MEETING HELD AT BREWERS' HALL, ADDLE STREET, E.C., ON MONDAY, APRIL 18TH, 1904.

Mr. A. GORDON SALAMON (Vice-President of the Institute) in the Chair.

The following paper was read and discussed:—

On a Method for the Application of Hansen's Pure Yeast System in the Manufacturing of Well-Conditioned English Stock Beers.

By N. HJELTE CLAUSSEN (Director of the Laboratory of the New Carlsberg Brewery).

AT the present time there hardly exists on the Continent, a brewery of any importance which has not adopted, directly or indirectly, Hansen's pure yeast system. The larger brewing establishments are provided with yeast propagating machines in which they cultivate the yeast proceeding from a single cell, and these breweries, or special zymotechnical laboratories, supply the smaller breweries with pitching yeast. And this is the case, not only with bottom, but also with top-fermentation breweries.

Moreover the benefits of the pure yeast system have long ago been utilised by other fermentation industries besides brewing. Pure yeast of a single methodically selected race or species is also used to a great extent in distilleries, in the manufacture of yeast, and in the fermentation of wines, including those derived from fruits other than the grape. In respect of its applicability no less than in other respects, this system has proved to be remarkably fertile and capable of development, so that, beginning with breweries, it has found its way not only into all the other branches of alcoholic fermentation industry, but also—and with great success—to such fermentations in a wider sense of the word as are used, for instance in tobacco manufacture and in dairies.

Such being the state of things, it seems rather difficult to account for the fact that there is a branch of the brewing industry itself—and a very important one—in which the system has not hitherto been able to gain a footing. I refer to English brewing practice.

At the outset, one would be inclined to take it for granted that the brewers of Great Britain would be sure to derive quite the same advantages as their Continental colleagues from the use of pure yeast. Indeed, during the years immediately following 1883 (when Hansen introduced, with success, his pure yeast system in the bottom-fermentation brewery, Carlsberg, near Copenhagen), the first trial was made at Burton-on-Trent to ferment English beers with single-cell yeast. I refer to the experiments made at that time by the late Dr. Morris in conjunction with your celebrated chemist Dr. Horace T. Brown. The results of these and several subsequent experiments, however, fell short of the expectations.

In the year 1894 Alfred Jörgensen published a communication "On Hansen's System of Pure Yeast Culture in English Top Fermentation" (*Transactions of the Institute of Brewing*, **7**), and he has since then, from time to time, reverted to this subject. Jörgensen comes forward as an ardent advocate of Hansen's principles and Hansen's methods. In his paper, just referred to, he states that he has succeeded in isolating from English top-fermentation yeast, several races which prove to be capable of carrying through the entire fermentation. He is, therefore, strongly of opinion that English top-fermentation brewers ought to adopt Hansen's system in that simple form in which it is in use on the Continent, that is, as single-cell yeast. The same train of reasoning is repeated in a paper written by Jörgensen in conjunction with Mr. Walter A. Riley and recently read before this Institute. But on going through the experiments made by the latter, I have nowhere found it stated, that these experiments relate to stock beers, that is to say to the real type of English beers. Accordingly it seems to me to be doubtful whether Riley agrees perfectly with Jörgensen, in whose opinion it is a positive fact that single-cell yeast is just as well suited to stock beers as to running beers.

On the part of English brewers and brewing chemists it has been repeatedly stated that their attempts at the introduction of single-cell yeast resulted in failures and, accordingly, they deny that single-cell yeast is capable of carrying through the whole work of fermentation.

In the face of these assertions Jörgensen holds that those failures may have been due to peculiar causes which have nothing to do with the single-cell yeast itself. Thus he sets forth a series of surmises and especially that the cultures of single-cell yeast may have been propagated in an unsuitable manner in the breweries, or that they may have degenerated in consequence of the shaking to which they have been subjected before being taken out of the flasks in the laboratory. But as will be shown hereafter, the solution of the question is to be sought at quite another point, and the real truth is that Jörgensen is completely mistaken.

As matters stand at this moment, the great majority of English brewers are doubtful, or prefer to wait and see, which course to take in regard to the pure yeast question, feeling that the latter has not yet been sufficiently cleared up, as regards the conditions obtaining in English breweries.

As the employment of single-cell yeast was found often to entail deficiency of secondary fermentation, the latter was generally supposed to be carried on by a peculiar yeast different from the one which is active in the case of primary fermentation. But no positive proof was ever adduced in support of such a view. With regard to the nature of the so-called secondary yeast, the brewing scientists also confined themselves to mere conjectures. The opinion has been advanced that it was a true Saccharomyces, a wild yeast belonging among the species described under the old designations *Saccharomyces pastorianus* and *Saccharomyces ellipsoideus;* indeed, the only attempt hitherto made at isolating and utilising this yeast was made on this assumption by van Laer (*Transactions of the Institute of Brewing*, 7).

Now, I have succeeded in proving experimentally that the "secondary yeast" exists, indeed, but it is not as was heretofore presumed a Saccharomyces, but a peculiar, non-sporulating budding fungus, which has not been isolated and described up to the present. It belongs to the group Torula. As, however, in contra-distinction to the other hitherto known species of this group, it is one of great practical importance, I have thought proper to propose a particular name for it, and with regard to its close connection with British brewing industry I have called it Brettanomyces.

As to the manner of isolating and propagating this new micro-organism and concerning its specific properties, I shall not anticipate

a complete scientific investigation on the subject which is being made in the Carlsberg laboratory by Professor Hansen's well-known assistant Mr. H. Schiönning, and which will be published before long. In this paper I shall only make some statements relating to the industrial use of the new fungus.

Brettanomyces produces a slow fermentation in wort or in beer fermented with ordinary brewer's yeast. The carbonic acid developed by its action is retained very firmly, and when set free by agitation, forms a copius and lasting foam. In the course of the fermentation rather a considerable amount of acid is formed, accompanied by ethereal substances, the taste and flavour of which cannot fail to attract the attention of any connoisseur by their striking resemblance to the flavour of stored English beers.

Like Saccharomyces, Brettanomyces appears in a large number of varieties. In English breweries as well as anywhere else the primary fermentation is carried on by Saccharomyces, whereas the secondary fermentation of the typical English beers, as being due to Brettanomyces, essentially differs from those secondary fermentations known on the Continent. In other words, *the action of Brettanomyces is absolutely necessary to bring English stock beers into proper cask and bottle condition, and to impart to them that peculiar and remarkably fine flavour which in a great measure determines their value.*

It is very easy to furnish a direct and striking evidence of its being so. If we add to pasteurised beer a slight portion of a Brettanomyces culture in wort (say a few drops to a bottle of the beer), and if we then leave the beer to stand in well-corked bottles at a temperature of 75—85° F. during 10—14 days, a slight deposit will be observable, and at the same time the beer will assume an *unmistakable English character*, both in regard to its content of carbonic acid gas and to its taste and flavour.

Under the influence of Brettanomyces, however, the great majority of bottom-fermentation beers assume a somewhat impure taste, and the same is true of most of the Continental top-fermentation beers. More particularly, attenuation must have proceeded to a certain limit during the primary fermentation if Brettanomyces is to yield a pure flavoured product. The fact is that when Brettanomyces is added to beer fermented with the yeast of very feeble attenuative power in common use in Danish top-fermentation breweries, the beer thus infected possesses a

peculiar impure and sweet mawkish taste, whilst at the same time an "English" character becomes apparent especially to the nose, and a very similar impure taste is the result if the primary fermentation has been partially carried out by English top yeast, but interrupted at an early stage by filtering and pasteurising, and if then the Brettanomyces is added.

On the other hand, by using a suitable English single-cell yeast and adding a pure culture of Brettanomyces after the termination of the primary fermentation, a remarkably good result will be obtained. Thus, for instance, I have fermented with English single-cell top yeast a Danish stout wort prepared by decoction mashing, and after addition of Brettanomyces and 2 or 3 weeks' storage, I bottled the beer which was then left to stand for a fortnight at a temperature of 77° F. According to the verdict of several connoisseurs, the product thus obtained was in no way inferior to the best sorts of London stout, whilst parallel bottles which did not contain Brettanomyces entirely lacked the English character.

Hence it is evident that the *secondary fermentation effected by Brettanomyces is indispensable for the production of the real type of English beers.*

This fact being established it gives no difficulty to account for the contradictory results of the attempts hitherto made at using single-cell yeast in English breweries.

Brettanomyces exists as a general infection in these breweries. I suppose it invariably forms a minor constituent of English pitching yeast, and it may probably be found in all such places in the pipes, utensils, and vessels of the breweries, where such infections may creep in and get fixed. If now, in the experiments on single-cell yeast, sufficiently effective measures have been taken to avoid the disturbing effects resulting from accidental infections, then the Brettanomyces is kept off, and consequently the production of stock beers of the quality wished for is rendered out of the question. As to the running beers, however, which do not undergo any secondary fermentation, they may very well turn out satisfactory. If, on the other hand, the experiments on single-cell yeast are made while using the plant of the brewery in its usual state without subjecting it to any particularly radical cleansing, then a sufficient quantity of Brettanomyces has been present, and consequently the experiments have been able to yield satisfactory results, and, in fact, they have sometimes proved to do so. This has been

erroneously interpreted, as if in practice Saccharomyces were capable of carrying through the whole fermentation.

A state of affairs in which the success of a process is dependent on fortuitous infection, which is beyond control, is obviously unsatisfactory. In fact, in those places in a brewery where the Brettanomyces necessary for the production of typical stock beers lives, there may, of course, at the same time exist a lot of pernicious disease germs, and the ways by which, depending upon an experience handed down from generation to generation, the brewer has learned to introduce, blindly as it were, the indispensable Brettanomyces in his manufacture offer the inevitable drawback of opening an easy access to all sorts of noxious organisms. By way of example, I would only mention the current procedure of dissolving isinglass in returned beers, which by a long exposure to the action of air have become converted as far as possible into acid, which solution is then added to sound beers as finings. If subjected to biological analysis, the solution referred to will doubtless be found to contain a good quantity of Brettanomyces, and in so far it will have a profitable effect and contribute towards bringing the beer into condition, but it is likely to shelter at the same time numerous disease germs.

If once the fact is established that the secondary fermentation of typical English beers cannot possibly be effected by Saccharomyces alone, but that Brettanomyces is quite indispensable for its being carried duly through, you will clearly see how important it is that, in regard to this part of his manufacture, the brewer should no longer trust the chapter of accidents, but walk upon sure ground in making use of methodically selected and prepared cultures of Brettanomyces. By this means alone considerable advantage can already be obtained, but full certainty and constancy is only attainable if the pure yeast system is brought to bear upon both the primary and upon the secondary fermentation.

In what manner then are the principles laid down by Hansen to be applied to the English art of brewing?

In the first place the pitching yeast must be selected single-cell yeast suited to local requirements. I need not here trench on the question of the advantages of single-cell yeast, this question having been so often discussed before the Institute of Brewing, that from a general point of view, there may hardly be anything to add to it. I

only wish to say that, as the secondary fermentation peculiar to English beers is to be effected by other means, there is no reason whatever why the use of single-cell yeast should not yield the very same advantages to the English brewers as those which Continental brewers have attained several years ago in using it.

It is most probable that a suitable single-cell yeast will prove fully to suffice for the fermentations of all sorts of running beers. These beers do not get sufficient time to go through a secondary fermentation, and consequently Brettanomyces will hardly be able to influence upon them to an appreciable extent. But this holds good only for such beers which are drunk very soon after their being racked off. If the beers are kept for a longer period than a few days, either on the premises of the brewery or at the customers, their content of Brettanomyces may have some influence upon their character. This is a question, however, which can only be definitely settled by experiments on an industrial scale.

At any rate, with the various sorts of stock beers the case is quite different. In the case of these beers the action of Brettanomyces is a necessary condition for the production of a beverage possessing the properties wished for, and it is therefore necessary, after the yeast has done its work and has been separated off, to add a pure culture of Brettanomyces. This will give no trouble in practice, because the quantities required are very small. A pure culture of Brettanomyces may conveniently be propagated in wort of 1055 specific gravity at a temperature of 75—80° F. Brettanomyces grows like a bottom-fermentation yeast at the bottom of the vessels, and at the end of about a week it will have formed a deposit which in each pint of wort will be sufficient for at least five barrels of beer. A general rule cannot be given for all cases, but the quantity of Brettanomyces to be added must be regulated by local circumstances, more especially by the time the beer has to be stored and by the temperature of the storing room.

As was said before, there are different varieties of Brettanomyces which attack the fermentable substances with unequal energies and which settle more or less readily and completely in the fermented liquid. Hence it follows that here also a pure culture must be made, starting from a single cell. This is the more necessary because there also exist noxious forms of Brettanomyces, and among them several varieties which form films on the surface of the beer in the bottles.

By means of single-cell cultures of Brettanomyces the secondary fermentation can be effected with the same certainty as the primary fermentation. Most of the difficulties connected with conditioning the beer can doubtless be avoided, as well as the work done for this purpose, such as rolling the casks. But still more important it is that the brewer is enabled to regulate the secondary fermentation and to materially shorten the storage.

Thus, the peculiar character of English stock beers renders it necessary to make two separate pure-cultivations, whereas one pure-cultivation suits the purpose of Continental beers. It is true that the mode of working is rendered a little more complex, but in return insecurity is removed.

It must be admitted that the judgments passed against the applicability of Hansen's pure yeast system to English beer brewing by eminent English brewing chemists were essentially sound, in so far as trials were made to use single-cell yeast, by itself, but through our knowledge of the Brettanomyces and its action such facts have been brought to light as compel a revision of those judgments. I have no manner of doubt that a different judgment will be pronounced as regards the mode of working, the outlines of which I have just described and which is perfectly in accordance with the principles laid down by Hansen. The primary fermentation is effected by putting a selected single-cell yeast to a liquid sterilized by boiling. This is an ideal case where the single race used is allowed to operate without any competition on the part of other ferments. In the secondary fermentation this is, no doubt, not the case to the same degree, in so far as the beer always contains rather a considerable quantity of yeast from the primary fermentation, however bright it may appear to the naked eye. But this yeast has virtually performed the whole amount of work which it is capable of doing in the liquid concerned and, consequently, it will not offer any appreciable competition to the Brettanomyces culture. Thus, the latter is enabled to produce, without let or hindrance, the effect which is characteristic of it and on account of which it was selected. But this is the very principle of the pure culture system: to preclude, as far as practically possible, the co-operation of, or competition between, different organisms and to make exclusive use of the particular species which best serves the purpose in view.

Discussion.

The Chairman said he felt sure that Mr. Claussen would feel that the best way of rewarding him for the trouble he had taken in preparing his paper, would be to discuss it in such a manner as might best assist him in the continuation of the research he had undertaken. There were many present who were well qualified to give an expression of opinion upon this most interesting question. Letters of regret at not being able to attend had been received from several gentlemen, including Dr. Horace T. Brown and Mr. Walter A. Riley. It appeared to him that this paper might be divided into two phases, the one purely scientific, in which they welcomed the acquisition of another form or variety of ferment which it was predicted would be of use in the brewing industry. Knowing that Mr. Claussen had worked for so long in the laboratory of Professor Hansen, it would be at once agreed that the work he had brought forward was sound and valuable. That it was full of interest, went without saying, for to think of this stranger lurking in their midst for these hundreds of years and not to have known that it had this power of imparting its peculiar flavour to the secondary fermentation went far to prove the necessity of continuous research upon the subject. In the second phase the paper seemed to him to be purely industrial and commercial, and the question was, could they in the future avail themselves of the investigation which Mr. Claussen had submitted. If he would kindly throw a little further light on one or two points which appeared to require elucidation he would render the task of judging as to the suitability of his method a little easier. In the first place he referred to the action of this variety of Torula as applicable to black beers, and he noticed that his experiments were made upon black beer worts and not upon ale worts. Of course, what was applicable to an after fermentation of black beers was conceivably applicable to the fermentation of ale, but one would like to know whether these ferments carried, as some of this variety did, a haze—whether, in other words, the action of this variety of Torula would affect the brightness of the beer, and if so, would it do so permanently. Again, he had referred to sediment as being deposited as fermentation progressed, and, of course, one would have to determine the extent and character of that sediment, whether, for instance, it would affect bottled beer by giving a greater

sediment than they had to cope with already, and whether it would by itself constitute the haze to which he had referred as being possible. Then one would like to know the gravity of the wort on which he had operated, and whether this after-fermentation could be produced with light gravity ales which required conditioning before they were sent into consumption, or whether it only applied to what *used* to be known as "stock ale"; in other words, they would like to know what Mr. Claussen really regarded as stock ale and what were the limits of functioning of this ferment in respect of the other ales which he might not include in that category. It would also be interesting if Mr. Claussen could give some account of the action which this organism had upon the carbohydrates in effecting the after-fermentation, whether any selective or preferential action. Did it, for instance, break down some of the carbohydrate combinations which the ordinary *Saccharomyces cerivisea* would not readily break down, and if so, what was approximately the time limit of its action? It seemed to him of importance to know how long this organism must be given to effect its influence, as if it was going to be a question of months or a year, as might have been the case with old stock ales, if it were going to be slowly progressive all that time, then they might have to judge as to its practicability. He would also ask Mr. Claussen if he considered that this Brettanomyces was solely responsible for really satisfactory secondary fermentation. He knew there were other ferments which would bring about a secondary fermentation, and did he consider that as regarded a true satisfactory after-fermentation this new organism could alone account for the result which they desired to achieve? He hoped many members would join in the discussion, and that there might be a useful addition to the stock of knowledge as the result of that evening's work.

Mr. C. H. Babington (President of the Institute) said he was glad to have the opportunity of adding his quota of gratitude to Mr. Claussen for coming so far and giving them so interesting a paper. If there were nothing else in it, it would be easy from the scientific point of view to observe his acumen from two sentences in which he remarked on the attitude of English brewers to scientific questions—the attitude which had hitherto prevailed being that of waiting to see what was going to turn up; and in another sentence where he pointed out how the English brewer was too apt to trust to the chapter of accidents.