The Yeasts: a Chapter in Microscopical Science.[1]

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The word "fermentation," from *fervere*, to boil or seethe, was at first applied to all cases of chemical change the cause of which was unknown, and which were accompanied by the formation of large quantities of gas, giving the liquid the appearance of boiling or seething. In its widest sense the word is still occasionally applied to a number of chemical processes in which micro-organisms are the active agents, such, for example, as the souring of milk, the conversion of alcohol into vinegar, the production of butyric acid, and similar processes. In its restricted sense, however, it is applied to the conversion of sugar into (mainly) alcohol and carbon dioxide gas by means of the organism known as yeast.

In 1680 Leeuwenhoek addressed to the Royal Society a communication headed "De Fermento Cerevisiæ," in which he announced that he had discovered that yeast consisted of small ovoid globules. Of these, which he appeared to regard as consisting chiefly of batches of six, he gives several excellent drawings. When we remember the nature of the magnifying apparatus with which he had to work, and that the average diameter of the yeast-cell is only $\frac{1}{120}$ millimetre ($\frac{1}{3000}$ in.), it will, I think, be realised that Leeuwenhoek had accomplished a very remarkable feat. He did not, however, push the discovery any further, and in this position, curiously enough, the matter remained for more than a century.

In the year 1814 Kieser, in the course of a paper by Döbereiner, described yeast as consisting of small spherical corpuscles, but this statement does not appear to have attracted attention, and about the year 1837 the microscopical character of yeast was again made the subject of investigation, and the true nature of the yeast organism was definitely and independently discovered by three observers, Cagniard de Latour, Schwann, and Kützing. These observers recognised that yeast is composed of a vast number of small transparent globules which reproduce by budding, and consist of a cell wall with granular contents. A year or two later Schwann appears also to have observed the formation of ascospores. These observers, and Cagniard de Latour in especial, put forward the view that it was owing to the vegetation of these cells that the disengagement of carbon dioxide gas and the formation of alcohol were due.

The microscope having definitely shown yeast to consist of minute living cells—that is to say, of a living organism—it became of high interest and importance to study its life-history, and to ascertain what connexion, if any, there was between the vital functions of the organism and the phenomena of fermentation.

In 1897 Buchner made the very important and interesting observation that the liquid contents of the yeast cell, when added to a fermentable liquid, are able to excite fermentation without the presence of any cells at all. He showed that the production of alcohol and carbon dioxide were the result of the activity of an enzyme secreted by the cell, to which he gave the name zymase. As in the case of other enzymes, zymase is very sensitive to external conditions, and is also highly selective in respect of its chemical activities. Thus, so far as is known, the hexoses alone, and of these, only four (*d*-glucose, *d*-mannose, *d*-galactose and *d*-fructose) are directly fermentable; and before the fermentation of other sugars, such as maltose and cane sugar, can take place, it is necessary that they should be converted into one or other of these hexoses. This is, in all cases, effected by enzymes which are secreted by the yeast, and it is very interesting to note that certain yeasts, whilst secreting invertase, and therefore capable of fermenting cane sugar, do not secrete maltase, and are therefore incapable of fermenting maltose. Then again, there are a few yeasts which, in addition to secreting invertase and maltase, secrete lactase, and are therefore capable of fermenting milk sugar.

We will now turn for a moment to the consideration of yeast as a living organism. The yeasts, as is well known, belong to the great family of the fungi, and may be described as unicellular fungi, reproducing by budding, and capable also of forming ascospores. This latter function is of importance from the point of view of classification, as it serves to differentiate between what are regarded as the true yeasts and certain other closely allied organisms, such as the torulæ and mycoderma. In the common process of budding, the bud, which occurs first as a small protuberance on the surface of the cell, quickly increases in size until it has attained roughly the dimensions of the parent-cell, after which it usually becomes detached, leading a separate existence, and reproducing in turn by the same process. It often happens that before the offspring cell has separated from the parent-cell it has itself commenced to bud, and so chains or clusters of connected cells may frequently be seen.

In the second mode of reproduction to which reference is made above, the yeast cell becomes changed into an asc, in which are formed a number of spores which may vary from one to as many as twelve, but is usually from two to four. The conditions which favour this mode of reproduction are the employment of young and vigorous cells, a moist surface, plenty of air, and a suitable temperature, usually about 25° C. The line between budding and ascospore formation is not very sharp, and it often happens that budding and sporulation may be taking place simultaneously. As a general rule the spores are spherical, but in some of the yeasts they have very characteristic forms. It would seem that spore formation is a provision on the part of Nature for securing the persistence of the species under conditions in which active budding is impossible. It appears, at any rate, to play an important part in the hibernation of yeasts, rendering it possible for them to live through the winter in the soil, or on surfaces from which very little nutriment can be extracted.

In addition to reproducing by budding and by ascospore formation, yeasts are capable of reproducing by still a third method, namely, that of true conjugation. In these yeasts, constituting the genus Zygosaccharomyces, certain of the cells form, instead of

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ordinary buds, long beak-like processes. When the "beaks" of two adjacent cells touch one another a union takes place, the tips of the "beaks" disappear, and a tubular connexion is established between the two cells, one or both of which then proceed to produce ascospores. Of these conjugating yeasts a number of different species have been described, and this sexual process in one form or another appears to be much more common than was until recently supposed.

Finally, there is a group of organisms, usually included among the Saccharomycetes, which are capable of reproducing by the process of fission. In these so-called Schizosaccharomycetes the fission of the cell, often accompanied by conjugation, is preceded by the formation of a septum which at once commences to divide into two lamellæ. Budding does not occur, but the cells form spores, usually from two to eight. It will be seen, therefore, that in the great family of the yeasts many types of reproduction are exhibited—from true conjugation (heterogamic and isogamic) in the case of some, through isogamic conjugation of ascospores formed in the same asc, in others, to complete parthenogenesis, as in the case of many of the better known cultivated yeasts. The industrial yeasts, which appear to be entirely asexual, may perhaps be regarded as retrograde forms descended from higher types in which sexuality was quite clearly marked. On this point I do not consider myself qualified to express an opinion.

As may well be supposed, in the case of a group of organisms which, although presenting some very important differences, are yet so closely allied, and in which there are very many transitional forms, a great deal of confusion exists in respect of their classification. The system at present generally adopted is one based upon that suggested by Hansen in 1904, but it is customary to include the Schizosaccharomycetes which he excluded, and there has been, of course, a natural tendency to include a number of subdivisions. The great family of the Saccharomycetes is capable of being subdivided into a number of groups or genera, each of which in turn includes a number of species, considerably more than one hundred of which have been described.

From the foregoing it will have been gathered that the division of the yeasts into more or less well-defined genera has been based almost entirely upon differences in their morphological and physiological characters. For the further differentiation into species it was found necessary, in many cases, to adopt other methods of investigation, such as the behaviour of the yeasts towards certain selected carbohydrates, and observations on the optimum conditions required for the formation of ascospores and of films.

Of the very large number of yeast species known, it may be said at once that only a comparatively few are of industrial importance, and it is customary to divide the various yeast species for technical purposes into the "cultivated" and the "wild" yeasts. The former include brewers' and distillers' yeast in all its varieties—that is to say, yeast which has from the earliest times been used for the production of alcoholic beverages, and has in a sense been cultivated for the purpose. This yeast represents, so far as is known, one species, namely, *Saccharomyces cerevisiæ*, although there are many races and varieties which differ considerably in certain respects, as, for example, in the rapidity with which they bring about fermentation, the degree of attenuation which they can effect, and the flavour of the finished product.

The "wild" yeasts are yeast which occur wild in Nature, frequently having their habitat on the surface of ripe fruits, and often finding their way into the brewery. Some of these yeasts, such as the wine-yeasts, are capable of fulfilling useful functions; others again are, so far as is known, without effect good or bad; whilst others are industrially pathogenic—that is to say, give rise to products which are unpleasant in respect of flavour or smell, or exhibit some other defect, such as pronounced and persistent turbidity.

The importance of these observations in connexion with industrial fermentation processes may easily be imagined. Prior to the isolation and study of the various yeast species, and to the microscopical control to which it naturally led, industrial fermentations were very largely a matter of chance. Sometimes the results were good, sometimes they were bad, but none could say precisely why. Now all that is changed, and when it is remembered that the industrialist who is concerned with any fermentation process is threatened on all sides by intruding organisms which may have the effect of reducing his yields or spoiling his products, the need for scientific control and for the constant employment of the microscope will be evident.

I now propose to consider briefly the cytology, or, if the expression may be permitted, the anatomy of the yeast cell. For a great many years after yeast had been subjected to microscopical examination, there was much uncertainty as to whether the cell did or did not contain a true nucleus. Although the existence of a nucleus is now well established, there is still some doubt as to the precise nature—to say nothing of the functions—of certain of the internal structures which the microscope reveals. Wager and Peniston, Guilliermond, Fuhrmann, Henneberg, Meyer and others have published important papers dealing with the cytology of the yeast cell, and have shown that it possesses a well-defined and complex internal structure.

In addition to a nucleus with a clearly differentiated structure and a nucleolus, the cell contains cytoplasm, a chondrium, metachromatic granules, a nuclear and other vacuoles, and certain thread-like structures. The cell wall, about which a good deal of uncertainty exists, appears to consist as a rule of a single membrane, and to have a complex chemical composition.

In addition to these elements, which may be regarded to some extent as structural, there exist in the cytoplasm accumulations of materials concerned in the nutrition or metabolism of the cell, such, for example, as glycogen and fat.

With regard to the functions of these various cell elements it is not yet possible to speak with very great certainty. As in all cells, the nucleus is the main seat, or rather the directing organ, of the physiological functions of the cell. It is all-important in cellular reproduction and division; it plays apparently a prominent part in nutrition, and doubtless in it reside the properties which are hereditary, and in virtue of which one species may be distinguished from another. The chondrium, consisting of two forms of mitochondria, appears to be concerned in processes of nutritional elaboration, and the nuclear or main vacuole appears to be largely concerned with metabolic processes, and is, according to some observers, the seat of fermentative activity. This latter function has, moreover, been observed to be dependent on the amount of metachromatic granules contained in the cell, the larger the amount of metachromatin (volutin) the greater the fermentative activity; and Henneberg has gone so far as to suggest that the metachromatic granules may be the parent substance from which the enzyme zymase is derived. From this necessarily brief and sketchy account of the yeast-cell anatomy, it will at least be gathered that our knowledge is very imperfect and that we have much to learn, and it may be hoped that expert cytologists may be induced to turn their attention to the elucidation of the subject. There can be very little doubt that the results would be of important industrial as well as of purely biological value.

The ordinary microscopical examination of cells which have been subjected to the drastic processes of fixing and staining obviously has its limitations, and modifications of structure, such as must almost inevitably be brought about by the above processes, may very easily give rise to incorrect conclusions in regard to the internal structure of such a delicate organism as the yeast cell. It would almost appear, in fact, that we have gone as far as it is possible to go in this direction, and some improved method of investigation will have to be resorted to if many of the questions which are at present in doubt are to be satisfactorily solved. It is possible, for example, that a very careful microscopical study of the unstained cell by means of ultraviolet light may be helpful in giving us a better insight into its internal structure, and Mr. Barnard has already carried out some interesting experiments of a preliminary character in this direction.

When one remembers that the whole of a miniature solar system is comprised within the compass of an atom, it is not, perhaps, altogether fanciful to suppose that the yeast cell—small as it is—may have a much more highly developed internal organisation than has been revealed with our present imperfect means of investigation, and that there may be more or less distinct localisation of the different functions of the cell. In this connexion two sets of facts may be briefly referred to.

In the first place, it is well known that the yeast cell, like other living organisms, may be made to perform different functions according to the conditions under which it is compelled to carry out its activities. Thus, whilst the ordinary *Saccharomyces cerevisiæ* normally decomposes sugar with the production of alcohol and carbon dioxide, and only about 3 per cent. of glycerin, it has been found that when the fermentation is conducted in the presence of a considerable quantity of sodium sulphite, the main products of the fermentation consist of acetaldehyde and glycerin in roughly equal molecular proportions, and that instead of the normal 3 per cent. so much as 36 per cent. of glycerin can be produced. In other words, it would appear that the well-known equation representing fermentation, namely,

$$C_6H_{12}O_6 = \underset{\text{alcohol}}{2C_2H_6O} + 2CO_2$$

has, when the process is carried out in the presence of sulphite, to be written in the following very different and unfamiliar form,

$$C_6H_{12}O_6 = \underset{\text{acetaldehyde}}{CH_3COH} + CO_2 + \underset{\text{glycerin}}{C_3H_8O_3}.$$

In the next place, it is of considerable interest to note that the behaviour of the enzymes within the cell appears to differ materially from that of the same enzymes in the expressed juice. Thus, the acceleration of fermentation by the addition of aldehydes is much greater in the expressed yeast juice than in the case of the living cell, and there are other respects in which the actions proceeding in the juice differ from those occurring within the cell. This seems to suggest that the mechanism of fermentation is in some way directly connected with the organised structure of the cell. In the living cell, again, the velocity of fermentation is much greater than in the expressed juice, and it would seem that, in its natural surroundings within the cell, zymase is free to act without the disturbing influences which probably exist in the expressed juice where all the cell contents are mingled, and some substances may well interfere with the activity of others.

Cramer (Proc. Roy. Soc., 1915, 88, B, 584) has dealt with this important and interesting point, and has shown that the most striking difference between the action of enzymes within the living cells and their action after extraction is the extreme sensitiveness with which, in the former case, they respond to very slight changes in the surrounding medium, being sometimes retarded, sometimes accelerated, and sometimes reversed. According to Cramer, surface tension would appear to be an important factor, such surface tension being operative, for example, at the periphery of the cell and at the boundaries of the nucleus, vacuoles, granules, colloidal aggregates, etc. Thus the conditions for enzyme action may be very different in one part of the cells from those occurring in another part. Under the influence of very slight changes in external conditions there may, for example, take place within the cell a movement of the cytoplasm, or changes in the concentration of the cell constituents which, by altering the surface tension at different parts, may altogether change the conditions for enzymic action.

Even assuming Cramer's explanation to be correct, it still means that the great variations in the physiological and chemical activities of the cell are dependent on internal structure, and it is to this problem that future research may usefully be directed. Any great increase in our knowledge of this subject might prove to be of the highest importance, not merely in regard to industrial operations, but also as affording a deeper insight than we yet possess into the true character of the vital activities of the living cell. The results of such an investigation might well prove to be of fundamental importance. In the living cell we have, in fact, a chemical laboratory of the highest efficiency, and of the most remarkable character; and could we but understand and imitate artificially the processes of building-up and breaking-down which are so quietly and so regularly occurring in a single cell of yeast, we should be not only within measurable distance of a new organic chemistry, but we should also be appreciably nearer to an understanding of that greatest of all problems, the nature of life.