The Alcohol-producing Enzyme of Yeast.

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IN a paper published in this Journal last year[1], I gave an account of some researches which I carried out with the object of confirming the discovery which had been announced some few months earlier by Dr. E. Buchner, that the alcoholic fermentation of sugar is effected by the activity of an enzyme or soluble ferment which, by appropriate means, can be extracted from the yeast-cell.

My experiments were made upon both high and low fermentation yeasts, procured without any special precaution from breweries actually at work, so that my inquiry might show whether in ordinary brewing operations the fermentation of the wort is due to an enzyme.

The yeasts I used were taken after the greatest activity of fermentation had subsided, and were kept in the laboratory for a day or two before using, so that they may be spoken of as being in the resting condition.

I followed closely the method of preparation described as his own by Dr. Buchner. Though I succeeded in pre-

[1] Annals of Botany, XI, No. XLIV, Dec. 1897, p. 555.

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paring an extract which agreed in most particulars with his, my results were distinctly opposed to his as regards the existence of an enzyme. The liquid had the odour and the specific gravity of Buchner's, answered the same chemical tests, and deposited a heavy proteid coagulum in heating to 45°–50° C. It would not, however, set up even a small evolution of CO_2 when kept in contact with solution of cane-sugar at any temperature, nor would it manifest any other sign of the desired activity. It was capable, on the other hand, of inverting cane-sugar, and it digested its own proteids.

As a result of my inquiry made upon resting yeast under these conditions, I came to the following conclusion, which was given in my paper:—'For the present, therefore, I must contend, in opposition to Buchner, that at any rate our English yeasts do not contain any alcohol-producing enzyme.'

My researches during last year were made, as I have already said, upon yeast in the resting condition. During the present year I have carried out further experiments to supplement the former ones, and have worked with yeast taken for extraction at the moment of its greatest activity.

I obtained, through the kindness of Messrs. Combe & Co., a sample of a pure culture of *Saccharomyces Cerevisiae* from Hansen's laboratory. I cultivated this in beer-wort in an incubator, using about a kilogramme. The fermentation was conducted at a temperature of 20° C., and after a few hours it was very vigorous, the liquid frothing very energetically. While it was at its height, I removed the yeast from the wort, and rapidly strained it through a calico filter. The resulting pasty mass was then subjected to pressure in a screw-press till it was dry enough to crumble between the fingers. So prepared, the kilogramme of moist yeast originally employed weighed 100 grammes.

I had found in my former experiments that it was very difficult to grind the yeast-cells in the presence of sand, the latter being coarse enough to protect them from contact with the grinding surfaces: I therefore used no sand in the next stage of the process. Instead, I mixed the nearly dry

yeast with kieselguhr till the resulting powder was of the consistency of flour. This was then ground in an agate mill, procured recently from Lautenschläger of Berlin, which is used in the Pathological Laboratory here for grinding Bacilli. The mill is driven by water-power, and is capable of disintegrating the Bacillus of diphtheria.

This new apparatus materially shortened the operation of grinding, so that I was able to carry out the whole operation, from the collection of the yeast to the disintegration of the cells, in a few hours. I lay considerable stress upon this, for reasons which will appear later.

The absence of any enzyme in the resting yeast made it probable that, if present in the cells during active fermentation, it would not be very stable in the extract. In his publications Buchner has pointed out that this instability is one of the features of the enzyme as he prepared it. He speaks of it as being decomposed after exposure to the laboratory temperature for 24 hours, though he found it capable of greater resistance if kept at 0° C. He says, however, that he has kept it for a week without damage if it has been in contact with sugar. In order therefore to avoid every possibility of losing the enzyme, in case only a little should be present in the yeast under experiment, I took the precaution of extracting the ground mass, now a fine dry powder, with 100 cc. of a 10 per cent. solution of cane-sugar. During the progress of the grinding I examined every charge of the mill microscopically at intervals, and continued grinding until no intact yeast-cells were visible. It is of course unlikely that all were disintegrated, but certainly not more than 10 per cent. escaped intact.

On mixing the ground powder with the sugar-solution, and stirring it into a thin paste, I was struck by the fact that within five minutes an evolution of bubbles of gas took place in the paste, much after the manner in which yeast causes the fermentation in dough to occur. The bubbles rose slowly but constantly all over the surface of the paste. I rapidly mixed some unground yeast with kieselguhr, and

added some of the same solution of cane-sugar to compare the behaviour of the ground and unground yeast. In this control, a certain amount of gas appeared after a while, but it was much longer in being generated, and the amount was much less than in the other case.

I transferred the paste in a stone mortar to a refrigerator, in which I left it all night. The next morning the paste had become very porous, and had risen like so much dough, forming a dome-shaped mass nearly three times as large as the original volume of the paste. The control did not show at all an equal activity.

The paste was then wrapped up in a piece of fine sail-cloth and submitted to pressure. It was first squeezed in a screw-press, the weight being added very gradually till it reached 5–7 atmospheres per square inch. About 80 cc. of a yellowish liquid were thus extracted. It was then transferred to a hydraulic press, and the squeezing continued till a weight of about 500 atmospheres per square inch was applied to it. This great pressure only extracted about a further 15 cc.

My preparation so far agreed with Buchner's in the method of obtaining it, but the quantity of extract was very much less than his. I did not recover even all the extracting liquid, probably owing to loss by evaporation during the night.

The two extracts were kept separate during the further operations. Both were shaken up with a little kieselguhr and filtered through fine Swedish filter-paper. The resulting liquid gave the same reactions as Buchner has described for his own preparations.

Both before and during the filtration the extract was giving off small bubbles of gas, which formed a ring round the edge of the liquid in contact with the beaker.

I ascertained by microscopical examination that the filtrate was free from yeast-cells, and then I added to the 80 cc. of the first (lightly pressed) extract 50 cc. of a solution of cane-sugar of 40 % concentration. I put this mixture into a flask fitted with a mercury manometer; and to protect it from

disturbance by yeast-cells, if any should have escaped detection, I added an excess of chloroform, shaking it well. The flask had a side-tube, closed by a clip, by which I was able to equalize the pressure within and without in case any difference should appear. The outlets were closed with stoppers of sterilized cotton-wool, and the whole apparatus was sterilized carefully before putting the extract and sugar-solution into it.

After letting it stand about half an hour to recover from the shaking with the chloroform, the pressure was adjusted, and the flask left at the temperature of the laboratory. In less than another half-hour there was an internal pressure in the flask which displaced the mercury in the manometer ·5 inch. As time went on, this displacement increased until all the mercury was driven into the distal limb; and then, on gently shaking the flask, the generated gas escaped through the mercury-column.

At the end of twenty-four hours the chloroform had produced a copious precipitate of proteid matter in the flask. I rapidly extracted some of this precipitate, fearing it might be a growth of yeast. Microscopic examination showed that it was free from yeast-cells, and consisted of a fine amorphous powder. It is well known, of course, that yeast-cells will not grow in a saccharine liquid saturated with chloroform.

On the third day I divided the contents of the flask into two, and filtered half of it through filter-paper to get rid of the proteid precipitate. Fermentation was active in the liquid before opening the flask, the pressure supporting 1·5 inch of mercury in the manometer. I then put the filtered and the unfiltered halves side by side in similar flasks, and left them again at the temperature of the laboratory. Next morning the flask containing the filtered liquid showed very little alteration of level in the manometer, and on shaking only a slight amount of gas escaped from the liquid, only enough indeed to displace the level of the mercury about ·25 inch.

On shaking the flask containing the unfiltered liquid, on

the other hand, there was a copious escape of gas, and the mercury showed a permanent displacement of 1·5 inch.

It is interesting to note from this experiment that whatever gave rise to the evolution of gas was associated with the precipitate to a very large extent as soon as the latter was formed. The precipitate did not, however, remove from the solution all the gas-generating power, as the filtered liquid continued to give off gas, though in greatly reduced amount.

The evolution of gas in these two flasks was still going on at the end of a week from the beginning of the experiment. I then drew the gaseous contents of both flasks through baryta-water by means of an aspirator, and, as I expected, there was an immediate formation of barium carbonate, showing that the gas was CO_2.

I then distilled the contents of the flask, and found the liquid to have a specific gravity corresponding to the presence of about 1·5 % of alcohol. The presence of the latter was confirmed by the iodoform reaction. The original extract, as prepared from the yeast, contained about ·3 % of alcohol, so that during the fermentation rather more than 1 % of spirit was formed.

The second extract, which was prepared by the heavier pressure as stated above, measured only about 15 cc. I filtered this through a porcelain filter under pressure and mixed it with sugar-solution, as in the other case: but instead of fixing a manometer to the flask I attached the latter to an arrangement by which the evolved gas was led through a tube containing calcium chloride to a set of potash-bulbs, so that its weight might be ascertained. Escape of water from the flask was guarded against by filling the connecting arm with sterilized cotton-wool. It gave off gas much more slowly than the liquid in the other flask, but there was still a noticeable activity. The flask and the bulbs were both weighed at the commencement of the observation, and again as the experiment proceeded. There was a continuous diminution in the weight of the flask, and a corresponding gain in that of the bulbs.

From these experiments I think there can be only one conclusion drawn. While the yeast-cells are active they secrete an enzyme, as Buchner says, which enzyme can be extracted by appropriate means. When so extracted it sets up fermentation in sugar-solutions under conditions which prevent the activity of living yeast. All the conditions of such fermentation were observed—the diminution of the sugar, the production of CO_2, and the coincident formation of alcohol.

The enzyme is easily decomposed ; hence the necessity for rapid manipulation during the process of extraction. It possesses one of the characteristic properties of enzymes in general, in that it is largely thrown out of solution by the formation of an inert precipitate in the liquid which contains it.

The secretion of the enzyme by the cell is now shown to be intermittent, only taking place during actual fermentation by the yeast. It is soon decomposed when this activity ceases, so that resting yeast does not give it up to an extracting solvent. The completeness with which it can be extracted from the yeast-cell depends upon successful disintegration of the cell. I did not find that the enormous pressure employed by Buchner was necessary ; indeed, the extract obtained by the comparatively low pressure of five atmospheres to the square inch, was more active than that obtained later by the use of the hydraulic press.

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