

Alcoholic fermentation without yeast cells

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A SEPARATION OF THE FERMENTING power from living yeast cells has until now never been done. In the following a method is described which can be used to this end.

1000 g. of pure brewer's yeast cells, taken before the addition of potato starch, is mixed carefully with 1000 g. of quartz sand and 250 g. of Kieselguhr, and ground until the mass has

become moist and plastic. 100 g. of water is added to the paste and the mixture wrapped up in a filter cloth and gradually placed under a pressure of 4-500 atmospheres. The result is 300 cc. of expressed juice. The residual material is ground again and filtered, and 100 g. of water added. This is then also pressed in the hydraulic press under the same pressure, to yield another 150 cc. expressed juice. From one kg. of yeast can be obtained 500 cc. of juice, which contains 300 cc. of cell contents. To remove a slight turbidity, the pressed juice is finally shaken with 4 g. of Kieselguhr and after several infusions, the first portion is filtered through filter paper.

The expressed juice obtained in this way is a clear, opalescent, yellowish liquid, which has the pleasant smell of yeast. The specific gravity was found to be 1.0416 (17°C.). When it was boiled, an extensive coagulum separated out, so that the liquid almost completely solidified. The formation of insoluble flocks began to occur at 35-40°. Even before this the formation of gas bubbles could be noticed, which were proved to be carbon dioxide. These were formed because the fluid was saturated with carbon dioxide. The juice contained about 10 per cent dry material. In an earlier batch prepared by a poorer technique, from 6.7 per cent dry material, 1.15 per cent ash was present, and 3.7 per cent protein, as calculated from the nitrogen content.

The most interesting property of this juice is that it is able to bring about the fermentation of carbohydrates. When an equal volume of the juice is mixed with a concentrated solution of cane sugar, after only one-fourth to one hour the regular formation of carbon dioxide bubbles begins, and this may last for days. Glucose, fructose and maltose show the same

phenomenon. However, no fermentation occurs when saturated solutions of lactose and mannose are added. These two latter sugars are also not fermented by living beer yeast cells. After a number of days of fermentation in the ice box, a mixture of yeast juice and sugar gradually becomes turbid, without any microscopic organisms being visible. At 700 times magnification, a rather large coagulum of proteinaceous substances can be seen, which probably are formed by the acid developing during the fermentation. The saturation of the mixture of yeast juice and sucrose with chloroform has no effect on the fermentation, but causes an earlier formation of a small amount of proteinaceous coagulum. The fermentation is not prevented by the filtration of the yeast juice through a sterilized Berkefeldt-Kieselguhr filter, which is able to retain all of the yeast cells. The mixture of this completely clear filtrate with sterilized cane sugar undergoes fermentation after perhaps a day, even in the ice box, although the beginning of fermentation is somewhat delayed with this sterile filtrate. When a parchment tube was filled with yeast juice and placed in a 37 per cent cane sugar solution, after several hours the surface of the tube was covered with a large number of tiny gas bubbles. Naturally there was also a lively development of gas within the tube, as the result of the inward diffusion of sugar. Further experiments will be necessary to decide if the agent causing the fermentation is able to diffuse through parchment paper, as it seems from this experiment. The fermenting power of the yeast juice is gradually lost with time; after five days in ice water in a half-full flask, a yeast juice was inactive against sucrose. It is remarkable that as opposed to this, the fermentation of yeast juice with cane

sugar continues for at least two weeks in the ice box. Perhaps the unfermenting juice is affected by oxygen from the air, which would not be present in the fermenting juice due to the formation of carbon dioxide. It could also be that the easily assimilable sugar is able to maintain the fermenting agent.

Only a few experiments have been carried out so far on the nature of the active material in the yeast juice. When the juice is heated to 40–50°, there is first the development of carbon dioxide, and then gradually the separation of coagulated protein. After an hour this was filtered. The clear filtrate had only weak activity against cane sugar in one experiment, while in another it had none. The active substance seems therefore either to lose its activity at this surprisingly low temperature, or to coagulate and precipitate. In another experiment, 20 cc. of the juice was added to three times this amount of absolute alcohol, and the precipitate which formed was removed under vacuum and dried over sulfuric acid. This yielded 2 g. of dry material which was only very slightly soluble in water. The filtrate from this had no activity against cane sugar. This experiment must be repeated. Especially, it must be attempted to isolate the active substance by the use of ammonium sulfate.

The following conclusions can be drawn concerning the theory of fermentation. First it has been shown that for the introduction of the fermentation process, no apparatus so complicated as the yeast cell is necessary. The bearer of the fermentative power of the expressed juice is more on the contrary a soluble substance, and more than likely it is a protein. This same will be called zymase.

The view that a special proteinaeous substance derived from the yeast

cell caused the fermentation, was first announced as the enzyme or ferment theory as early as 1858 by M. Traube, and was later especially defended by F. Hoppe-Seyler. The separation of this kind of an enzyme from the yeast cell had been until now unsuccessful.

The question still remains if zymase can be identified directly with the already known enzymes. As C. v. Nägeli has already emphasized, there are important differences between the action of fermentation and the action of the ordinary enzymes. The latter action is merely hydrolytic, and this action can be reproduced by the simplest chemical agents . . . but the breakdown of sugar into alcohol and carbon dioxide is one of the most complicated of reactions. Carbon-to-carbon bonds are broken in such a way that has not been obtained with other methods. There are also significant differences in the heat stability.

Invertase can be separated from yeast killed by dry heat (one hour at 150°), by water, and can be precipitated with alcohol to obtain a powder easily soluble in water. In the same manner, the fermenting substance cannot be obtained. . . . Therefore it seems reasonable that zymase belongs to the true protein substances, but is more closely related to the living protoplasm of the yeast cell than invertase. . . .

The fermentation of sugar by zymase can take place within the cell, but it is more probable that the protein substances are excreted into the sugar solution, and the fermentation occurs there. The process is then perhaps a physiological act only in so far as it is the living yeast cell which excretes the zymase. . . . It seems evident, as the above observations have shown, that the zymase can diffuse through parchment paper.

In one experiment the gas which

developed was allowed to pass into lime-water, and in this way identified as carbon dioxide. In two other experiments, after three days' fermentation at ice box temperature, the alcohol formed was determined. In one experiment with 50 cc. sucrose solution and 50 cc. of yeast juice, 1.5 g. ethyl alcohol was formed. In another experiment with 150 cc. of sugar solution and 150 cc. yeast juice, 3.3 g. ethyl alcohol was formed. . . . In all cases the alcohol was identified by the iodoform reaction and was finally salted

out of the aqueous solution with potassium carbonate. The material collected in this way in the latter experiment showed a boiling point between 79–81° (734 mm.). The distillate was colorless, combustible, and possessed the odor of ethyl alcohol. . . .

It has been shown that the above described method for the expression of yeast juice is also suitable for obtaining the contents of bacterial cells, and experiments on this are in progress, especially with pathogenic bacteria, at the Hygienische Institut in Munich.

Comment

This paper by Buchner is a fitting close to this section on the nature of fermentation and spontaneous generation. The early opinion that fermentation was not a vital process gave way to the opinion that fermentation only occurred when a microorganism developed. Now we see that the whole cell is not necessary for this process, but that a proteinaceous material can be extracted from the cell which carries out the process.

It is obvious that Buchner's work marked the beginnings of biochemistry and has led directly to our current vast store of information about enzymes and enzyme reactions. Those workers who believed that all of the properties of a living organism could be explained in terms of chemistry and physics could begin to find their ideas justified by the work of Buchner.