We are here, again, brought face to face with the old question as to whether the ***Saccharomycetes*** are independent organisms, or only forms of development of the higher fungi. Should the latter prove to be the case, we should naturally also have to take into consideration these original forms, indeed it might even be possible that these would prove of importance in clearing up the problem. These purely theoretical investigations, regarded from this point of view, thus become also of practical interest. Although several of the most famous investigators have been at great pains to discover these supposed progenitors, yet hitherto no trace of them has been found. Recently attention has again been drawn to this question, especially by Brefeld. The position of the matter at the present time is such that we must still regard the ***Saccharomycetes*** as independent organisms.*

* In a paper on this subject (1890) Müller-Thurgau agrees with the View which I put forward. In one point, however, he has misunderstood me, as shown above, in assuming that in my opinion the dissemination of the yeast cells is effected only through the agency of the wind.

* Addition 1895. For the present no decisive conclusion can be drawn from Juhler's and Jörgensen's observations mentioned on page 81.

From the foregoing investigations it is clear that, at all times of the year, atmospheric dust may contain cells of true ***Saccharomycetes***, and amongst these disease yeasts. The soil of fruit gardens offers the greatest danger in this respect. The production of new generations of cells thus occurs in nature at the time when the sweet succulent garden fruits are ripe; in Denmark this is especially the case in August and September. In these months, therefore, the cells will not only be most abundant in the dust particles, but they will also be comparatively less enfeebled than at other times of the year. The clouds of dust which are blown up from the soil of fruit gardens in these months often contain an abundance of young vigorous cells.

My analyses of the micro organisms of the air showed that, in the year 1879, the ***Saccharomycetes*** gradually became more and more plentiful from June to August, so that "the infection to which they gave rise reached its maximum in the latter month. After that there was again a decrease. In the years 1878 and 1880 the infection was greatest in August and September, the maximum occurring at the beginning of the latter month. At other times of the year yeast cells were very scarce. ***August and September are, as regards" infection with wild yeasts, the two most dangerous months for breweries.***

The open coolers afford the means by which these harmful organisms generally gain access to the brewery; sometimes, however, they can gain admission directly to the fermenting cellar. More rarely they become introduced into the beer in the lager casks; but

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even when this occurs, the infection will, under normal conditions, be of no consequence, as we have seen above; this at least holds good for the species which I investigated. As long as the temperature of the wort on the coolers is at its highest, the yeast cells are either killed, or, at any rate, their further development is prevented. It is only when the temperature falls that budding can commence. When the aerated and cooled wort is run from the coolers into the fermenting vessels, living yeast cells will become deposited in the pipes, and will be able to multiply in the small quantity of wort which remains in the latter. In this manner a multitude of disease germs may be produced. The next portion of wort will thus become more strongly infected than the first, and the importance of a frequent and thorough cleansing of the pipes and their unions is evident, and this, also, of course applies to the coolers and filter bags. The dangers of the latter have been clearly demonstrated by Will in the "*Zeitschr. für das ges. Brauwesen*," 1892. It is of great importance to add the pitching yeast to the wort in the fermenting vessels as soon as possible, in order that the struggle with the dangerous foreign organisms may be commenced at once.

The dust from fruit gardens is, however, not the only source from which disease yeasts become introduced into the brewery; ***the deposits in the lager tanks form another source of infection.*** This will almost always contain more or less wild yeast, even in breweries which are kept in good order; and when the beer has been attacked by disease yeast such deposits become especially dangerous. Formerly this point was generally much neglected in breweries. The cask deposit was thrown into the yard, and a portion was then carried on the boots of the workmen into the fermenting room; a large portion would dry up to dust, and be carried by the wind on to the coolers and into the fermenting room. Some years ago I drew the urgent attention of brewers to this source of danger. At the present time greater attention is certainly paid to this point than was formerly the case, but it may not be superfluous to again draw attention to it.

Most frequently, however, a brewer introduces wild yeast into the brewery when he obtains his pitching yeast from another brewery; there is always more or less danger in this. On this account the more important breweries have now adopted the system of pure yeast culture.

Mixtures Of Different Species Of Brewery Yeasts

Each of the two bottom yeasts which I introduced into the Old Carlsberg brewery in 1883 and 1884 gives a good and satisfactory product, yet, as pointed out on p. 86, they differ widely from each other. When regarded from a purely practical point of view, we find in the first place that the beer produced with the help of ***Carlsberg bottom yeast No. 2*** is fuller and contains more carbonic acid gas than that obtained with ***Carlsberg bottom yeast No. 1***; the latter beer, on the other hand, is much more stable. These results naturally led to the idea of experimenting with mixtures of the two species.

Captain Kühle, the director of the brewery, kindly consented to the carrying out of these

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experiments on a large scale in the brewery. In some cases the beers produced with the help of the two yeasts were mixed after the completion of the primary fermentation, so that the lager casks contained both kinds of beer; in other cases we employed a composite pitching yeast consisting of the two species. As it had been previously found that in spite of its deficiencies the No. 1 yeast was best suited to the Old Carlsberg brewery, this yeast was the chief constituent of the composite yeast in all cases; and where the two beers were mixed, that fermented with this yeast also formed the main bulk of the mixture.

I have omitted to make notes of the details of these experiments; ***the main result was a negative one - this mixed beer, as it may be called, did not attain the desired fullness, and was in all care: much less stable than the beer produced exclusively with Carlsberg bottom yeast No. 1.*** The term stability has reference here, 8 in the foregoing chapters, to the behavior of the finished stored beer with regard to the formation of yeast sediment, and does not refer to bacterial diseases. The experiments were, indeed, always carried out with pure cultures of well-known yeasts. By stable lager beer, I mean lager beer which can be kept in well-corked bottles for two to three weeks at the ordinary room-temperature, without forming any appreciable yeast sediment, and which will not become cloudy when well shaken after the lapse of the period mentioned.

As these experiments with mixtures led to no satisfactory result, they were discontinued, and the No. 1 yeast was employed in by far the greater part of the brewery; some years ago, the employment of the No. 2 yeast was completely given up, and the fermentations at Old Carlsberg are now exclusively conducted by means of the first-named species.

I did not however, by any means, regard the question of yeast mixtures u definitely settled. My numerous experiments with disease yeasts led me involuntarily to new investigations in this direction, although from a different point of view. The investigations may here be described, which were undertaken with a view to decide the question of the influence of mixtures of brewery yeasts on the stability of beer.

I. Series of Experiments

Four two-necked flasks of a liter capacity, A, B, C, D, each of which was charged with 660 cc. of the same sterilized and aerated lager beer wort, were infected with young vigorous growths of the following yeasts:

A with 1 cc. of ***Carlsberg bottom yeast No. 1;***

B with 1 cc. of the ***bottom yeast from the Tuborg brewery,*** and which was mentioned above;

C with 1 cc. of the same Tuhorg yeast and } cc. of ***Carlsberg bottom yeast No. 1;*** and

D with 1 cc. of ***Carlsberg bottom yeast No. 1*** and 1 cc of the ***Tuborg yeast.***

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The yeast was in all cases of a thick consistency. The primary fermentation took place at the ordinary room-temperature, and when this was finished, it was found that the beer in A and B had brightened satisfactorily, whilst the mixed yeasts employed in C and D gave a less satisfactory result. The beer was now transferred to other flasks, and stored at a temperature of 7° C.

After remaining at this temperature for about a month and a half, A and B were perfectly bright, and C and D, on the other hand, were opalescent. The beer was drawn off into small bottles in the manner already described. After standing twelve days in bottle at the ordinary room temperature, A and B were bright and showed no yeast turbidity, C and D were still slightly opalescent, and in both yeast turbidity was beginning to manifest itself, and was distinctly noticeable in D. After two months' storage C and D were less opalescent, but otherwise had remained practically unchanged.

Thus, whilst in the ease of each of the two brewery yeasts, when employed separately and in pure culture, the brightening was faultless, both as regards primary and after-fermentation, and the beer was also stable, this was not the case when they were mixed.

A still more marked effect of the same character was produced when the fermentation was effected by means of a mixture of the **Carlsberg bottom yeast No. 1**, and the top yeast which I have named **Saccharomyces cerevisiae I.**

After the laboratory experiments had thus shown that, under certain circumstances, a good brewery yeast can play the part of a disease yeast, the next problem was to determine whether this was also the case under the conditions obtaining in the brewery. With this object I undertook the two following Series of Experiments, in which the method adopted corresponded with that of the experiments described above. The plan of the experiments has, in fact, been practically the same throughout, and is described in greatest detail in that part of the chapter which deals with **Saccharomyces ellipsoideus II.** and **Saccharomyces Pastorianus III.**

II. Series of Experiments

Two of the wooden vessels, B and D, which have been already mentioned, were charged each with 1.5 hectoliters of lager-beer wort (14.4 per cent. Ball.) in the fermenting cellar of the Old Carlsberg brewery. The temperature of the wort when the yeast was added was 7° C.

B was pitched with 400 grams of **Carlsberg bottom yeast No. 2.**

D was pitched with 360 grams of **Carlsberg bottom yeast No. 2** and 40 grams of **Carlsberg bottom yeast No. 1.**

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The yeast was of fairly thick consistency, and consisted of young vigorous growths which had been grown in wort at about 10° C. After nine days the extract was 7.96 per cent. Ball. in B, and 8.04 per cent. in D. B was bright, and D fairly bright. The beer from each vessel was run into two casks, and these were then placed in the lager cellar, the temperature of which was about 2° C.

When the beer had been stored for one-and-a-quarter months, a large number of bottles were filled from one cask of each beer, and these were put away in a dark cupboard at the ordinary room-temperature. The extract of B was 7.23 per cent. Ball., and that of D 6.90 per cent. Both beers were perfectly bright. After eleven days there was still no sediment. After fifteen days B was still free from yeast turbidity, whilst D showed commencing turbidity. After three months' storage samples were taken in the same manner from the second series of casks. The extract of B was 6.49 per cent. Ball., and of D 6.41 per cent. The beer was perfectly bright. After standing ten days there was still no sediment. Five days later there was a very slight sediment in B, insufficient to cause yeast turbidity when shaken up; in D there was slightly more sediment, and on agitation the beer became a little cloudy. ***The difference noticeable after one-and-a-quarter months' storage had thus almost completely disappeared when the storage period was prolonged to three month.***

III. Series of Experiments

The wort employed in this Series of Experiments had an extract of 14 per cent. Ball; the fermentation was carried out in four vessels.

C was pitched with 400 grams of ***Carlsberg bottom yeast No. 1.***

D was pitched with 380 grams of ***Carlsberg bottom yeast No. 1*** and 20 grams of ***Carlsberg bottom yeast No. 2.***

E was pitched with 400 grams of ***Carlsberg bottom yeast No. 2.***

F was pitched with 380 grams of ***Carlsberg bottom yeast No. 2*** and 20 grams of ***Carlsberg bottom yeast No. 1.***

Whilst in the second Series of Experiments the ratio between the two species in the mixture of yeasts was 9:1, in this case it was 19:1; otherwise the conditions were the same. The primary fermentation was finished at the end of eleven days, when it was found that the extract was 7.31 per cent. Balling in C, 7.64 per cent. in D, 7.39 per cent. in E and T64 per cent. in F. The clarification in C and D was rather unsatisfactory, but was good in E and F. D was, perhaps, a little worse than C, and E was a little better than F. In accordance with the previous Series of Experiments, the attenuation in D was slightly less than in C, and in F slightly less than in E. The conditions of storage were the same as in the last series.

After 1.75 months a considerable number of bottles were filled with the beer from one series

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of the casks. The beer was bright in all cases; its extract was 6.58 per cent. Balling in C, 690 per cent. in D, 6.25 per cent. in E, and 6.33 per cent. in F. After standing fourteen days in bottle C and E showed a very slight sediment, which, however, on agitation, did not cloud the beer; in D and F, on the other hand, there was a more pronounced sediment, which, on agitation, rendered the beer slightly cloudy. Thus, in this Series of Experiments there was also an appreciable difference in the stability of the beer which had been fermented, on the one hand, with pure culture: of either of the two brewery yeasts, and on the other hand with the mixed yeasts. After about three months' storage the beer of the second series of casks was drawn off into bottles. The extract was found to be 6.17 per cent. Balling in C, 6.33 per cent. in D, 6.25 per cent. in E, and 6.33 per cent. in F. In all cases the beer was perfectly bright and stable, the only difference as regards stability being that the beer from D became very lightly cloudy on agitation, which was not the case with the others; there was, however, no question of yeast turbidity.

The addition of a low-fermentation yeast to ordinary lager beer, at the stage when it is removed to the lager cellar, had no injurious effect on the stability of the beer; this also holds good when the addition is made, after storage, to the beer in the small casks and bottles. These experiments were carried out in the same manner as those described above. In all cases the normal, favorable conditions of the brewery have been maintained.

Main Result. In the cases investigated it was, therefore, found that the beer was less stable when the pitching yeast employed consisted of a mixture of two brewery yeasts, than when only one species, no matter which, had been employed. In these mixtures, the species present in smallest proportion played the part of a disease yeast. The experiments teach us that this not only occurred when the ratio of the two mixed species was 9:1, but also when the proportion was 19:1, that is to say when only one-twentieth of the pitching yeast consisted of a foreign brewery yeast. We are thus confronted with the curious fact, that good brewery yeasts can, as it were, modify their nature, and play the part of disease yeasts.

When we recollect that the **Carlsberg bottom yeast No. 2** is a species which does not yield particularly stable beer, there appears, indeed, nothing remarkable in the fact that the addition of this species to a pitching yeast, consisting mainly of **Carlsberg bottom yeast No. 1**, has the effect of rendering the resulting beer less stable than when the fermentation has been effected by the latter species alone.

On the other hand, it is remarkable that the addition of **Carlsberg bottom yeast No. 1** which, indeed, is characterized by its yielding a stable beer to a pitching yeast consisting mainly of the other species mentioned, should also cause the resulting beer to be less stable than when fermented with Carlsberg bottom yeast No. 2 alone.

The effect described was only produced when the storage of the beer was discontinued at a rather early stage, namely, after 1.25 to 1.66 months; after three months' storage there was at most only a faint indication of instability. After 11 months, however, the beer was bright in all cases, and where the No. 2 yeast had been employed it had the appearance of a finished

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stored product.

In breweries where yeasts are employed similar to the last mentioned, and where the storage period is therefore brief, mixed pitching yeasts may give rise to the irregularities described. In an earlier part of this book I have in several places discussed the question of the employment of mixed yeasts from different points of view.

These investigations furnish a further proof, that in the brewery we should employ a pure culture of a single selected race or species.

Mycoderma cerevisiae

As is known, this name is used to designate the yeast cells which readily form films on beer and other alcoholic liquids, but which do not develop endospores, and, therefore, do not belong to the *Saccharomyces*. As with several other names, so with this, the advance of science has shown that the systematic name embraces not one but several species. Some of these excite alcoholic fermentation, although far less vigorously than the majority of the *Saccharomyces*. There are also some species of *Mycoderma* which, according to recent investigations, produce sickness in low-fermentation beers. I have been requested from several directions to give an opinion on this question, and I believe this can best be done in connection with the above investigations.

When experimenting in the lager cellars of the Copenhagen breweries, *Mycoderma cerevisiae* is everywhere met with. This I pointed out, in 1878, in my investigations on the micro-organisms of beer. In the years following I undertook a very comprehensive study of the beer in the lager casks of the Old Carlsberg brewery, including both the ordinary lager beer and the export beer. The contents of every cask contained a growth of the cells named, but, nevertheless, there was in no case any indication that the beer had acquired any disease whatever from this cause. The cells were, indeed, also frequently found when the beer was especially characterized by a high degree of stability and by a good flavor.

Similar experiments have been recently carried out by Mr. A. Petersen, who is at the head of the laboratory of the Old Carlsberg brewery, and these have led to the same result; and the same may be said of Professor Gr"c5nlund's investigations at New Carlsberg. Further, Director Alfred Jörgensen has also informed me that several hundred samples of sick beer are sent in the course of a year to his laboratory for examination, but that in no case have either he or his associates detected *Mycoderma cerevisiae* as the cause of disease. The same result had been arrived at by him and his associates in their investigations on the irregularities in the brewery itself.

The beers of Old and New Carlsberg are amongst the stronger beers, the lager beer wort having an extract of about 14 per cent, and the export beer wort about 16 per cent. Balling. Most of the beers examined in Mr. Jörgensen's laboratory belong to the same class. It might

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be imagined that the reason why the *Mycoderma* cells had produced no disease in the beer in which they were found, was due to the high extract of the wort. On the other hand, it was also conceivable that the cause was quite a different one namely, that the species or races occurring in the two above mentioned great breweries are not at all capable of producing any appreciable injury or sickness in the beer. As we shall see in the following pages, both these views have found supporters.

If we regard the growths of *Mycoderma cerevisiae* which occur normally in the Copenhagen beers as essentially of a harmless nature, this naturally only applies on the assumption that the beer has not been subjected to any unfavorable treatment. However, if it is left for any length of time in a warm place, in imperfectly closed casks or badly corked bottles, the surface will rapidly become coated with a film of *Mycoderma cerevisiae*, and under such conditions this growth will be sufficient to completely spoil the beer.

Bêlohoubek was the first to express the opinion that *Mycoderma cerevisiae* can, under certain conditions, cause injury in the brewery. Four years later, in 1889, Kukla published some papers on beer turbidity in the "*Berichte der VersuchsAnstalt für Brauindustrie in Bohmen.*" This disease occurred in two ways. After the beer had been three to four weeks in the lager casks, it became charged, so to speak, with a fine dust, which increased from day to day. In the second case the beer was bright after the completion of the fermentation in the lager cellar, and it only became "dusty" after it was drawn off, and had been some time in the consumers' cellars. Both forms of the disease were attributed to *Mycoderma cerevisiae* having been present and multiplied during the primary fermentation. Kukla further expresses the view that the weak wort of 10 per cent. Ball., generally employed in the Bohemian breweries, affords an especially favorable medium for the fungus in question. He also believes that at the time when his experiments were made, the malt was abnormal in its composition in respect of the ratio between the different albuminoid matters, and that the proportion of sugars to non-sugars in the wort was an unfavorable one. Kukla does not, however, give any scientific confirmation in support of his views. He promises to do so in a special treatise, and until this appears it is not possible to decide these questions.

In the treatise in which I, some years ago, studied the behavior of the yeast fungi with reference to the different sugars, I suggested that the name *Mycoderma cerevisiae* included not merely one, but several species. Lasche's investigations were, however, the first to give us definite information on this point. In his "*Mitteilungen aus Wal und Henius' Versuchsstation für Brauerei in Chicago,*" 1891, he describes how he separated from cloudy beer four different varieties or species, all of which may be included under the old systematic name *Mycoderma cerevisiae*. He conducted experiments with them in flasks charged with wort at 10° C., and he states that these species multiply freely during the primary fermentation when they are present in conjunction with a brewery yeast. Unfortunately nothing is said as to what took place subsequently in the infected beer during or after storage. In another experiment, made by Lasche, some bottles were filled with bright faultless beer, which had just completed its primary fermentation. In some cases, however, the beer was first passed through filter paper. Each of the bottles was then inoculated with one of the four species of

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Mycoderma mentioned. In one series the bottles were well corked, and in the other the necks were merely plugged with cotton-wool. It was found that in most of the bottles of the latter series the **Mycoderma** cells developed vigorously, whether the temperature was 10° or 4-6° C., and that the beer was cloudy after five days. In the bottles which had been well corked, only two of the species developed. The experiments were discontinued at this point, and we, therefore, do not learn in what condition the beer was after the completion of a normal storage.

These, and the foregoing investigations, deserve to be again taken up, and the experiments should be carried out in the fermenting and lager cellars, and under conditions exactly corresponding with those ordinarily prevailing in the brewery. There are certainly considerable difficulties connected with such experiments in the brewery, but the trouble will be well repaid, for only in this way is it possible to solve these practical questions.

Finally, Lasche states that when beer, which had been prepared with the help of pure cultivated yeast, was inoculated with his species of **Mycoderma**, and then set aside at the ordinary room-temperature, three of these varieties produced cloudiness in from four to seven days, whilst in a fortnight the beer had deteriorated as regards both taste and odor. The fourth species, on the other hand, did not affect the beer in any way under the conditions named. If, as I assume, the beer in question was low-fermentation beer, which had completed its storage period, and had been drawn off into bottles and well corked, these three species of **Mycoderma** must certainly be regarded as very dangerous at this stage of the fermentation.

The form of **Mycoderma cerevisiae** investigated by me and which, at least some years ago, formed the main part of the **Mycoderma** growths in the beers of Old and New Carlsberg, and is certainly also at the present time the most essential constituent differs from Lasche's species or races, not only in that it produces no disease in beer as already stated, but also in that it produces no alcoholic fermentation in beer wort. One of Lasche's species yielded 0.26 per cent., two of them produced 0.79 per cent, and the fourth as much as 2.51 per cent. of alcohol (by volume) in wort. The growths examined by Lasche and myself are, therefore, distinctly different from each other.

The main result arrived at is that, amongst the film forming species ordinarily described under the old systematic name **Mycoderma cerevisiae**, there is, at least, one species which must be regarded as harmless in the manufacture of beer. This species is very abundant in the Copenhagen breweries, and it formed the subject of my investigations.

The **Mycoderma** diseases, which have been recently reported from the Stations in Prague and Chicago, are caused by quite different species; at all events, this applies to the species investigated by Lasche.

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CHAPTER VII. ON THE PRESENT POSITION OF MY SYSTEM OF PURE YEAST CULTURE.

1. The object of this review.

IN papers published in the "*Compte-rendu des travaux du laboratoire de Carlsberg,*" and likewise in the first part of this book, I have given an account of my methods for the pure cultivation, analysis and treatment of yeast, both in the brewery and in the laboratory.

Every investigation which seeks to radically change an old deeply-rooted opinion will, of necessity, meet with opposition, and this will be redoubled when the subject treated of is not only of theoretical, but also of practical interest. Nothing, however, will advance a matter of this kind more than favorable practical results. The investigator who desires to bring about reforms of a practical nature must not consider it beneath his dignity to assist in the practical work itself; it is indeed here that the battles are to be won. Mere theoretical deductions and proofs are of little avail. It is, of course, evident that the work must have a scientific foundation.

In 1888 I gave an account of the position to which my system had then attained, and in this I enumerated the breweries in which the pure yeast propagating apparatus had been introduced. This publication produced a good effect, especially on account of the exact addresses of the breweries in question, which it contained. Practical brewers were thus enabled to ascertain for themselves the results which had been obtained in breweries of high repute, and thus to control the correctness of my statements. In the following review I have adopted the same plan, in that I have again thought it of significance to give a list of the breweries and distilleries which employ the said apparatus. In the present position of things it would be too much to attempt to name all the manufactures in which pure yeast has been adopted, for in most of these my old method is made use of namely, by growing the pure cultures in small fermenting vessels of the ordinary form.

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A list which, as in this case, includes firms in nearly all parts of the globe, will naturally always be incomplete, however much trouble has been bestowed upon it. If any names which should have been included have been omitted, the omission is not from any want of courtesy.*

* The following list is, in the main, only a reprint of that published in 1892 in the German edition of this book; it was made up partly from journals and mainly through communications from the following gentlemen to whom I again offer my best thanks: Prof. Dr. Aubry (Munich), S. Baumann, manufacturer (Vienna), Inspector Bischoffl (Frederica), Burmeister and Wain, manufacturers (Copenhagen), Ebbensgaard, manufacturer (Handbjerg near Struer, Jutland), Dr. Eckhardt (Augsburg). Director Holten (Wandsbeck), W. Jensen, manufacturer (Copenhagen), Director Alf. Jörgensen (Copenhagen), Dr. Koltosinski (Lille), Director Kukla (Prague), Prof. Dr. Van Laer (Ghent), Dr. P. Lindner (Berlin), Director Olesen (Copenhagen), Pest, manufacturer (Berlin), Dr. Prior (Nuremberg), Schneider, manufacturer (Hamburg), Professor Thnusing (Vienna), Professor Dr. Vuylsteke (Löwen, Dr. Wahl and Dr. Henius (Chicago), Dr. Wichmann (Vienna), and Mr. Wilson (London).

In most of the breweries mentioned below, the apparatus devised by Captain Kühle and myself is employed (for description see p. 40); in others, one of the modifications is used, and in some the apparatus of Bergh and Jörgensen, or that of Marx. In those marked with a star, Lindner's small apparatus is employed.

The pure yeast propagating apparatus is also used in a large number of laboratories in which pure yeast is prepared for employment on the large scale. The names of such.

Laboratories are, however, omitted here, as the list is intended to include breweries, distilleries and yeast factories only.

The list published in 1888 gave the names of low-fermentation breweries only; in the present list the number of these has very considerably increased, a tangible proof of the advance made by the new system of fermentation during recent years. This is likewise seen in the fact that it has also been introduced into high fermentation breweries, distilleries, yeast factories, and for wine and cider fermentations; in short, into all branches of the great fermentation (alcoholic) industries.

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This review will enable practical men, who are still doubtful of or opposed to my attempts at reform, to acquaint themselves more readily than was formerly possible with the practical results obtained. As I have not taken out any patent or sought any pecuniary gain from my work, the object of this list will not be misunderstood. *

* On account of enquiries often addressed to me, I take this opportunity to again state that the laboratory under my direction is for ***scientific investigation only***, and that, therefore, analyses, the preparation of pure cultures, or other technical work cannot be undertaken.

2. Low-fermentation breweries.

In the following breweries, one or more propagating apparatuses are employed. At the Old Carlsberg brewery, and also at the New Carlsberg brewery, three fermenting cylinders are made use of.

LIST OMITTED

Only in comparatively few of the breweries where my system has been adopted, are the above-mentioned propagating apparatus employed; ***in the majority of case: my old method is still adopted***, small fermenting vessels of the ordinary form being used for the propagation of the pure culture. ***The breweries employing this method number several hundreds***, and they are to be found in all countries where low fermentation is adopted. Director Jörgensen tells me that he supplies 66 such breweries in different countries annually with pure yeast from his laboratory in Copenhagen. Dr. Prior, director of the experimental station in Nuremberg, states ("Bayerisch. Brauer-Journal," April 21, 1894) that the station sends out more than 100 samples of pure yeast annually to small Bavarian breweries. Several of the other stations where the preparation of pure yeast is undertaken for breweries, could, probably, also show equal activity.

In the above list only five breweries are named in Sweden in which the propagating apparatus is employed, but according to the Swedish catalog of the International Exhibition (Chicago, 1893), my system has been introduced into most of the other breweries of that country. As examples of large world-famed breweries which also apply my system without the apparatus, Spatenbräu and Lowenbräu, in Munich, may be mentioned.

It may also be stated here that breweries which will have nothing to do with my reform, do, nevertheless, in many cases derive benefit from it in that they obtain at least a nearly pure yeast from other breweries where pure cultivation has been adopted.

The position of my system in Bohemia is curious. As is known, the brewing industry has attained to a prominent position in that country; yet it was late before pure yeast culture was introduced, and long after it had gained recognition in several other countries. Professor

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Bêlohoubek was, indeed, an early advocate of the reform, and at the same time he proposed the founding of a brewing station in Prague; but it was only after this plan had been realized that the reform made progress, especially owing to the efforts of Director Kukla. In his report of 1891 (*"Oesterreich. Brauer und Hopfenzeitung"*) on the work of the station, he states that of the breweries supplied with pure cultures of yeast, there were twenty in which pure yeast exclusively was employed. Some of these were foreign breweries, but I assume that the majority were in Bohemia. It is a curious fact that all the Bohemian breweries in which my system has been adopted are small ones. The propagating apparatus has nowhere been introduced, and, as far as I have been able to learn, none of the large breweries have adopted pure yeast. The position in Bohemia thus differs from that in other countries. To attempt to trace the cause of this anomaly would lead us too far from our subject.

Before concluding this chapter, it will be of interest to turn to North America. Here the pure yeast system has made the greatest advance in recent years, and a glance at the list will show that it has been adopted in the largest and most famous breweries. Drs. Wahl and Henius state that pure yeast has now been introduced with great success in more than sixty North American breweries. In February 1894 they wrote as follows in the American "Brewers' Review": "Pure yeast has already conquered a wide territory, but is far from being mistress of the entire industry. From the view point of the brewer, who is perfectly right in being conservative, it would be a great misfortune if so radical an innovation could conquer the world so quickly. We were the first in the United States to point out the importance of pure yeast, and have defended our position against all attacks. We have predicted final triumph to pure yeast in America, and we are confident that this will come about, although in some instances the attempts to introduce it may have been balked by ignorance or jealousy. The results obtained by firms of world-wide renown, as the **Pabst Brewing Company**, the **Anheuser-Busch Brewing Association**, and the **Schlitz Brewing Company**, not to mention breweries outside of the United States, gives eloquent testimony for the virtue of the innovation."

In that country things are done on a larger scale than in Europe. The **Pabst Brewing Company** in Milwaukee, e.g., produces annually about 1,100,000 barrels (1,293,000 hectoliters), **Jos. Schlitz**, in Milwaukee, about 800,000 barrels (944,000 hectoliters), and **Anheuser-Busch**, in St. Louis, about 900,000 barrels (1,062,000 hectoliters). For comparison, it may be mentioned that the annual production of **Old Carlsberg brewery** amounts to 290,000 hectoliters. It is thus seen that we have to do here with an enormous industry, and that we are dealing not with thousands, but with millions.

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3. high fermentation breweries.

The pure yeast propagating apparatus is employed in the following breweries:

LIST OMITTED

After I had succeeded, in 1883 and 1884, in introducing pure yeast into some Danish and German low-fermentation breweries, I requested Director Jörgensen to make similar experiments in Danish high fermentation breweries. It was then found that the choice was to be made between the feebly attenuating species and those which clarified rapidly. As early as 1885, pure yeast was successfully introduced in the Wiibroe brewery at Elsinore by Jörgensen and J. Wulff, the director of the brewery. Subsequently, the new system gained a firm footing in the Rahbeks Allee brewery at Copenhagen. This was in 1891, when Mr. W. Haurowitz took over the direction of this and the other breweries of the United Breweries Company at Copenhagen. With the help of two fermenting cylinders, this brewery not only supplies itself, but also gives pure yeast to the other high fermentation breweries of the company. At about the same time, Jörgensen also introduced a pure top-yeast at Messrs. Baartz & Sons' brewery, d'Oranjeboom, at Rotterdam. Mr. Grimmer, the technical director of this brewery, published an account of this in 1890, according to which the new system had yielded good results. He stated (*"Oesterreich. Brauer und Hopfen-Zeitung,"* 1892, No. 15) that pure culture had been thoroughly adopted in this famous brewery, with the help of three propagating apparatuses. Arminius Bau has also been successful in introducing my system in Dutch high fermentation breweries. A large number of foreign high fermentation breweries have in recent years also received supplies of pure cultivated, systematically selected species of yeast from Jörgensen's laboratory.

Dr. Olsen introduced a pure cultivation of a high yeast in Ringnes & Co.'s brewery at Christiania. Ehrlich stated in his journal, in 1894, that many breweries had for years obtained pure cultures of high fermentation yeast from his station at Worms, that these had proved successful, and that the arguments raised against pure yeast were entirely unsupported. Aubry, in Munich, expressed himself in like manner a few years ago.

Experience has thus shown that, as in the case of low fermentation yeasts, there are also different species or races of high fermentation culture yeasts, several of which differ in several respects in their properties. In order to satisfy the different requirements with respect to the character of the beer, it is just as necessary to make a systematic selection of the species as in the case of low fermentation. In consequence of the great differences which exist in various species and races, the same treatment will not suit all. My remarks referring to low-fermentation yeast in this connection (see Chapter I.) also apply here; no general rule can be given.

The advance soon found its way to Australia. In the Australian *"Brewers' Journal"* (Melbourne, Dec. 20, 1888, and Jan. 20, 1889), Mac Cartie and De Bavay state that the

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system of pure yeast has given good results in several high fermentation breweries in Australia, and not only for running ales, but also for stock beers. They say that they have met with no difficulty in obtaining a proper secondary fermentation, and that the beers of Melbourne are, in the main, similar to those made in England. This is especially of interest, since in the latter country brewers have been inclined to believe that a pure cultivated yeast, consisting of a single species, would be unable to produce the necessary secondary fermentation. The experiments made in Australia were carried out partly with a species which formed the main constituent of a high fermentation yeast from a Burton brewery, and partly with species obtained from Australian breweries. The former pure culture was prepared in J. G. C. Nsen's laboratory, and the latter in De Bavay's laboratory.

Pure yeast culture has been introduced with success in high fermentation breweries in North France by Dr. Kokosinski, the director of the brewing station at Lille. In his treatise entitled, "***Application industrielle de la méthode Hansen à la fermentation haute***" (Station scientifique de brasserie, 'Comptes rendus, Gand, 1890, p. 13), he states that he commenced his experiments in 1888 in a brewery in Lille, and that two years later my system had been introduced in fifteen high fermentation breweries in North France. In further confirmation of the results obtained in that country, Professor Grönlund, of New Carlsberg, has told me that on Nov. 16, 1891, MM. Dazin frères, Brasserie de Beaurepaire in Roubaix, wrote to him as follows: "Since 1888 I employ pure cultivated high fermentation yeast, and after several more or less successful trials, I have found a race which suits our brewery; the quality of the beer has thereby been greatly improved." The new system has recently been introduced into a large number of French high fermentation breweries through the brewing station at Nancy.

In Belgium pure culture was introduced by Dr. J. Vuylsteke, Professor at the University of Löwen, and by Dr Van Laer, Professor at the brewing station at Ghent. The latter founded La Société des Ferments purs in 1891. The manufacture of the yeast of this society is carried on in two of the breweries mentioned above namely, Caulier's, in Brussels, and Spreux, in Tournai. ***At the Brewers' Congress at Client in July, 1892, M. Spreux stated that during the previous month the society had supplied 75 Belgian breweries with pure yeast, that they had 60 regular clients in Belgium, Holland, and France, to whom the necessary yeast was sent every week or fortnight.*** From Van Laer's publications it is seen that, until 1894, he held the view that single cell yeast was applicable to Belgian high fermentation breweries, and he and his society published several communications on the good results thereby obtained. Subsequently he came to the conclusion that a mixture of several species is better still. La Société des Ferments purs continues to advertise "le système Hansen"; whether it is my system in its simplest form, or in the more complicated form dealing with mixtures, I do not know. Later on I shall return to Van Laer's composite yeast.

In Belgium the manufacture and supply of pure yeast is thus carried on at two breweries, whilst in other countries this is undertaken at the zymotechnic laboratories. In connection with the Berlin Station, however, a brewery has been erected, and a part of it is arranged for this manufacture.

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It is thus seen that the system of pure culture has attained a wide application in high fermentation breweries, and also in this branch of the industry it has been pronounced a great advance by the highest authorities. It is, therefore, all the more remarkable that it has not yet been adopted in England, where high fermentation has prevailed from time immemorial, and where there are the greatest breweries of the world. When in 1889 read my paper in London "On my System of Pure Yeast Culture and its Application in Top Fermentation Breweries" (see "Transactions of the Laboratory Club"), the subject had certainly excited considerable interest in England, but there was more inclination to discuss the matter than to make experiments. In his Cantor lectures, Gordon Salomon had given an account of my investigations, and had recommended English brewers to make trials in accordance with the method which I described; but his suggestion met with little encouragement. As far as I am aware, experiments in this direction had at that time only been made by H. T. Brown and Morris, at Worthington's Brewery. These experiments led to no decisive result, yet these two chemists were still of opinion that the new reform would in the end make its way into the English brewing industry, as it had already done abroad. Most English zymotechnologists at that time certainly held the opinion that pure yeast culture might be employed in the case of light running beers, but not for the heavier stock beers requiring an after fermentation. With regard to this secondary fermentation, it was held that this depended upon maltodextrin, and certain dextrins which could not be attacked during the primary fermentation, but were converted into maltose during storage, and were then fermented. In order to bring about this change it was assumed that certain species of wild yeast must also be present. No experimental proof was given in support of this doctrine, although the subject was frequently discussed in the different journals.

As will be remembered, Captain J. C. Jacobsen, the late owner of the Old Carlsberg brewery, held a similar view with regard to low-fermentation beer, assuming that the wild yeasts which I wished to exclude were necessary to the production of a secondary fermentation. He considered that this view was supported by certain statements in the works of Reess and Pasteur, and which in fact might be understood in that sense. That this view was incorrect we have heard in the first chapter. In agreement with this are the publications from the Berlin Station mentioned above (p. 16), which show that single cell yeast gives excellent results both in high and in low-fermentation breweries. In the lecture which I delivered in London I referred to my experiments in this direction. The favorable results obtained in breweries in Australia were likewise opposed to the objections raised in England. It is here worthy of note that the methods adopted in the Australian breweries were essentially the same as in England. As there appeared to be an inclination to experiment with a mixture of yeasts, I showed how this could be carried out. In this case, also, pure culture and systematic selection of the distinct species is, of course, necessary if certainty is to be secured. But since this would present great difficulties in breweries, I advised against the employment of mixtures, and recommended experimenting with single species according to the methods which I had elaborated.

The reasons for adopting the new system are the same in the case of the English high fermentation breweries as in the other branches of the fermentation industry. The

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fermentations in English breweries are, as elsewhere, exposed to the danger of attack by bacteria and wild yeasts, which may cause sickness in the beer, and thus give rise to great disturbances and heavy loss. English brewery yeast, moreover, usually contains not one, but several culture species; there is no certainty at all that the most favorable species preponderates, and it is not even certain that it is present at all. Everything depends here on chance; the brewer knows really nothing about the yeast which he puts into his fermenting vessels.

In many of the old breweries in England, which I had an opportunity of visiting, the fermenting rooms were badly arranged, and for want of space these could not be improved. Dust could enter freely, and every current of air brought infection with it. Under such conditions, it is especially desirable to introduce large quantities of a satisfactory species in pure culture throughout the brewery. By this means, both bacteria and wild yeasts are suppressed in the most effective manner. If it is not possible to introduce pure culture entirely, we still have it in our power to maintain a preponderance of the desired species.

After my visit to England, experiments were commenced in some of the breweries there, and new advocates of my system came forward, and amongst them Hagen-Schow, Dr. Sykes and Dr. Stanley Smith. For some years several breweries in different parts of England have had regular supplies of pure cultivated high yeast from A. Jörgensen's laboratory at Copenhagen. I conclude from this that these must have given satisfactory results, as otherwise the supplies would scarcely have been continually renewed. Recently a company, called The British Pure Yeast Company, has been started at Burton on-Trent. The technical director is Professor Van Laer, who is also director of the Belgian company mentioned above; the object of the company is to supply pure cultivated yeast to the breweries of Great Britain.

As far as can be seen from statements emanating from the Burton company, I must assume that the yeast which it supplies is a composite one, consisting of several species. In his paper in the "Transactions of the Institute of Brewing," 1894, Van Laer postulates that the yeast in English high fermentation breweries must necessarily consist of several species, as, according to his view, a secondary fermentation will not otherwise be obtained. In one of my treatises, published in 1883, I showed that on inoculating beer with a species of yeast which under ordinary circumstances causes beer sickness, it is possible under certain conditions to obtain a good effect. Van Laer has now adopted this idea, and carried it out further than is admissible, in that he assumes that beer fermented with a single species is less resistive to infection than beer fermented with several species. Every experienced investigator knows, however, that such generalizations are never correct. If a "mixed yeast" is to be safe, the ratio between the different species present must remain constant during the fermentation in the brewery. It is at once evident that this is inconceivable.

When Horace T. Brown and Gordon Salamon drew attention to this difficulty, in the discussion following the reading of the paper, Van Laer was unable to defend his position. Finally, I may also mention here my experiments described above, in which it was shown that **there are cases** in which low-fermentation yeasts, each of which, when used alone, gives an excellent

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product; but which, when mixed, may give rise to diseases in beer. Similar results were obtained by Jörgensen in his experiments with some high fermentation yeasts. On the other hand, it is a universally known fact that a good product can, in many cases, be obtained by means of a mixture of several species of yeast. An intelligent director of a large brewery in Austria told

me, some years ago, that he had noticed that he obtained the best beer when the bacteria were not over scarce in his pitching yeast. Before I introduced pure culture, mixtures were, indeed, employed everywhere. The point is, therefore, not whether mixtures can or cannot be used, but whether a single species can effect the desired fermentation; if this should prove to be the case, this method is the most rational, the simplest and the safest; in this alone lies the real advance.

The untenable points in Van Laer's arguments were opposed especially by W. R. Wilson, Miller, Hyde and Jörgensen. As early as 1892, Wilson stated (*"The Brewers' Journal,"* p. 527) that he had obtained the most satisfactory results at Messrs. Combe & Co.'s brewery, London, by the employment of a pure culture of a single selected species. He subsequently stated that pure yeast was employed throughout the whole of the brewery, one species being employed for running ales, porters and stouts, and another species for pale ales. He specially emphasizes the fact that a normal after-fermentation was obtained. He cultivates his yeast in a propagating apparatus which is constructed essentially on the model devised by Kühle and myself, but modified in some respects. The manager of the brewery is Mr. Frank Wilson, the President of the Institute of Brewing.

Miller and Hyde have given an account of their experiments in the *"Transactions. of the North of England Institute of Technical Brewing,"* 1894. In this paper they say: "There is no doubt whatever, that there are a number of yeasts, and possibly a large majority, which do not give any satisfactory cask or secondary fermentation, and, as far as we are aware, neither Hansen nor any one else has stated anything to the contrary. We have come across several such yeasts, but as soon as we had determined their properties, we put them aside and continued our search for a more suitable species, and it did not take us long to find one which answered our expectations and gave a good secondary fermentation. This yeast has now been in use at the brewery (Chester's brewery, Manchester), for slightly more than a year, and during that period it has been employed with uniform success for the production of the whole output of the brewery." They also state that the beer was superior to, and more uniform than a beer produced with a composite yeast.

Jörgensen made a vigorous attack against Van Laer's attempt to introduce my system in England in a complicated and uncertain form. Jörgensen's paper appeared in the *"Transactions of the Institute of Brewing,"* 1894, p. 227. It contains a review of the position to which my system has attained, and a discussion, from different points of view, of the questions relating to the controversy. The most important points in this paper relate to the experiments made by Jörgensen with Van Laer's own composite yeast, obtained from the Burton company. The result arrived at by these experiments was, *"that it is not able to*

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preserve the constancy of ratio between the species of which it is composed, but has to be renewed continually if wanted to keep unaltered." He justly emphasizes the difficulties which may thus arise. In many cases such a composite yeast originally introduced in a brewery, may in a shorter or longer period become a more or less pure culture of a single species, owing to the other species originally present becoming suppressed. If such a yeast proves satisfactory, it is not because it was at the commencement a composite yeast, but because the conditions of cultivation brought about the suppression of the useless varieties. The main result arrived at is that also in the case of English high fermentation, it will prove most rational to adopt my system of pure culture in its simplest form.

The following is a statement made by Dr. Sykes during the discussion which followed the reading of Jörgensen's paper: "Not long ago I visited a brewery some distance from town, where I found a Hansen yeast-cultivation apparatus in full working order, and a large quantity of beer being produced with the single cell yeast which had been grown in it. On tasting the beers so produced, I found the flavor satisfactory, and the after fermentation all that could be desired. One thing that particularly struck me was the excellent conditioning in a cask of lightly-hopped beer, which had been removed from the growing chamber of the yeast apparatus. This beer had been fermented under conditions which rigorously excluded all contamination from without, and it had been afterwards placed in a perfectly clean cask. These observations, coupled with the results obtained at Messrs. Combe's brewery, irresistibly led me. to the conclusion that beer could be successfully produced with single cell yeast."

In conclusion, I will only give the two following statements by Professor Petit, director of the École de brasserie de Nancy, and Professor C. J. Lintner, of the Polytechnic at Munich. Petit expresses himself as follows: "I am rather distrustful of mixed yeasts Moreover, such a yeast keeps by no means constant, the relative proportions of the different races changing rapidly, and in different directions, according to the composition of the wort. It follows that every renewal of the yeast necessitates a new acclimatization, and, therefore, one of the great advantages of single race yeast is lost, namely, the permanence of the race, and, in consequence, the constancy of the product." In his "*Handbuch der landwirtschaftlichen Gewerbe,*" Berlin, 1893, Lintner describes (p. 466) my system of pure yeast culture. and mentions that a mixed yeast can be prepared consisting of several pure yeasts; he adds, however, "but a method of this kind **is not to be recommended**, as the conditions would become unnecessarily complicated." Prior, also, recently opposed the employment of symbiotic fermentation in breweries.

The English zymotechnologists have generally inclined to the view that everything depends upon the chemical composition of the wort, and this was to be so regulated that the fermentation would proceed as desired. When, further, all possible precautions were taken to restrict the development of bacteria, it was considered that everything had been done that was necessary. In short. brewery yeast was not regarded from a botanical point of view. If my investigations have not found the same great practical application in England as elsewhere, they have at least not been passed over without producing some effect. An opponent of my

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system, Mr. Lott, recently expressed himself as follows: "Hansen's investigations into the question of pure yeast have emphasized, amongst others, one fact of considerable importance to brewers, namely, that there are a great variety of pure yeasts and that some of these varieties are suitable to one process and some to another." Interest, indeed, is aroused in the varieties of yeast, and in the different biological problems relating to them. In every course of instruction for zymotechnologists and brewers, the results of investigation in this field now play an important part. If the review which I gave in 1892 of the position of the question at that time, be compared with the present summary, it will also be seen that a considerable advance has, in fact, been made since then.

4. Distilleries and yeast factories.

It is well known that the production of spirits and of pressed yeast is carried on in the same factory, and that sometimes the one and sometimes the other is the main product aimed at, and on this account I have made no distinction between distilleries and yeast factories. The pure yeast propagating apparatus is employed in the following:

LIST OMITTED

It is only in recent years that the new system has been introduced in these manufactures. In the distillery and yeast factory Handbjerg, in Jutland, a pure cultivated yeast obtained from Jörgensen's laboratory has, according to the owner, Mr. Ebbensgaard, long been employed with success (1892). The company "De danske Spritfabriker," was, however, the first to introduce the propagating apparatus, and, in fact, to systematically adopt pure yeast culture. The director of the company, Mr. Olesen, and Inspector Bischoff, to whom I am indebted for the information, have stated (1892) that they consider pure yeast culture a decided advance also in the case of yeast manufacture. To this Danish company the honor is due of being the first to carry out the reform in this branch of industry.

In a paper in the "Zeitschrift für Spiritusindustrie," 1892, No. 6, and "Ergänzungsheft," p. 24, Professor Delbrück draws attention to the complete success attained in the brewing industry as the basis from which to start. He then points out that Dr. P. Lindner has proved that in the German distilleries an impure yeast is employed, which consists of a large number of races differing in their reproductive and fermentative powers, and that some of these are especially suitable whilst others are useless. The yield is not only reduced, owing to the employment of unfavorable yeasts, but also through the effect of contamination with bacteria. In consequence of this, and at Delbrück's suggestion, the association of German distillers resolved to introduce pure cultivated yeast of a suitable race in the distilleries, and for this purpose to establish an institution for pure yeast culture.

After some unsuccessful trials, favorable results have been obtained in German distilleries. In No. 25 of the journal mentioned, Dr. G. Heinzelmann reports that "in the laboratory of the station a race of yeast has been isolated which promises to satisfy the conditions required in

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practice. With this yeast he has experimented on a large scale in Mr. Otto's distillery at Schlagenthin, near Arnswalde, N.M. Before the introduction of the pure yeast the mash tun and the pipes were well disinfected with lime, but the usual method of working was followed. The material used was maize." The advantages gained in this distillery by the introduction of pure yeast were essentially that the formation of acid was less than it had been previously; the fermentation was more satisfactory, the yield of alcohol being 1 per cent. (by volume) higher than when the impure yeast was employed. Finally, the spirit obtained by means of the pure cultivated yeast had a more agreeable taste and odor than usual.

Experience subsequently recorded was equally favorable (see No. 28 of the same journal). In a distillery where the material employed was a mixture of maize, rye, oats and green malt, a good yield had hitherto been obtained by means of the ordinary impure yeast, but nevertheless, when a trial was made with the pure cultivated yeast, it was found that the yield was increased by 0.20 - 0.25 per cent. A still more favorable result was obtained when rye was used alone. In the case of a large distillery, in which maize was used, the effect of the employment of pure yeast was to raise the percentage yield from 11.4 to 11.66. Very satisfactory results are also recorded in the case of a distillery where molasses was used, and of a yeast factory. The results obtained with pure yeast in another yeast factory have, on the other hand, not given satisfaction. Hitherto this is the only unfavorable instance. The same pure cultivated yeast also appears to give a good result in the manufacture of potato spirit. It should be stated that in the cases mentioned, only one race is referred to; this is called No. II. at the station where it is in continuous cultivation for the supply of pitching yeast to the distilleries belonging to the members of the association. In the distilleries the yeast is always cultivated in the ordinary vessels, but at the station a pure yeast propagating apparatus is of course employed. In a paper published by Delbrück, it is stated that in the year 1893 the station supplied no less than 2647 kilograms of pure yeast to distilleries. In "**Wochenschr. f. Brauerei,**" 1895, he further states: "The pure yeast which I have introduced in Germany has given the most satisfactory results, and it may be stated that all the larger German distilleries of our Association, to the number of 800, employ pure yeast of the race No. II."

The success of the new reform in distilleries and in yeast manufacture must therefore be regarded as secured. In the sixth edition of his famous "**Handbuch der Spiritusfabrikation,**" 1894, Maercker mentions the satisfactory results which my reform has effected in this field, and he advocates its general adoption. In his book he gives instructions for the introduction and employment of pure yeast culture in distilleries, and those who are interested in this subject are referred to the work in question.

It would carry me far beyond the limits of the present work to attempt to correct the erroneous views which are rooted in these factories concerning the yeast question. Before concluding this chapter I must, however, emphasize one point that is of especial importance in connection with the reaction which has now set in. It is the same error against which I had to contend when I commenced my attempts at reform in the brewing industry. It is, namely, that more is expected of pure selected yeast than from its nature it can accomplish. When we have an ordinary more or less impure yeast which is found to work satisfactorily, the yield will

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not, as a rule, be increased by the employment of a pure culture prepared from it. To condemn the new system on this account is a mistake. The importance of it is the certainty which it gives. Assuming that the yeast race has been properly selected, it will give the most favorable result, and will continue to do so so long as the conditions of cultivation remain fairly constant. On the other hand, there is no certainty in the employment of the ordinary impure yeast; its composition may change in a short time to such an extent that it will give anything but a satisfactory result; in short, with impure yeast we are always dependent upon chance, and we do not in fact know what we are adding to our mash. In the present position of things, ***the cause of most, and of the worst, irregularities in practice must be looked for in the fermentation. By the introduction of a pure cultivated systematically selected species of yeast, certainty is secured in that respect, and also a rational made of procedure.*** This it is which constitutes the advance. There are, however, several other sources of irregularity and danger, and in which the fault cannot be attributed to the pure yeast. As I have previously strongly emphasized, pure yeast cannot do everything. The requirements as to the good quality of the raw products, and a proper and uniform method of working, are the same as before. Another great obstacle to the advance in distilleries and yeast factories is the traffic in secret recipes. In the case of more than one factory, where pure selected yeast has been adopted with success, statements have been made with a view to mislead.

Before closing this chapter, the following instances of fermentation may be briefly mentioned as bearing some relationship to the above.

In the preparation of black bread, as carried out in Denmark, so-called leaven (*Sauerteig*) was hitherto employed. In 1892, however, Schiöttz-Christensen, of Copenhagen, prepared a pure cultivated yeast, which is now employed in several bake houses in place of the leaven.

In 1893, Percival H. Grey showed that only some of the species of *Saccharomyces* occurring in the yeast employed in the manufacture of Jamaica rum are suitable for this purpose, and that the others are injurious. He therefore recommends the employment of pure cultures of those species which impart to the rum a fine aroma and good flavor. Not only will greater certainty be thereby attained in the manufacture, but, according to his experiments, it will also be possible to some extent to improve the character of the product.

Went and Prinsen Geerligs obtained similar results in their experiments on the manufacture of arrack in Java. In some distilleries the chief ferment was a mold ***Monilia Javanica***, in others *Saccharomyces Vordermannii*. The arrack obtained by means of the former was inferior to that yielded by the latter ferment. The experiments showed that a pure culture of ***Saccharomyces Vordermannii*** yielded a very fine arrack, which, unlike the ordinary commercial product, did not contain fusel oil.

In east and south Asia, mold fungi have from olden times been employed on a large scale in the preparation of fermented liquors, and mainly as diastatic agents. In the above, we have seen that this is the case in the manufacture of arrack in Java; a better known example is afforded in the manufacture of Japanese sake. Certain species of ***Aspergillus*** and ***Mucor***

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play an important part in this connection. Great interest has been excited by Jokichi Takamine's communications, according to which he obtains diastatic and yeast ferments from different molds. Some more or less detailed accounts of these have recently appeared in some English newspapers and technical journals. Takamine desired mainly to elaborate a method which would do away with the present process of malting, which he regards as objectionable. He sought a diastase producer amongst the micro-organisms, and found one in one of the species included under the collective name *Aspergillus oryzæ*. According to his statements, this species gives an abundant growth on wheat bran at about 32° C.; the newly-formed unripe conidia develop his yeast cells when sown in the mash, and at this stage the mycelium is also rich in diastase. His description of this fungus and its properties is, however, not very clear, and in all essentials he only repeats what has been said before concerning the old Japanese process. The view held by him is that this diastase not only should do away with the malting process, but that it also will be of service in medicine. The *Aspergillus* yeast is stated to excel all known yeasts in its power of producing a rapid and vigorous fermentation, and is said to be capable of yielding as much as 20 per cent. of alcohol. Moreover, the same has been said of certain wine yeasts long before anything was heard of Takamine's yeast. It is also assumed that this ferment will become of importance for use in bake houses. In 1891, Takamine obtained his first patent in connection with the above statements, and he has since obtained a number of patents in different countries. It is said that The Manhattan Distillery, Peoria, Ill., U.S.A., now runs daily 7500 gallons of alcohol by the Takamine process with satisfactory results. His and Juhler's statements concerning a development of yeast-cells of the *Aspergillus* species seems, however, to be based on a great mistake (see p. 81). We are here, in fact, only dealing with diastatic action.

5. Wine, cider, and fruit-juice fermentation.

Pasteur's investigations on wine fermentation led him to the view that the must could without danger be left to the spontaneous fermentation caused by the yeast fungi existing on the surface of the grapes. On p. 4 of his "*Études sur la bière*," he again gives expression to this view. In fact, the custom of leaving this fermentation to chance continued to prevail, and no one thought of looking for a more rational process. At that time it was assumed that *Saccharomyces ellipsoideus*, or, as Pasteur called it, "*la levure ordinaire du vin*," was a single definite species. In 1883, I showed that this name included at least two species. In my investigations, published five years later, on the behavior of the yeast fungi with reference to the sugars, it was further shown that, in the soil under vines, and elsewhere, yeasts occur, the cells of which resemble those of *Saccharomyces ellipsoideus*, but which are distinguished from the latter in that they do not yield spores. Several of these non-*Saccharomycetes*: excite a vigorous fermentation in solutions of dextrose, and it is, therefore, not improbable that they often take part in the wine fermentation. They have, undoubtedly, been described as belonging to *Saccharomyces ellipsoideus*. From this it is evident that wine yeast is not a single species, but that it consists of several varieties and species.

After my system of pure culture had become recognized in the brewing world, attention was

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directed to the fermentation of wine. Up to that time the costly must, as stated above, had everywhere been left to a chance fermentation.

The first to submit this question to a scientific treatment was a Frenchman, Louis Marx (*"Moniteur scientifique,"* Paris, 1833). By means of my methods for the preparation of pure cultures and the analysis of yeast, he proved that several species occur in every wine yeast, that these often present a similar appearance under the microscope, but differ in other respects, and likewise exhibit a different activity in the must. As in the *Saccharomycetes* investigated by myself, it was found that also in the case of these wine yeasts the development of the spores afforded useful characteristics. Considerable practical importance attaches to the experiments made by Marx with several of the species which he isolated. Different portions of the same must were inoculated with different species, and it was found that these yielded wines differing in bouquet and taste. He, therefore, expressed the opinion that, by employing a pure culture of a certain selected species of yeast, it would be possible to obtain a better wine than otherwise, even though the must is inferior. This is essentially the same result as that obtained in the brewing industry in 1882-84 by my experiments. Marx described a method for the cultivation of large quantities of pure yeast, by means of which a satisfactory fermentation of the must could be secured in a convenient manner.

At about the same time another Frenchman, Rommier, gave an account of some experiments on wine fermentation in the *"Comptes rendus"* of the Paris Academy. He did not, however, work with pure cultures, but he either took his yeast in the impure state in which it occurred in the wine, or he merely employed methods similar to those proposed by Pasteur in 1876, for the purification of brewery yeast. Rommier attacks the question as if investigation had since then been at a standstill, and he appears to be partly in ignorance of, and partly to intentionally ignore, the advance made outside France. According to his statements, the bouquet of wine is dependent upon the yeast alone, and must from districts which only produce inferior kinds of wine, can be made to yield wine possessing in the main the same characters as that of any typical high-class wine by fermenting it with yeast from the latter. These observations are in agreement with the statements of Marx, but they are recorded as if they required no qualification. No importance is attached to the chemical composition of the must; it is assumed that the yeast will do everything. Similar loose statements have been made by several other wine technologists. As Rommier did not work with pure cultures of definite species, no definite conclusions could be drawn from his observations, as was justly pointed out by Wortmann.

In 1882-84, as already mentioned, I proved for the first time, by means of accurate experiments, that there are different beer yeasts, and that these give rise to fermentation products differing in their character. Whilst, in the accounts I published of my investigations connected with brewing, I urgently insisted on the importance of conducting the fermentation with a single selected species or race, I also emphasized the fact that the general character of the beer was dependent upon several other factors besides the yeast; the latter is certainly a very important factor, but it is only one of several. For instance, a beer of the Pilsener type will not be obtained by the employment of yeast from Pilsen in a brewery whose wort is prepared

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according to the Munich system. Every experienced brewer knows this; and in the brewing industry it is scarcely conceivable that such exaggerated statements as those of Rommier and his followers could be made. These statements have, indeed, been refuted; and some of the opponents have, again, gone too far in the opposite direction. A great fault in recent writers on wine fermentation is, that they have made themselves only superficially acquainted with the investigations which have been carried out in connection with the fermentation of beer; for these constitute the groundwork, the greatest advances having been made in this field.

In 1890 two other Frenchmen, Martinand and Rietsch, published their investigations, which closely followed the lines of those carried out by Marx; they prepared pure cultures in the same manner as the latter had done. They have established a laboratory for supplying wine-growers with pure cultivated wine yeast of selected races. Kayser subsequently published some valuable practical investigations in connection with this subject.

To Professor Müller-Thurgau is due the credit of having made the first step towards a rational method in wine fermentation in Germany. He was then director of the station for physiology of plants, at Geisenheim on the Rhine. A report of his work in this field appeared in "**Weinbau und Weinhandel**," published in Mayence. In 1889 some winegrowers of this district, at his suggestion, selected a portion of the sound undamaged grapes, and which were, therefore, also free from mold, and the must obtained from these was fermented in the ordinary manner. This fermenting must was then used to pitch the rest of the must. He obtained a rather good result, and thus an advance was made. In the autumn of 1890 the first experiments were made with a pure cultivated yeast and, in fact, with a species which Müller-Thurgau had separated from "Steinberg" wine. It gave a good fermentation, and Schlegel, who reported on it, therefore suggested that several trials should be made with it in practice. With regard to the bouquet of wine. Müller-Thurgau at first held exactly the opposite view to that of the French investigators, namely, that it was not in the least dependent upon the species of yeast with which the fermentation was carried out, but that it depended solely upon the grapes; subsequently he adopted, in the main, Wortmann's views mentioned below. In a letter which I received from him in December 1891 he expressed himself as follows: "Prompted by your works, I instituted in Germany experiments with reference to the pure fermentation of wines, and based upon these I carried out wine fermentations on a large scale with a pure cultivated yeast selected for this purpose. Although, for technical reasons, we were for the time being prevented from carrying out true pure fermentations, the results obtained are nevertheless of importance." The pure cultures were added to the ordinary must. As director of the experimental station at Wädensweil, he has now introduced the new system on a large scale in Switzerland.

In 1892, Professor Wortmann, Müller-Thurgau's successor in Geisenheim, published a treatise on the fermentation of must with pure cultivated yeasts; it appeared in No. 23 of "**Weinbau und Weinhandel**," Mayence. In this he strongly emphasizes that the method hitherto adopted for the fermentation of wine must is very crude, as it is entirely a matter of chance whether a good fermentation is obtained or not. He then indicates the different sources

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of danger to which the wine is thus exposed, and strongly recommends the introduction of such races of pure cultivated yeast as have been previously found to give a good result. Only in this manner is it possible to insure a good product, as has been done in the brewing industry. He says further, "When we ferment the same must with different races of yeast, we also obtain dissimilar products, and these will differ the more, the greater the differences are with respect to the properties and habits of life of the yeasts employed." Nevertheless, he advocates the view that the character of the wine is never determined exclusively by the yeast, but that it is dependent first and foremost upon the composition of the must. The yeasts exert an influence on the taste, bouquet, and on the whole character of the wine; but it is too much to say that a high-class Johannisberg wine, for instance, can be obtained from an ordinary must by fermenting this with the yeast from the wine named. According to Wortmann's more recent investigations, the specific activity of the yeasts is prominently noticeable in the formation of alcohol and glycerin. The view that the substances causing the bouquet were due to the yeast was held even by some of Pasteur's predecessors. But Wortmann's experiments were the first to make this clear. He showed that each race of yeast has the property of developing during fermentation its characteristic bouquet substances, and that one yeast will produce more and another less of these products. These being produced through the influence of the yeast, he terms them secondary products, in distinction to the primary substances which originate exclusively from the must. For the practical application of the system of pure yeast culture, it is important that the species of wine yeast show a similar constancy in their physiological properties as in the case of brewery yeasts. "It is thus possible," says Wortmann, "in institutions where pure yeast culture is carried on, to select a number of pure yeasts having definite properties, and such as are desirable in practice, and these can then be supplied at any time, and according to the purpose to which it is intended to apply them." By means of selected species of yeasts, inferior must may be made to yield better wines, in that a bouquet is imparted to them which they would not otherwise acquire. The line must from the best kinds of grapes already contains so much of the primary bouquet substances that there is here little room for improvement; the proper selection of the race of yeast is, however, of great value also in this case, especially as the valuable must is thereby insured against the destructive influence of injurious micro-organisms. It is stated that wines fermented with pure yeast are superior as regards odor and taste, clarification and stability, to wines fermented with ordinary impure yeast and from the same district. Dr. Schnell obtained a like result from his experiments.

With regard to the fermentation of sparkling wines (champagne) Wortmann states: "In this field, especially, the advantages offered by a fermentation with selected pure yeast are evident. For, in order to re-start the fermentation of these wines, the method hitherto adopted was almost more dependent upon chance than in other cases, for one had to be content with yeasts the properties of which often were very undesirable. But especially in the case of the fermentation in bottle, the yeast should possess certain properties, namely, in the first place, that of having a high fermentative power, in order that they may also be able to effect the completion of the fermentation in the presence of the high proportion of alcohol, and under the great pressure of the carbonic acid gas; in the second place, the yeasts must be heavy, in order that they may be readily shaken down on to the cork without producing permanent

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cloudiness. On the other hand, another property which is in other cases desirable, namely, that of giving bouquet, is of little moment here, where one wishes to obtain products as neutral as possible." With the help of his Johannisberg yeast, Wortmann was most successful in solving these practical problems.

Italian chemists and physiologists have also recently turned their attention to the question of pure yeast culture. Investigations in connection with this have been carried out especially by Forti and Pichi. Several French, and some Italian, investigators obtained no definite result in their experiments; there appear, however, now to be but few who are entirely opposed to the new system.

We shall next consider the conditions in the cider and fruit-wine industry. In 1890, Kayser gave an account of some experiments made with cider must. He made use of seven species of yeast, experimenting partly with each separately and partly with mixtures, and his experiments were arranged so as to approximate as nearly as possible the conditions obtaining in practice. The yeasts were obtained from different French ciders. It was found that some of them gave a good product whilst others did not, and that good cider could be obtained by the use of a pitching yeast consisting only of a single species.

Dr. Nathan of Rortweil was, however, the first to undertake experiments in practice in this field. He published his report on these in the journal "*Der Obstbau*" (Stuttgart) in 1891 and 1892. Nathan is not merely a theorist, but is also a practical man of great experience in this field. His experiments are all the more important since they were carried out on a very large scale. They show that the quality and the whole character of the different fruit wines and the cider depends more upon the species of yeast which plays the main part during fermentation than upon the must. In his report of 1892 he writes: "When I examined the 40 vessels which I had filled with the same must, either from berries, apples or pears, and afterwards inoculated, each with one species or race of yeast, the fermentation products differed in some cases to such a remarkable degree that no one would have thought that one and the same material had been dealt with. Whilst certain races of wine yeast, for instance, imparted a vinous taste and odor, it was found that others, again, had very little influence on the taste of the cider. Several imparted to the must a very disagreeable after-taste." Nathan found, further, that his yeasts not only differed from each other in that they imparted a different character to the product, but that when examined by my method of analysis they also showed good biological characters. The must employed in his experiments was to a great extent freed from germs by means of Bergh's centrifugal apparatus.

The result was, in short, so favorable that Mr. Duttenhofer, the owner of the cider and fruit-wine factory, requested Dr. Nathan **to introduce the system of pure yeast culture throughout his establishment.** In order to advance the production of fruit wines in Würtemberg, Mr. Duttenhofer undertook to supply pure cultures of good races of yeast to other establishments at a small cost. "**Pure yeast culture," writes Nathan, "is destined to bring about a great revolution in the production of fruit wines, and to raise this to a flourishing branch of industry.**"

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In a later communication, "**Fortschritte auf dem Gebiete der Fruchtweinbereitung**" (Stuttgart, 1893). Nathan describes the method he elaborated for using pure yeast culture in the preparation of fruit wines. He also states that he succeeded in finding a species of yeast which imparted to his wines a Fine bouquet (e.g., of a Riesling). On p. 7 he says: "What an important advance has been effected by pure yeast culture, has been amply experienced this year in our establishment at Rottweil. We have fermented 300 hectoliters of fruit wine exclusively with pure yeast, and in our cellar we have not a single wine which does not taste clean, or which has any sickness, although the fruit wines prepared in the same district are very subject to disease." He is of opinion that the preparation of fruit wine based upon the new system will be carried on on a much larger scale in all parts of Germany; and he therefore recommends the American "Mountain gooseberry," especially for general cultivation, for this purpose.

In the yearly reports of the stations at Geisenheim (Wortmann), Wädensweil (Müller-Thurgau) and Gratz (Hotter), similar favorable results are recorded. In a lecture delivered before the congress of wine-growers held in Mayence (1894), Wortmann pointed out that pure yeasts have proved especially successful in the preparation of cider. One of the largest producers of cider conducted experiments on a large scale with different selected yeasts from the station at Geisenheim, and he sent the following report of the results obtained: "The different races of yeast imparted to the cider, especially *during* the fermentation, a characteristic flavor indicative of the kind of wine from which the yeast was obtained. After the vigorous fermentation, the characteristic properties of the wines from which the yeasts were taken were no longer so marked in the cider. Yet the finished cider still unmistakably possesses the vinous taste. The yeast from Moselle wine gave a very delicious and pleasant cider. The yeast from Ahrweil red wine imparts fullness, and that from Wirzburg Stein wine gives fulness and an agreeable aromatic bouquet. The Rüdesheim and Johannisberg yeasts gave a very delicate, fragrant product"

It must be acknowledged that considerable impulse was given to these investigations after Wortmann took the matter in hand; interest has thereby been excited in wide circles where little or no attention had been previously given to the subject. Most practical men now perceive that they must keep pace with the new advance if they wish to make head against competition. The station for pure yeast culture in Geisenheim, founded by the German Association of winegrowers, supported by the Ministry for Agriculture, affords distinct evidence that this is the case; it is a result which will be gladly welcomed by every one who is interested in the advance.*

* In addition to the publications quoted, the reader may also be referred to the yearly reports of the stations at Geisenheim, Widensweil and Gnta, and to the journal "**Weinbau und Weinhandel.**" The more important publications of Wortmann appeared in the "**Landwirtschaftliche Jahrbücher,**" 1892 and 1894, which also contain an account of Aderhold's morphological investigations on the German wine yeast. Schnell's paper appeared in the "**Zeitschrift für**

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angewandte Chemie," 1894. Recently Wortmann published an interesting popular little book, "**Anwendung und Wirkung reiner Hefen in der Weinbereitung**" (Berlin, 1895), which in a short time gained much attention among the wine growers

A beginning has recently also been made in Denmark, a pure cultivated yeast from A. Jørgensen's laboratory having been successfully employed in Andersen's fruit-wine factory in Slagelse.

It appears that the matter is now being vigorously followed up also in Austria. The station at Gratz has already been mentioned; and at the-present time Professor Roesler, of Klosterneuburg, is engaged in fitting up a department for pure yeast culture, which will be under the charge of Dr. Seifert.

All the experimenters named adopt the new method of fermentation in its simplest form, that is to say, they employ in every case only a single species or race of yeast. In France, however, it appears that some are disposed to make use of mixtures. The numerous experiments mentioned above show that there is no necessity for the more complicated and less certain method; what has been stated in the last section with reference to high fermentation yeast also applies here.

6. Retrospect and concluding remarks.

In the course of the twelve years which have elapsed since my first practical experiments were made at the Old Carlsberg brewery, my system of pure yeast culture has gained a wide application, as shown above. It is now adopted in all branches of the great industry which is dependent upon the cultivation of yeast fungi, and it has gained advocates in all countries; several of my earlier opponents have become strong advocates of it. What a contrast between now and when I started!

Professor Aubry of Munich, who was one of the first to exert himself in favor of my reform, wrote as follows in one of his publications of 1891: "It was, indeed, at that time (1884) no light task to advocate a cause about which zymotechnologists of repute not only shrugged their shoulders, but which they openly and energetically attacked, and thus caused brewers to turn away from this innovation; more than this, failures which with a more intimate knowledge might easily have been shown to be immaterial, and due to other causes, were brought forward as arguments against selected pure yeast. Only the consciousness of being able to hold out a prospect of success to brewers, enabled the writer under these circumstances to continue to work unshaken for the good cause; and not without great exertion has the desired end been finally accomplished."

Whilst Aubry directs attention to the struggle against which the reform had to contend, Jørgensen, in his book quoted above, on the other hand, refers to the favorable reception

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which my investigations gradually gained. In fact, my cause has, up to the present time, experienced very different vicissitudes of fate, but, although strongly opposed, it has yet always advanced. Especially at the beginning it met with strong opposition, but eminent colleagues helped me to overcome this. I have elsewhere expressed, and I here repeat, my sincere gratitude for this support.

After the advance which the system of pure yeast culture has now made, it is scarcely too bold to assume that, within a generation, the progress will be such that the difficulties which my efforts at first encountered will be scarcely intelligible. The whole matter will then be regarded as self-evident, as has been the case for centuries with regard to the cultivation of the higher plants in horticulture and agriculture. The principle, in fact, is the same, and it is only the methods the technique which are different. The young science of microorganisms is a development of the older biological science of the higher organisms. Many of the problems of microbiology, which have only recently been taken in hand, were long ago thoroughly treated in the doctrine of the higher plants.

In those industries where alcoholic fermentation is carried on, the preparation of low-fermentation beers affords the simplest conditions with regard to the fermentation which in this case can be more easily controlled than in other branches. Thus it followed, as a natural consequence, that the pure culture of systematically selected species and races was also first introduced in the case of this branch of industry, and this again led to the introduction of appliances for purifying the air, and for cooling and aerating the boiling sterile wort without contact with impure air. In low-fermentation breweries not only was pure yeast culture first carried out, but the system has also attained to greater perfection in this than in any other branch. The experience gained here naturally afforded a basis for the experiments which were made in other branches of the fermentation industry.

The manufacture of low-fermentation beer is so nearly related to that of high fermentation beer that the application of the new system to the latter branch of the industry was a step which was quickly made. The slight modifications which were necessary, especially with regard to the construction of the propagating apparatus, were carried out by Jensen, Jörgensen, Kokosinski, Van Laer and Wilson.

In the case of distilleries, pressed yeast factories, and in the manufacture of wine, cider, &c., these branches of the fermentation industry were, from their nature, the last into which the new reform could gain admission. The methods employed are quite different from those obtaining in low-fermentation breweries; likewise the mash and the must are usually more highly infected than the wort in breweries in which even open coolers are used. Nevertheless, experience shows that also in the former case a vigorous pure growth of a good species of yeast will, in the majority of instances, crowd out the competing organisms present, and therefore a sufficiently pure fermentation of the desired character will also, under these circumstances, be obtained. As to the wine and cider, it is of importance that by properly selecting the yeast, we can improve these liquids as regards bouquet, and thus, for instance, from an inferior must obtain better wine than would otherwise be produced. The reports

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quoted show that in this field especially will the new system gain its greatest triumphs.

My investigations on the alcoholic fungi have likewise reacted _ on other branches of industry, although indirectly. In Professor Weigmann's address on the occasion of the opening of the experimental dairy station at Kiel in 1839, he alludes to the practical results obtained in the brewing industry by means of my pure yeast culture, and he designates it as a problem for the dairy, to strive for a similar result in those cases in which fermentation occurs. The question here is one dealing especially with the souring of cream, with the defects of butter and milk, and also with the ripening of cheese. Weigmann has made important contributions towards the solution of these questions; many German dairies have already been supplied from his laboratory with pure cultures of a species of bacterium which is successfully employed for the souring of cream.

In Denmark important investigations in the same direction have been published by Professor Storch; and Messrs. Chr. Hansen, Quist and Zoffmann have supplied several Scandinavian dairies with pure cultures similar to those of Weigmann. In Austria the matter has been taken up by Adametz; and the previous work of Duclaux and Hueppe may also be mentioned here.

A beginning has also been made, in the tobacco industry, to make use of the same principle. After the tobacco leaves have been gathered in, they are placed in deep layers to dry slowly. Here a bacterial fermentation takes place, which modifies the taste and odor of the tobacco. Dr. Suchsland, of Halle, has proved (1892) that the ordinary German tobacco may be made to acquire a superior aroma and a milder flavor by bringing about the fermentation mentioned by means of certain species of bacteria which occur in Havana and other superior kinds of tobacco. Suchsland employs a mixture of several species for this purpose. *In the case of certain fermentation in the dairy, the cooperation of several species also appears to be necessary. In all cases in which we fermentation can be carried out with a single species, as in tie brewing industry, this method is naturally to be preferred as the simplest and most certain."*

There is no doubt that other branches of industry more or less dependent upon a bacterial fermentation will follow suite in deriving advantage from the new advance. It is strange that in the manufacture of vinegar no step has yet been taken in this direction.

Nowadays it must be clear to every zymotechnologist who has made himself familiar with the results of recent investigation, that wherever fermentation organisms are made use of, the aim must be the same, namely, to give up the old traditional method which depended upon mere chance. In this entire field a new era has now commenced.