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[*Reprinted from* JOURNAL OF QUEKETT MICROSCOPICAL CLUB.]

YEAST AND OTHER FERMENTS.

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(*Read March 23rd*, 1867.)

In this paper I shall endeavour to lay before you some of those chemical changes which take place in certain substances when under the influence of other substances called Ferments. In some of these transformations the microscope shews us that there exists an intimate connection between the processes and the growth of some minute organisms, while in others the changes are purely chemical. The subject, which is of interest alike to the physiologist, microscopist, and chemist, has received great attention from many excellent observers; nevertheless, very little is known about it, and at present the whole matter is involved in great mystery.

I, therefore, feel considerable diffidence in addressing you on such a subject, and should not have attempted it had I not observed that many writers fall into serious errors when discussing the chemical operations of the Ferments.

I may at once tell you that the matter contained in this paper is perhaps more chemical than microscopical; but the fact is, these two investigations are inseparable if we desire accurate knowledge, and it is impossible to view Ferments broadly, if treated only as a chemical or only as a microscopical subject.

Fermentation is a term applied to various chemical transformations, which certain ordinarily stable compounds, such as starch and sugar, undergo when in contact with a small quantity of an azotised or albuminous substance, which is itself in an active state of alteration. This active substance is called a Ferment, and one of the peculiar properties of such a body is that it receives nothing from, nor imparts anything to, the matter which is undergoing fermentation, but is itself decomposed and destroyed as a Ferment in proportion to the matter fermented, which is gradually split up, or unfolded into two or more substances of simpler composition, sometimes with and sometimes without the assimilation of water.

This unfolding under the action of Ferments is totally different to that chemical change known as Catalysis, which takes place in one substance by mere contact with another, such as the unfolding of Alcohol into Ether, and Water, by contact with Sulphuric Acid; for although the acid causes such a wonderful change, it is not destroyed by the operation, and, consequently, when once the process is set going an unlimited quantity of alcohol may be converted by the original acid.

All the Ferments are highly complex azotised substances allied to Albumen; but while they possess this character in common, they may be divided into two groups—the one being living organisms, as Yeast, and the other substances derived from various organic sources, such as Albumen, Gluten, Casein, Diastase, Emulsin, and a variety of others—all of which decay most rapidly when in a moist state.

The authors of the "Microscopical Dictionary" would "exclude these substances from the Ferments, and desire that the term Fermentation be restricted to those changes which take place only through the agency of living organisms or Fungi;" regarding which, they also say, "A general law appears to prevail throughout the Fungi that their nutrition differs from that of all other plants in depending exclusively on the absorption and decomposition (with the evolution of carbonic acid gas) of organic compounds, therefore consisting of the performance of the operation of fermentatation on the organic matters on which they feed." But as the chemical operations of the Ferments are so similar, notwithstanding the wide difference in their organisation, I consider there would be no advantage in separating them as proposed, as they form a distinct class of chemical phenomena. I have also to observe that it is not true that carbonic acid gas is always given off during fermentation, nor is it proved that it is evolved during the growth of all the Fungi. The Ferments to which I desire to call your attention are—

Mycoderma Vini, or Yeast, which converts Sugar into Alcohol.

Boiled Yeast, which converts Sugar into Gum and Mannite—this transformation being called the Viscous Fermentation.

Casein, which converts Sugar into Lactic Acid and Butyric Acid; this last conversion, however, being attributed to the action of the Vibrio and Diastase, which converts starch into sugar.

I shall have a few words to say on M. Aceti, or the Vinegar Plant as some call it, which, although included by many among the Ferments, is not so considered by chemists, for reasons I will hereafter explain.

When a saccharine solution is left in contact with casein either in the form of fresh curd or cheese, the sugar is slowly transformed into lactic acid, according to the following equation :—

$$\text{Cane Sugar} \ldots\ldots\ldots \; C_{12} H_{11} O_{11} + HO = 2\, C_6 H_6 O_6 \; \text{Lactic Acid.}$$

In this fermentation water is assimilated, but no gas is evolved.

A solution of lactic acid, similarly treated, is transformed into Butyric acid thus :—

$$\text{Lactic Acid} \ldots\ldots\ldots \; 2\, C_6 H_6 O_6 = \underset{\text{Butyric Acid.}}{C_8 H_8 O_4} + \underset{\text{Carb. Acid.}}{4\, C O_2} + \underset{\text{Hydrogen.}}{4\, H}$$

In this fermentation, both carbonic acid and hydrogen gases are evolved. It is a question not yet answered, whether these chemical changes are induced by mere contact with the decomposing casein which is regarded as the ferment, or whether the minute organisms developed in these solutions are the real ferments living on the matters therein. One thing is quite certain, that in both fermentations living organisms abound, and they cannot grow without chemical changes taking place.

" M. Pasteur considers that a specific ferment is concerned in the production of the lactic acid fermentation, which spreads itself out as a grey substance over the surface of the sediment; and he asserts that this organism when once obtained, and a small quantity added to a solution of sugar, very rapidly converts it into lactic acid, provided the solution contain a small quantity of some nitrogenous substance. When this grey matter is examined by the microscope it is seen to consist of very small globules or very short articulations, either isolated or in threads, much smaller than Yeast, and to exhibit very rapid gyratory motion."

I have not succeeded in obtaining this grey matter, but as the lactic acid fermentation goes on very slowly, and as this season of the year is not favourable for experiments on fermentation, it may not have had time to make its appearance.

In order to observe the organisms which accompany the transformations of sugar, I watched the progress of the lactic acid

fermentation of cane sugar, that of milk sugar by the gradual decomposition of milk, and also the viscous fermentation of cane sugar; for although I have seen no notice of any living organism being concerned in this fermentation, I thought it likely that the viscid ropy matter which is formed therein was probably due to some organic growth.

Now in all these experiments I found that as soon as decomposition commenced, or at least was appreciable, but not until then, organic life was found in all the fluids; that in all cases they appeared on the surface before they were seen in the body of the fluid, and that when first discovered they were not in an active condition, but as the decomposition progressed they became so, and moved through the fluid with rapidity, but those at the surface continued to be the most active. These bodies are species of Vibrio and Bacterium.

The milk used in the experiments was obtained perfectly fresh, and divided into three portions—one containing the cream after the milk had stood 24 hours, the second was simply the skimmed milk, while the third portion was some of the same, with the addition of chalk to neutralise the lactic acid as it was formed. During four days the milk remained sweet, and I detected no organism in any part of it; but at the end of the fourth day the cream had a sour odour, indicating that lactic acid had been formed, and a small speck taken from the surface with a needle exhibited a mass of Bacterium like bodies which, when some distilled water was passed between the glass slide and cover, swarmed through the fluid with rapid and various capers.* On the fifth day the milk had become sour, and exhibited the same active organs, but in the portion to which the chalk was added they were neither so numerous nor so active. On the eighth day fungus spores and mycelia appeared on the surface of the cream, and the same was noticed, but in a lesser degree, some days afterwards on the two portions of milk; but as a considerable amount of lactic acid was formed before these objects made their appearance, I do not imagine they were concerned in the fermentation which was going on.

But it was in the mixture of Boiled Yeast and sugar solution to produce the viscous fermentation that I found these bodies developed most rapidly, for in 24 hours after the mixture was made,

* The motion here referred to is not due to the currents produced by capillary attraction, evaporation, &c.

the fluid was covered with a thin film, which proved to be entirely these organisms packed closely together, so that no motion could be seen until some distilled water was added, when their activity was fully displayed. In the course of a few days the film had become a thick viscid scum, consisting entirely of these minute bodies without a sign of any fungoid growth.

From the fact that these organisms grow most rapidly, and are in the greatest activity at the surface, it appears that air is necessary to produce these results, for in the mixture of milk and chalk from which carbonic acid was given off as the lactate of lime was formed, they were always in smaller quantity, and less active condition: this vessel, too, was covered with a plate of glass, while all the other solutions were covered with paper.

When starch or sugar is transformed* into butyric acid, Vibriones are sure to be found in the fluid, whether they produce this fermentation or not; and lately a most remarkable statement has been published by M. Bechamps regarding this matter. This gentleman asserts that he has discovered that there exist at the present time, in large blocks of chalk taken from a depth of 200 feet from the surface of the soil from a tunnel driven in a mountain, large quantities of microscopic animalculæ, which he has named Microzyma Cetæ; and he also states that if some of this chalk be placed in a saccharine solution lactic and butyric acid fermentation ensue.

Yeast is so well known that its description here is quite unnecessary, and the fact that it converts sugar into alcohol is patent to all. The chemical formula of this change is thus:—

Grape Sugar.		Alcohol.		Carbonic Acid.
$C_{12}H_{12}O_{12}$	=	$2\,C_4H_6O_2$	+	$4\,CO_2$

Yeast is supposed to be the conidial condition of Penicillium Glaucum, but much light is required to be thrown on this matter to raise it from its present obscurity.

The yeast cells consist of an outer membrane of cellulin—the

* During the transformations which took place in these experiments, I detected no organism having the slightest resemblance to Yeast; the only fungus being Oidium Lactis, which does not grow in the fluid, and, in my opinion, has no reference to the fermentation. In all the instances in which lactic acid was formed, I noticed only Bacteria or Vibriones, and while I admit that under more favourable conditions of temperature, other growths may appear, I do not consider any of these organisms to be the specific lactic acid ferment.

same material as the cellular tissue of other vegetables—in the interior of which is a highly complex gelatinous substance allied to albumen.

The appearance of Yeast under the microscope varies considerably with its condition ; when at rest, that is, when fermentation is arrested, its form varies from globular to ovoid, frequently with an uneven outline, as if the cells were very partially empty ; but when they are put into a fresh solution of sugar they swell out, and during active fermentation appear globular or nearly so, and more transparent than before.

When Yeast is added to brewer's wort it increases rapidly, and grows to six or eight times its original quantity during fermentation ; the wort being a solution which contains in abundance the elements required for its development, namely, grape sugar and some albuminous substances derived from the malt and hops.

During fermentation these albuminous matters disappear from the solution in proportion to the development of the Yeast, and the sugar also disappears in the same ratio. When the fermentation is complete, we find that in place of the complex albuminous matters in the wort we have simpler chemical combinations, such as salts of ammonia, and in place of the sugar we have alcohol. These chemical changes take place simultaneously ; but with this important difference, that the amount of nitrogen in the original wort is reduced by about one-half, while the alcohol and carbonic acid nearly correspond to the weight of the sugar, the remainder being converted into lactic acid, &c., a small quantity of which is always formed during vinous fermentation. But the Yeast consisting almost entirely of albuminous matters, and having increased to several times its original quantity, fully accounts for the disappearance of so large a proportion of the nitrogen from the wort.

Thus it will be easily understood, that Yeast, in order to grow, must be supplied with some soluble azotised matter, such as albumen ; and it is as easily proved that it will not grow without.

To ferment 100 parts of sugar, one part of yeast is required ; when the fermentation is complete, the yeast is exhausted, and in its place ammoniacal salts and cellulin are found. As the vinous fermentation takes place only during the growth of the yeast, it may be said that it will grow in simple saccharine solutions. In a certain sense this is correct, but such growth is degenerate and exhaustive, and not the healthy growth which increases and multi-

plies, for in such a solution the yeast positively lives on its own substance: this has been proved by Pasteur, in the following manner:—"He took a quantity of washed yeast, and divided it into two equal portions,—one of these was placed in a solution of pure sugar, the other portion was boiled in water, the decoction filtered, and the filtrate added to a similar solution of sugar, to which a very minute quantity of fresh yeast was added. In the first case 12 parts of sugar were converted into alcohol in six days, when the yeast became exhausted. In the second case the liquid became turbid; fresh yeast was formed at the expense of the azotised matter derived from the boiled yeast, and ten parts of sugar were fermented in nine days."

Some years ago, when experimenting on bread making, I was much puzzled by finding that when the yeast was thoroughly washed the sponge did not rise so quickly, nor was the bread so light as when made with yeast as received from the brewery. I have since learned that a portion of the yeast is soluble in water, and that when it has been dissolved out by washing, the yeast is less active; on exposure to the atmosphere, however, it recovers its activity.

Yeast causes a curious and important change to take place when added to a solution of cane sugar, converting it into fruit sugar by causing it to combine with one equivalent of water, during which operation the solution increases in specific gravity. This transformation is attributed to the soluble portion of the yeast; but be this as it may, some of it is evidently destroyed by the process, as a larger proportion of yeast is required to convert cane sugar into alcohol than grape sugar. It is a fact scarcely known to brewers, who use it, that cane sugar cannot be fermented into alcohol; for although when yeast is added to a cane sugar solution the vinous fermentation eventually ensues, it nevertheless does not commence until the yeast, without any apparent change in itself, has transformed the whole of the cane sugar into fruit sugar. The progress of this transformation may be witnessed by polarized light: the cane sugar producing a right hand rotation of the ray=73°, while the fruit sugar causes a left hand rotation of 26°.

I have one more observation to make in reference to yeast. When it has been kept some days, of course, according to temperature, it loses the pleasant smell it had when fresh, and acquires some fermentive properties, which, as far as I am aware, have not received much attention. It is well known to brewers that if the yeast be allowed to stand on the beer for a day or two after fer-

mentation has been stopped, a very disagreeable effect is produced; the beer is not acetified, but the flavour is entirely changed; it is unpalatable, and the brewers call it yeast-bitten.

Now I am not in a position to throw any light on this change; but if stale yeast be examined with the microscope, there will be found interspersed among the ordinary cells a large number of minute globular bodies, which are generally in motion; and I have also noticed a larger proportion of short, straight vibrio-like bodies, than are to be found in yeast during active fermentation.

Whether these organisms produce the disagreeable effects referred to, I am unable to say, and merely point to them as one of the changes which take place in yeast when left to itself.

Diastase is a ferment, which has the property of converting starch into sugar, by causing it to assimilate the elements of water without evolving any gaseous products.

The transformation is represented thus:—

$$\underset{\text{Starch.}}{C_{12} H_{10} O_{10}} + \underset{\text{Water.}}{2 H O} = \underset{\text{Sugar.}}{C_{12} H_{12} O_{12}}$$

Diastase is extracted from malt by soaking it in water, in which, at moderate temperature, it is soluble; it may be taken as the type of the ferments produced in all germinating seeds—for as all seeds contain starch, which must be rendered soluble in the form of sugar before it can become food for the embryo—so they all contain some azotised matters as albumen, gluten, &c., which are capable of passing into the form of a ferment, allied to diastase.

The action of diastase on starch is so well described in all chemical works—which treat of the vegetable products—that it seems strange anyone should attribute the conversion of starch into sugar, during germination, to any other cause, without assigning some sound reason. Yet, in a popular book by Dr. Carpenter, on "Vegetable Physiology," published a few years ago, he says:— "Starch differs but little from sugar, in chemical composition, except in containing one additional proportion of carbon. When germination commences, oxygen is absorbed by the seed in the substance of which it combines with the carbon that is to be set free from it; and a large quantity of carbonic acid is then given forth again to the air, whilst, in the same proportion, the starch is converted into sugar."

This implies that the conversion of the starch into sugar, and the evolution of $C O^2$ gas in germination, are the results of the

same process; but if you will refer to my diagram, you will see that starch does not contain an additional proportion of carbon, as compared with sugar, but that it requires two equivalents of H O to equal it; and that were one or two equivalents of carbon to be oxidized and abstracted, we should not have sugar as the result.

It is a well-known fact that, in germination, the starch is converted into sugar by the diastase, which is probably formed from the azotised matters by the vital action of the embryo. The oxidation of some of the carbon contained in the seed is more likely to be due to the decomposition of the sugar and other matters by the growth of the embryo, the cells of which appear to me to perform chemical functions similar to some of the fungi, for at this period of its growth it must be remembered the vegetable action is reversed, that it is now living on organic compounds and evolving $C\ O^2$ gas; whereas, when it has expanded its leaves to the light and atmosphere, its food must be reduced to simpler forms before it can assimilate it, and it will then construct organic compounds, and decompose $C\ O^2$ gas, eliminating oxygen.

Malt contains about $\frac{1}{500}$ part of its weight of diastase, and as one part of diastase will convert 2,000 parts of starch into sugar, it evidently contains a much larger quantity than is necessary for the conversion of the remaining starch in the grain. This is taken advantage of in various ways by distillers, &c., for the purpose of converting unmalted grain and starch from other sources into sugar.

The action of diastase and other similar soluble ferments is supposed to be instantaneous when the matters on which they act are also made soluble.

As an illustration of this, I will tell you what is done at one of the large distilleries in the North.

Starch and grain are ground into a fine powder, and put into a mash tun capable of holding several hundred quarters, and heated till the starch granules burst, and a thick paste is formed. When at the proper temperature, an infusion of malt is run in and agitated, and in about two minutes the whole of this stiff mass becomes perfectly fluid, the starch being at once converted into sugar by the diastase in the infusion.

In the instances of fermentation I have brought to your notice, I have shewn only the chemical transformations of the matters fermented, these changes resulting in the re-arrangement of the atoms or the molecules of which those matters are built up, thereby giving rise to entirely new structures.

The ferments themselves suffer differently, being always reduced to the simplest combinations.

Looking at the result of a fermentation, it would appear that the ferment and the matter fermented did not enter into combination, but that its transformation is due to the force generated in the decomposition of the ferment with which it is in contact. It is, however, clear that the changes which take place in the two substances are collateral, for the same ferment will produce various chemical transformations of a substance according to the phase of its own decomposition. "Thus diastase, when fresh, converts starch into sugar; if kept for a few days, it converts it into gum instead of sugar; while at another period it converts the starch first into sugar, and then transforms it into lactic acid."

Therefore the transformations always depend on, and are relative to, the peculiar changes which take place in the ferment.

The commercial production of vinegar appears to be due to the agency of one or more microscopic organisms, the mass being called the vinegar plant, which, as I have said, is not regarded as a true ferment by chemists, and for this reason; all the ferments proper, such as I have described, produce the transformations entirely within the solutions, receiving nothing from, nor imparting anything thereto; but the conversion of alcohol into vinegar is a case of simple oxydation, in which the oxygen is derived from the atmosphere, each equivalent of alcohol absorbing four equivalents of oxygen to become acetic acid, according to the following formula:

$$\overset{\text{Alchol.}}{C_4H_6O_2} + 2O = \overset{\text{Aldehyd.}}{C_4H_4O_2} + \overset{\text{Water.}}{2HO}$$

$$\overset{\text{Aldehyd.}}{C_4H_4O_2} + 2O = \overset{\text{Acetic Acid.}}{C_4H_3O_3} + HO$$

In countries where no duty is imposed on the manufacture of alcohol, it can be made into vinegar cheaply and rapidly. The alcohol diluted with water, and a small quantity of some azotised substance added, is allowed to trickle over beech shavings placed in a vat, so arranged, that a current of air circulates freely throughout.

For some days the process goes on very slowly; but the shavings become gradually covered with a slimy fungus, called mother of vinegar, and then acetification proceeds much more rapidly.

Pure dilute alcohol, exposed to the air, undergoes no chemical change; and its conversion into vinegar is undoubtedly due to some

complex action of the growth of the fungus on the matters in solution; but the exact chemical operations of this vegetation are unknown.

Since writing the above, my attention has been called to some observations on this plant by Mr. Slack, (Vol. V., p. 2), and published in the "Microscopical Transactions." He states—and I have no doubt of the truth of the assertion—that, "If some of the gelatinous portion of the plant be examined with high powers, it will be found to contain millions of minute bodies, resembling bacteria, some of them not exceding $\frac{1}{10000}$ of an inch in length.

I have recently examined a dilute solution of alcohol, which is being converted into vinegar, and find these bacteria in abundance. They may be seen distinctly when magnified 250 diameters, though a high power must be used to resolve their structure.

The study of these minute organisms, though very uninviting to the general microscopist, would richly reward any patient investigator—for until we know more of the chemical processes which take place in and through them, the subject of putrefactive decomposition must remain a blank, as it is at present.

The vinegar plant and yeast are said to be different conditions of the same vegetation ; the Brothers Tulasne have shewn us that these lower species of vegetation pass through various phases during their growth, each having previously been considered as a distinct plant ; and I see no reason why these minute organisms should not produce different chemical combinations at the different stages of their development, since we see, in the higher order of certain plants, that some of their chemical processes are reversed at points of their existence, namely, during germination, flowering, and the ripening of the fruit, when they absorb oxygen and give off carbonic acid to the atmosphere.

In conclusion, allow me to observe, that I am fully aware of having written a paper with a very slender knowledge of the microscopic organisms, whose chemical operations I have discussed ; therefore, I hope those parts which I have left in darkness will now receive the light of your experience and knowledge as microscopists. I am very anxious to obtain information concerning the part which those minute vibriones and bacteria play in nature's economy, for there can be no doubt that those remarkable bodies, appearing everywhere, and springing into active existence almost at a moment's notice, must perform some important part in many of the changes which surround us.

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