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Review

Beginnings of microbiology and biochemistry: the contribution of yeast research

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With improvements in microscopes early in the nineteenth century, yeasts were seen to be living organisms, although some famous scientists ridiculed the idea and their influence held back the development of microbiology. In the 1850s and 1860s, yeasts were established as microbes and responsible for alcoholic fermentation, and this led to the study of the rôle of bacteria in lactic and other fermentations, as well as bacterial pathogenicity. At this time, there were difficulties in distinguishing between the activities of microbes and of extracellular enzymes. Between 1884 and 1894, Emil Fischer's study of sugar utilization by yeasts generated an understanding of enzymic specificity and the nature of enzyme–substrate complexes.

Overview

Early in the nineteenth century, even the existence of living microbes was a matter of debate. This article describes how biologists, studying yeasts as the cause of fermentation, came to recognize the reality of micro-organisms and began to characterize them. However, it was not until the last quarter of the nineteenth century that people knew with any certainty that microbes are important causes of diseases. The researches on fermentation by both chemists and biologists generated the beginnings of biochemistry; although the existence of intracellular enzymes, fundamental to that subject, was not completely established until the twentieth century. Indeed, the great physiologist, Eduard Pflüger (1878), commented that the existence of intracellular enzymes was 'not only unnecessary but highly implausible'.

Apart from the somewhat larger moulds, yeast was one of the first microbes to be studied scientifically. This was probably because (i) its cells are much bigger than those of most bacteria and (ii) there was a great deal of financial backing from the alcoholic fermentation industries.

During the period I am considering, there were various notable discoveries arising from research on yeasts, as well as some unseemly wrangling. Between 1789 and 1815, the first major chemical analyses of ethanolic fermentation were published. That yeasts are certainly microbes and cause fermentation was demonstrated early in the nineteenth century; but these microbiological findings evoked a remarkable attack by some of the most influential scientists of the time who disputed that yeasts were living organisms. The second half of the century saw the establishment by Louis Pasteur, once and for all, that alcoholic fermentation was a microbiological occurrence. And, as for many years there had been a widely accepted analogy between fermentation and disease, the germ theory of fermentation implied a germ theory of disease. There were, however, passionate controversies arising from the difficulty of distinguishing between microbial action and enzymic action and towards the end of the nineteenth century, work on yeast sugar fermentations gave convincing evidence of the specificity of enzyme action.

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Early chemical analyses of alcoholic fermentation, 1789–1815

Chemists, not biologists, made the first scientific studies of alcoholic fermentation and in this, Antoine Lavoisier (1789), one of the founders of modern chemistry, was a pioneer. He described the phenomenon of alcoholic fermentation as '*one of the most extraordinary in chemistry*' and made a series of analyses, estimating the proportions of the elements in sugar, water and yeast paste. To ascertain what happens during the production of wine, he determined the composition of both the fermentable substances and the products of fermentation. As a result Lavoisier was able to publish the first clear account of the chemical changes occurring during fermentation. He describes how sugar is converted into carbonic acid gas and spirit of wine, saying the latter is 'more appropriately called by the Arabic word *alcohol* since it is formed from cider or fermented sugar as well as wine'. Here he seems to be the first person to describe a chemical reaction by means of an equation, writing '*grape must* = *carbonic acid* + *alcohol*' and explained: 'In these experiments, we have to assume that there is a true balance or equation between the elements of the compounds with which we start and those obtained at the end of the reaction.'

Lavoisier estimated ethanol by distilling, and CO_2 by dissolving it in alkali. Twenty-six years later, another great

Table 1. Analysis of alcoholic fermentation by Lavoisier (1789) and Gay-Lussac (1815)

Figures are parts by weight.

	Lavoisier (1789)	Gay-Lussac (1815)	Today, assuming $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$
Sugar	100	100	100
Alcohol	60·1	51·34	51·19
Carbonic acid	37·0	48·66	48·90

French chemist, Joseph Gay-Lussac (1815), revised Lavoisier's figures. Gay-Lussac's findings are astonishingly close to present-day estimates (Table 1), partly because he made ingenious assumptions about the precise composition of sucrose (F. W. Lichtenthaler, personal communication).

The overall equation for alcoholic fermentation, $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$, is often misattributed to Gay-Lussac, in particular to his paper of 1815. However, Gay-Lussac could not have written it, because the empirical formula for glucose was not established until Dumas (1843) published it. Furthermore the molecular formula was not known before the publications of Baeyer (1870) and Fittig (1871). Gay-Lussac died in 1850 and the equation was not in fact worked out until early in the 20th century.

Despite executing Lavoisier (for his rôle in collecting taxes under the previous régime), the French government of the early nineteenth century nonetheless attached importance to understanding the scientific basis of alcoholic fermentation. France was then the world's greatest wine producer (Redding, 1833) and vast quantities of wine became spoilt for unknown reasons. Much turned into vinegar (by the action of acetic acid bacteria, as we now understand) or had bad flavours. So, in 1803, the *Institut de France* offered a medal worth one kilogram of gold for an answer to the question: *What are the characteristics which distinguish vegetable and animal substances acting as ferments from those that undergo fermentation?* However, no satisfactory answers were submitted (Cagniard-Latour, 1838).

In 1810, François Appert, a French manufacturer of food products, had described a way of preserving food by putting it into tightly closed vessels, which were then heated in boiling water (Appert, 1812). This proved to be the beginning of the canning industry. In the same year, Gay-Lussac (1810) observed that air left in the heated vessels lacked oxygen, and fermentation of grape juice or putrefaction of other foods started only after air was admitted. Hence, he concluded, oxygen is necessary for fermentation and putrefaction.

Consequences of improvements in microscopes

Early in the nineteenth century microscopes were greatly improved: Giovanni Amici (1820), professor of astronomy at Modena, made some of the first microscope objectives to be corrected effectively for chromatic and spherical aberrations. These corrections gave higher numerical apertures and, hence, better resolution for magnifications of up to 600 diameters. One of Amici's microscopes, made in 1837, had a maximum numerical aperture of 0·54 and a resolution of about 1 μm (van Cittert & van Cittert-Eymers, 1951) (Fig. 1). Such improvements opened up the possibility of seeing small microbes clearly for the first time and so were of prime importance for the development of microbiology. At that time, Louis Mandl, a professor at the Paris faculty of medicine, wrote that hitherto microscopy had been a dubious business:

> *...towards the end of the last century, the microscope experienced the fate of so many other new things; having exaggerated its usefulness and used it to support lunatic flights of fancy, people went to the other extreme and exaggerated its inconveniences and hazards; then its use was almost completely neglected, and results obtained with it were only spoken of with mistrust. Even the existence of blood corpuscles was doubted, and what Leeuwenhoek and his successors had described were attributed to optical illusions* (Saint-Hilaire & Edwards, 1838).

These improvements in microscopes enabled three independent scientists to go a long way towards answering the question put by the *Institut de France*. The three were Charles Cagniard-Latour, a physicist and engineer of Paris, Friedrich Kützing, an algologist from Halle and Theodor Schwann (Fig. 2), the great physiologist of Berlin. It would be fair to say that research on alcoholic fermentation, which led to understanding the rôle of enzymes in cell metabolism, started with the discovery by these three scientists that yeasts are living organisms.

Cagniard-Latour (1936a, b) examined beer and wine yeasts,

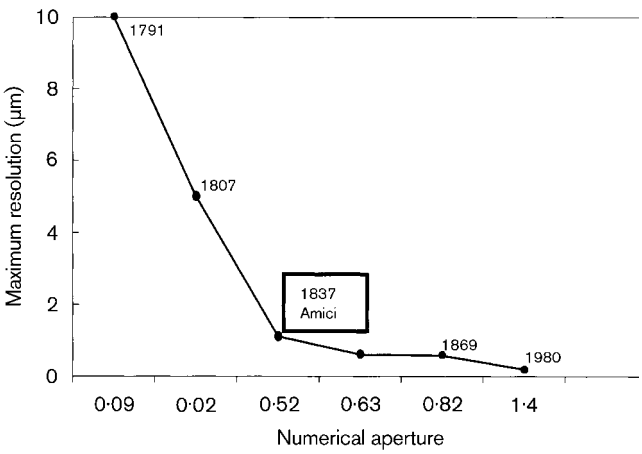

Fig. 1. Improvements in light microscopes since 1791. Results of examining microscopes in a Dutch museum (van Cittert & van Cittert-Eymers, 1951).

Fig. 2. Portrait of Theodor Schwann, from Frédéricq (1884).

describing them as composed of globules which he considered to be of the vegetable kingdom, as they are not motile and are formed by enlargement of other globules. He even described external features of the cells, such as bud scars, which are formed as part of the cell wall when a bud separates from its mother cell. His description of these scars was ignored until rediscovered by Barton (1950). Cagniard-Latour (1837) summarized his findings, writing: beer yeast is part of the vegetable kingdom and not, as had been supposed, an inert or purely chemical substance. Yeast seems to break down sugar, only when it is alive, liberating CO_2 from this breakdown and converting the sugar into a spirituous liquor.

Kützing (1837), the second of the three pioneers, published clear descriptions and drawings of yeast cells. His suggestion that different kinds of fermentation, such as vinegar fermentation, were due to different organisms was confirmed a quarter of a century later in the 1860s, by Louis Pasteur.

Schwann, the most illustrious of these three, is famous for developing a 'cell theory', namely, that living structures come from formation and differentiation of units (the cells), which then constitute the bodies of organisms (Schwann, 1839). His paper on fermentation (Schwann, 1837) was entitled 'A preliminary communication concerning experiments on fermentation of wine and putrefaction'. Using a microscope, Schwann examined beer yeast and described it as resembling many articulated fungi and 'without doubt a plant'. His conclusions from his observations and experiments were unequivocal, revolutionary and correct:

> *The connection between wine fermentation and the development of the sugar fungus is not to be underestimated; it is very probable that, by means of the development of the fungus, fermentation is started. Since, however, in addition to sugar, a nitrogenous compound is necessary for fermentation, it seems that such a compound is also necessary for the life of this plant, as probably every fungus contains nitrogen. Wine fermentation must be a decomposition that occurs when the sugar-fungus uses sugar and nitrogenous substances for growth, during which, those elements not so used are preferentially converted to alcohol.*

In one of his experiments, Schwann boiled some yeast in a solution of cane sugar in four stoppered flasks. After cooling, he admitted air into the flasks: for two flasks, the air was first passed through a thin red-hot glass tube (analysis showed this air still to contain 19·4 % oxygen); the other two flasks received unheated air. Fermentation occurred only in the latter two flasks. Schwann's conclusion was important:

> *Thus, in alcoholic fermentation as in putrefaction, it is not the oxygen of the air which causes this to occur, as previously suggested by Gay-Lussac, but something in the air which is destroyed by heat.*

In this notable 1837 paper, Schwann anticipated observations made by Pasteur over twenty years later, writing:

> *Alcoholic fermentation must be regarded as the decomposition effected by the sugar fungus, which extracts from the sugar and a nitrogenous substance the materials necessary for its own nutrition and growth; and substances not taken up by the plant form alcohol.*

Opposition from the 'Establishment': yeast as a physico-chemical phenomenon

Almost immediately, there followed a strident denunciation of the concept of yeast as a living organism by three of the leading and most influential chemists of the day, Jöns Berzelius, Justus von Liebig and Friedrich Wöhler. This was a remarkable event in the history of science and probably held up the development of microbiology for about twenty years.

Von Liebig (1839) summarized their views as follows. (i) The agent which produces fermentation is formed as the result of the action of air on plant juices which contain sugar; (ii) decomposition of the sugar occurs because of instability transferred to it by the unstable ferment; the latter is not a substance but a carrier of activity; (iii) yeast is a decomposing body with molecules in movement.

Von Liebig and Wöhler went so far as to publish jointly in their journal, *Annalen der Pharmacie*, an anonymous skit mocking the microscopical findings they rejected. The skit,

entitled 'The riddle of alcoholic fermentation solved', described yeast under the microscope as a tiny animal, shaped like a distilling apparatus, swallowing sugar and excreting alcohol from an anus and carbonic acid from its genitals (Anonymous, 1839).

Berzelius (1839) entered the fray independently, stating that microscopical evidence was of no value and yeast was no more an organism than was precipitate of alumina. Schwann's controls, he wrote, were inadequate; his experiments were worthless; and his conclusions exhibited a frivolity which had long been banished from science. Fermentation, he said, occurred by means of catalysis. Berzelius (1836a, b) had been the first to use the word catalysis to refer to phenomena, such as the action of platinum in decomposing hydrogen peroxide and the action of amylase in decomposing starch. What gradually emerged, much later, from this controversy was the question whether analogous catalysts are present in yeast cells, and are responsible for the fermentation of sugars.

Why all this opposition? The hostility of these chemists may have come, at least in part, from their own and other chemists' impressive achievements in establishing organic chemistry as a science. Early in the nineteenth century, it had been generally held that substances, such as fats and sugars, associated solely with plants and animals, could be formed only by living things. But soon the chemists themselves did much to overthrow this belief. Wöhler (1828a) had been responsible for one of the earliest productions of an organic compound by chemical means, namely that of urea from ammonium cyanate. Appropriately, it was to Berzelius, who seems to have been the first to use the expression 'organic chemistry' in print (Berzelius, 1806), that Wöhler (1828b), wrote triumphantly: 'I can make urea without the necessity of a kidney, or even of an animal.'

Moreover, at this time, various chemists had begun to make preparations that had enzymic activity. For example, Wöhler & von Liebig (1836) prepared 'emulsin' from bitter almonds. Very little of this water-soluble powder, which contains several enzymes including much β-glucosidase, was needed to hydrolyse the glycoside amygdalin to glucose, benzaldehyde and HCN. Wöhler and von Liebig compared this activity to fermentation

> *to which Berzelius* [they wrote] *has attributed a peculiar, catalytic force ... the comparatively small amount of emulsin required for decomposing amygdalin, shows that this is not an ordinary chemical action; it has some resemblance to the action of yeast on sugar...*

Von Liebig (1839) commented on the findings of Cagniard-Latour, Kützing and Schwann:

> *When we examine strictly the arguments by which this vitalist theory of fermentation is supported and defended, we feel ourselves carried back to the infancy of science.*

Some writers, such as Bulloch (1938) and Keilin (1966), have held that von Liebig and his colleagues considered the publications on fermentation by Cagniard-Latour, Kützing and Schwann were reactionary and a blow against the idea that processes associated with living things were chemical ones. However, others (McKie, 1944; Lipman, 1967) have drawn attention to the same chemists' continued adherence to the concept of a 'vital force' (*Lebenskraft*). Indeed, there were amongst these scientists many contradictions about the meaning of this term and to exactly what it should be applied.

Von Liebig's passionate feelings on the subject were clearly expressed in a 44-page article (von Liebig, 1839) on fermentation, putrefaction and decay, and their causes. In this, he made a series of dogmatic assertions. Putrefaction consisted first of a decay in which the oxygen of the air took no part and secondly, of an oxidation, of one or more elements of the decaying substance, using the oxygen of that substance or of water, or both. Fermentation was putrefaction of vegetable material. The *ferment* itself (i) arose during a metamorphosis which began after the entrance of air into a plant juice which contained sugar, (ii) could continue without air, (iii) did not cause fermentation, (iv) was a substance undergoing putrefaction or decay. When beer or wine yeast was washed, the residue did not cause fermentation in sugar water. Although the residue could be seen as globules under a microscope, the globules were not living for they occurred in many non-crystalline substances. To summarize von Liebig's view: the 'ferment' is formed as the result of action of air on plant juices which contain sugar; and decomposition of the sugar is owing to its instability conferred on it by the unstable ferment. Von Liebig himself carried out few, if any, experimental investigations of fermentation to justify his grandiose pronouncements.

Nonetheless, some important scientists of the 1840s and 1850s, even certain chemists, accepted that yeast was a kind of plant. One of these was the distinguished German chemist, Eilhard Mitscherlich, famous for discovering isomorphism (Mitscherlich, 1820).

He found the globules of yeast to be so large that they would not pass through a fine parchment filter. With a suspension of yeast in a glass tube, closed at the bottom by the filter paper, he put the tube into a sugar solution (Fig. 3). The sugar passed through the filter and was fermented, but no fermentation occurred outside the tube, where there was no yeast. Although he considered yeast to be a microbe, Mitscherlich (1842) explained the rôle of the yeast solely in terms of contact catalysis of the yeast's surface, as Berzelius had proposed earlier.

Acceptance of yeasts as living organisms

Although many influential scientists still adhered to von Liebig's view that yeast was not a living organism, by the time Louis Pasteur began work on alcoholic fermentation in the late 1850s, others were beginning to accept Schwann's

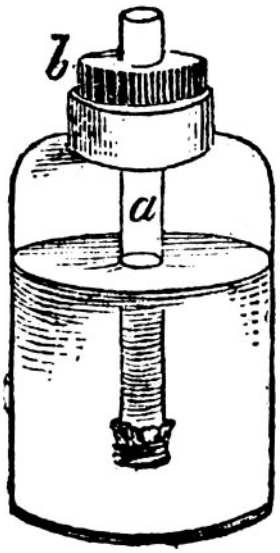

Fig. 3. The apparatus of Mitscherlich (1842) using parchment filter to divide a sugar solution into two compartments.

findings and those of Cagniard-Latour and Kützing. Between 1850 and 1880, yeasts became widely recognized as microbes. Different kinds of yeast were described, as were some bacteria, and their physiology began to be studied. At this time, those influenced by the earlier chemical approaches of Berzelius and von Liebig were in conflict with the newer biologists who followed Schwann. The chemists interpreted changes produced by microbes in terms of catalysis. Hence they helped to found enzymology. The biologists, in contrast, made advances in microbiology, especially microbial physiology.

With the acceptance of yeasts as living organisms which cause alcoholic fermentation, the major controversy shifted to another question, namely, should fermentation and other similar changes be attributed to intracellular activities of microbes or to the action of extracellular enzymes? Two major figures in this controversy were the French scientists, Louis Pasteur and Pierre Berthelot.

From his initial successes as an outstanding research chemist, Pasteur subsequently became one of the most distinguished microbiologists of all time. A master of experimental research, both academic and applied, he is described as an exceedingly serious man, totally obsessed with his scientific work, humourless, politically conservative, royalist and a Catholic by convention. He publicized his researches brilliantly but was sensitive to and highly intolerant of adverse criticism (Geison, 1995). Berthelot was a leading chemist who made major contributions to synthetic organic chemistry. Although brought up a Catholic, Berthelot became a sceptic, even rather anti-clerical, and a republican (Partington, 1964).

Pasteur began work on sugar fermentation by yeast in the late 1850s. Between 1855 and 1875 Pasteur established, unequivocally, (i) the rôle of yeast in alcoholic fermentation, (ii) fermentation as a physiological phenomenon, and (iii) differences between the aerobic and anaerobic utilization of sugar by yeasts. He invented the terms aerobic and anaerobic:

> *I propose with all kinds of misgivings these new words aerobic and anaerobic, to indicate the existence of two classes* [of microbe]*...those which survive only in the presence of free oxygen gas, and those which can multiply without contact with free oxygen* (Pasteur, 1863).

From chemistry to microbiology: Pasteur's conversion

By the age of 25, Pasteur (1848) had already reported the connexion between enantiomorphism (crystal structures that are mirror-images) and optical activity. In 1853, he gives a clear and interesting account of why he changed from chemistry to microbiology. His researches on the optical activity of organic compounds such as tartrates, asparagine and malic acid had led him to believe that all organic compounds with optical activity were formed by living organisms. He writes:

> *...never, in any circumstances, is an optically active compound produced by a non-living body, while almost all the substances elaborated by nature in vegetable organisms are asymmetrical, in the manner of tartaric acid* (Pasteur, 1853).

Lactic acid fermentation

Pasteur's first communication on microbial activity concerned lactic acid fermentation; he explains how he came to be interested in fermentation at all (Pasteur, 1858). Having shown one of the amyl alcohols formed during the fermentation of beet juice to be optically active, Pasteur argued that although derived from sugar, which is also optically active, the difference in structure between the sugar and the alcohol was too great for the asymmetric arrangement of the atoms to have been retained. This observation persuaded Pasteur that it would be of special interest to study how the 'ferment' produces this optically active alcohol. (This inference eventually turned out to be bogus, since these amyl alcohols are now known to be formed from amino acids and not from sugars.) He wrote 'I hope to be able to show the connexion between fermentation and the molecular asymmetry characteristic of substances of organic origin' and commented '...I have discovered a mode of fermentation of tartaric acid, which occurs very easily with ordinary dextro-tartaric acid and very badly or not at all with laevo-tartaric acid.' Pasteur had made the first steps towards the concept of enzymic stereospecificity, as he found that from a mixture of the two tartaric acids, only the dextro acid is fermented (possibly by a *Penicillium* sp.), so this was a way of separating them.

Now in the first fifteen years of the nineteenth century, during the Napoleonic wars, the British blockaded French ports, preventing the importing of cane sugar from their West Indian colonies, so beet began to be grown extensively in Northern France and many sugar-beet factories were built (Stein, 1988). By 1854, over 40 years later, when Pasteur became professor of chemistry at Lille, there was a flourishing beet sugar fermentation industry nearby for producing alcohol (mainly for industrial use).

Monsieur Bigo, a local alcohol producer, had serious failures of fermentation and consulted Pasteur. Bigo's son, who studied with Pasteur, wrote that Pasteur examined the fermenting liquor with a microscope and found the globules were round when the fermentation was satisfactory; but they tended to become elongate as it deteriorated and became a lactic fermentation (Valery-Radot, 1909). The papers Pasteur (1858, 1859) published on lactic acid fermentation indicate four general requirements for such research. (i) For the fermentation to be studied, optimum conditions must be found; (ii) the simplest possible substances must be used; (iii) the organisms that appear during the fermentation must be examined with a microscope and their appearance shown to be constant; (iv) a minute trace of the presumptive cause must be able to produce the characteristic fermentation.

Alcoholic fermentation

Pasteur (1857) published his first paper on alcoholic fermentation. Were the catalytic theory of Berzelius and von Liebig valid, he said, then during fermentation the 'ferment' would give up nothing and take nothing from the fermentable material. On the contrary, weighing the ingredients before and after fermentation showed the yeast to be taking something from the sugar; and Pasteur associated the breakdown of sugar to alcohol and carbonic acid with the living processes, the sugar providing part of the material of the yeast.

In 1860, Pasteur (1860a) affirmed the rôle of yeast in alcoholic fermentation. Contrary to von Liebig's assumption, only 95 % of the products of fermenting 'invert sugar' (glucose/fructose mixture) proved to be ethanol and carbon dioxide: the other 5 % included glycerol, succinic acid and 'cellulose'. Pasteur wrote '...we see that the yeast takes something from the sugar...' and declared unequivocally that alcoholic fermentation is a physiological process:

> *The chemical changes of fermentation are associated with a vital activity, beginning and ending with the latter. I believe that alcoholic fermentation never occurs without either the simultaneous organization, development and multiplication of cells or the continued life of cells already formed. All the results in this paper seem to me completely in opposition to the opinions of Liebig and Berzelius...Now...in what does the chemical act of decomposing the sugar consist; and what is its precise cause? I confess that I simply do not know.*

Furthermore, Pasteur produced a crop of yeast in a chemically defined medium of sugar, ammonium tartrate and inorganic phosphate. Nothing was present that *could* be putrefied by oxygen and extend its instability to the sugar, as von Liebig and his colleagues held. Thus Pasteur had finally refuted von Liebig's assertion that yeast originates from the action of oxygen on the nitrogenous matter of fermentable liquid.

Distinguishing between activities of whole organisms and of enzymes

In the same paper, Pasteur (1860a) acknowledges implicitly the problem of distinguishing between enzymic action on the one hand and fermentation by intact cells on the other. He wrote:

> *Should we say that yeast feeds on sugar and excretes alcohol and carbonic acid? Or, should we say, on the contrary, that yeast...produces a substance such as pepsin, which acts on the sugar and is soon exhausted, for no such substance is to be found in fermented liquids? I have nothing to say on the subject of these hypotheses. I neither accept them nor dismiss them and always wish not to go beyond the facts. And the facts tell me only that all true fermentations are associated with physiological phenomena.*

The long-standing difficulty in distinguishing between enzymic action and fermentation, and the confused controversies this caused, was given particular emphasis by Pasteur's controversy with Berthelot, one of the most powerful members of the French scientific establishment. In the year of Pasteur's big paper on alcoholic fermentation, Berthelot (1860) published a lucid and important account of his work on how beer yeast breaks down sucrose to glucose and fructose.

Previously, Mitscherlich (1842) had found yeast extract could convert cane sugar into a laevorotary sugar

$$\underset{+66{\cdot}5^\circ}{\text{Sucrose}} \rightarrow \underbrace{\underset{+52{\cdot}5^\circ}{\text{D-Glucose}} + \underset{-93^\circ}{\text{D-Fructose}}}_{-20^\circ}\Big\}\text{specific rotations}$$

which Dubrunfaut (1847) then showed to be a mixture of glucose and fructose. In his paper of 1860, Berthelot described the isolation of invertase (β-fructofuranosidase), an enzyme that hydrolyses sucrose, and disputed Pasteur's views.

Pasteur had stated unequivocally that the sucrose was broken down by the action of the succinic acid, which he had shown to be formed during fermentation.

> *...I think that the formation of grape sugar* [D-glucose and D-fructose, which Berthelot called 'inverted sugar'] *is due simply to the constant production of succinic acid, that this is only an incidental phenomenon and that it is never necessary that cane sugar must first become grape sugar to undergo fermentation...I do not think that yeast cells have*

any particular ability for transforming cane sugar into grape sugar. But succinic acid is a constant product of alcoholic fermentation, and the sugar must undergo in its presence the change that it undergoes generally owing to the action of acids.

Berthelot's experiments showed that, on the contrary, in conditions identical with those that held during fermentation, succinic acid hardly inverted sucrose at all and furthermore, that inversion could occur in an alkaline medium (Table 2). To 500 ml of 20 % sucrose solution (A), he added 0·8 g of succinic acid, much more than the yeast would produce during fermentation. To another 500 ml (B), he added 10 g of pressed beer yeast. After 16 h at 15–20 °C, solution B was in full fermentation: it reduced cupropotassium tartrate and showed a big change in optical rotation. Solution A, on the other hand, gave barely perceptible reduction. In solution C, which was the same as B except that it also contained 10 g $NaHCO_3$, there was slow fermentation and the solution then gave a positive Fehling's reaction.

From his results, Berthelot concluded:

It is not to succinic acid that one must attribute the inversion which follows the yeast's action...These facts prove that beer yeast inverts cane sugar by its own action and independently of the acidity of the solution.

In further experiments, Berthelot mixed pressed yeast with twice its weight of water, then filtered the mixture and obtained a solution containing 1·5 % of dissolved solids. This (presumably cell-free) filtrate rapidly inverted sucrose in the presence of 0·24 M $NaHCO_3$. He wrote:

The yeast extract thus contains a particular ferment, soluble in water and capable of changing cane sugar into invert sugar.

Furthermore, he found this *ferment* to be still active after redissolving and reprecipitating with alcohol. He had made the first isolation of invertase from brewer's yeast.

But he took his conclusions even further.

One knows that the researches of Cagniard Latour and especially those of Pasteur, have established that beer yeast consists of a mycodermic plant. From the new experiments that I am going to report, I have shown that the plant does not act on sugar physiologically, but simply by the ferments it secretes, in the same way as germinated barley secretes diastase, almonds secrete emulsin, the pancreas of an animal secretes pancreatin, and the stomach of the same animal secretes pepsin.

Pasteur's immediate response to this attack was semantically adroit, but disingenuous:

One can see...from Monsieur Berthelot's own words, that he calls substances soluble in water and capable of inverting sugar 'ferment'. Now everyone knows that many substances have this property, for example all the acids...When, however, we are concerned with cane sugar and beer yeast, I call only that which ferments the sugar 'ferment', that is, that which produces alcohol, carbonic acid, etc. As to inversion, I have not concerned myself with it. With respect to what causes it, I have only raised a doubt in passing in a note where I summarize three years of observations on alcoholic fermentation.

Consequently, the contradictions that Monsieur Berthelot believes he has found, between my statements and the true facts, hold only because of the wider definition he gives for the word 'ferment', whereas I have always applied it only to substances that produce true fermentations (Pasteur, 1860b)

It is striking how these two brilliant experimental scientists were both right and wrong and would not see the strength of each other's observations and arguments. Pasteur had thought solely in terms of *intracellular* activities and Berthelot of *extracellular* phenomena.

But, by the 1870s, even von Liebig had undergone a partial *volte face*, for he now acknowledged yeasts as living beings. However he still rejected the concept of fermentation as a physiological phenomenon. He seems to have had logical difficulties in interpreting the experimental evidence: he was unable, for example, to distinguish between growth of the yeast and its utilization of substrates. He wrote many boring and long-winded expositions. In the following, he failed to discriminate between growth and fermentation:

Table 2. Results of experiments of Berthelot (1860) on sucrose inversion by yeast

Each solution contains 500 ml 2 % sucrose (58 mM) incubated at 20 °C for 16 h.

	Solution...	A	B	C
Substance added	Succinic acid	0·8 g (14 mM)	–	–
	Pressed yeast	–	10 g	10 g
	$NaHCO_3$	–	–	10 g (0·24 M)
Results	Fermentation	–	+	+
	Optical rotation	+28·9°	−9°	+9°
	Fehlings reaction	Trace	+	+

> *The opinion that the decomposition of sugar during fermentation depends on the development and multiplication of yeast is incompatible with the fact that the yeast produces fermentation in a pure solution of sugar; the yeast consists of substances mostly rich in nitrogen and containing sulphur; it also contains a significant quantity of phosphate, and it has been difficult to understand how, in the absence of these elements in the pure sugar solution undergoing fermentation, the number of cells can be augmented* (von Liebig, 1870).

Between 1860 and 1880, there was a shift in attitudes about yeasts, fermentation and the activities of enzymes ('ferments' or 'diastases'), of both the 'chemists' in the tradition of von Liebig and the 'biologists' associated with Pasteur. These changes in theory were forced on the scientific community by experimental findings, especially those of Pasteur and Berthelot. The confusion produced by the double meaning of the word 'ferment' led Wilhelm Kühne (1878) to propose 'enzyme' (arbitrarily from the Greek *ἐν ζύμη* meaning 'in yeast') for the soluble ferments. He explained that his purpose was to distinguish between 'ferments', meaning microbes, and 'ferments' meaning chemical substances, like ptyalin, pepsin and so forth, which have catalytic properties.

Sugar metabolism and enzyme specificity

Emil Fischer (Fig. 4) was the founder of carbohydrate chemistry and the leading organic chemist at the end of the nineteenth century. His study of yeast sugar metabolism generated the first ideas about the molecular mechanism of enzyme specificity. Fischer & Hirschberger (1888) had prepared a new hexose (mannose), commenting: 'Mannose is avidly fermented by beer yeast at room temperature...' Fischer had encountered yeast previously, as his father had invested in a brewery in Dortmund and here young Emil tested the ability of beer yeast to ferment various sugars, such as glucose, mannose and galactose. Only the D-sugars were fermented, so he could separate them from the L-forms in racemic mixtures, as Pasteur had done with the tartaric acids. Accordingly, Fischer was able to characterize the hydrazones and osazones of the corresponding L-sugars. He had, himself, previously discovered the reaction of phenylhydrazine with sugars (Fischer, 1884), which he had used in establishing their configurations. Osazone crystals are often characteristic of the sugar from which they derived; progress in sugar chemistry had previously been handicapped by difficulties in obtaining sugar crystals.

Somewhat reminiscent of Pasteur's conversion from chemistry to biology, Fischer (1894b) wrote that, having established the configurations and hence, the classification of the monosaccharides, he could now apply his findings to biological research. As the preparation of sugars was often laborious and the experiments had to be varied frequently, he used a fermentation tube (Fig. 5) which was very small in order to save material (Fischer & Thierfelder, 1894). The bulb held as little as 70 mg sugar, 0·35 ml water, 0·35 ml

Fig. 4. Emil Fischer in 1889. (From the Institut für Organische Chemie, Technische Hochschule Darmstadt; reproduced by kind permission of Professor F. W. Lichtenthaler).

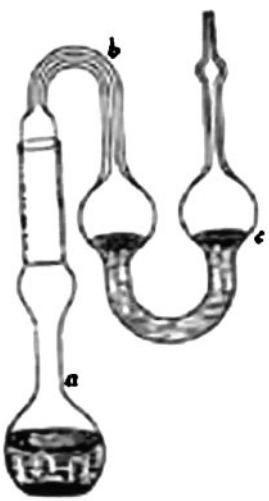

Fig. 5. Apparatus of Fischer & Thierfelder (1894) for measuring sugar fermentations by yeasts; total height about 6 cm. *a*, Fermentation flask; *b*, S-trap for CO_2 produced; *c*, aqueous $Ba(OH)_2$. The flask could contain 70 mg sugar in 0·35 ml H_2O + 0·35 ml aqueous yeast extract + 13 mg of yeast to be tested. Incubations were for 3–10 days at 24–28 °C.

sterilized yeast extract and 13 mg living yeast. The S-trap for evolved CO_2 contained aqueous barium hydroxide.

Pasteur's finding that microbes discriminated between D- and L-substrates had been given little attention until taken up by Fischer, who found that his yeasts fermented the D- but not the L-forms of glucose, mannose and galactose. He also found different yeasts to ferment different sugars and stressed that their structural characteristics might explain their fermentability:

> *D-Talose relates configurationally to D-galactose as does D-mannose to D-glucose. Because D-galactose already ferments less readily than the two others, any further small change in geometry eliminates fermentability altogether*. (See Fig. 6).

In a famous passage, Fischer summed up his interpretation of some of his findings with striking imagery:

> *The restricted action of the enzymes on glucosides could be explained by the assumption that only in the case of similar geometrical structure can the molecules approach each other sufficiently closely to initiate a chemical action. To use a metaphor, I would like to say that enzyme and glucoside have to fit together like lock and key in order to exert a chemical effect on each other* (Fischer 1894a).

He continued:

> *...the geometrical structure exerts such a profound influence on the chemical affinities that it seems legitimate to compare interacting molecules to key and lock...To cover the fact that some yeasts ferment more hexoses than others, the picture may be completed by the differentiation of a main key and special keys.*

Fischer's image of lock and key of 1894 led to the whole series of concepts of the enzyme–substrate complex developed by Henri (1903) and by Michaelis & Menten (1913) and eventually, to the idea of selective binding energy to stabilize transition states or destabilize substrates: that is, to J. B. S. Haldane's (1930) theory of substrate activation, Pauling's (1946) concept of enzyme-transition state complementarity, and to Koshland's (1958, 1994) induced fit theory. Each of these celebrated authors acknowledged the influence of Fischer's metaphor.

Summing up

1. Yeasts were the first microbes to be examined scientifically, because of their large cells and their obvious economic importance.

2. The research on yeasts was central to the early development of microbiology and biochemistry.

3. Since fermentation had already been equated with putrefaction and disease, finding that yeasts caused fermentation encouraged the search for other kinds of microbe, responsible for different kinds of fermentation, as well as different diseases.

4. Work on yeast fermentation by Schwann and Pasteur provided a model for future research on microbial physiology.

5. The study of yeasts' sugar utilization by Berthelot and by Emil Fischer provided foundations for studies of enzymes and their specificity.

6. The contribution of yeast research to biochemical knowledge continued through the twentieth century and included notable work such as those of the following: Buchner (1897) on fermentation by cell-free extracts, making research on glycolysis possible; Dienert (1900) on adaptation of yeasts to different sugars, leading to twentieth century concepts of enzyme induction (Monod, 1947); Harden & Young's (1905) discoveries of D-fructose 1,6-bisphosphate and coenzymes; and Hartwell (1991) and Nurse's findings (Forsburg & Nurse, 1991) on cell cycles.

Postscript

Material for the lecture on which this article is based came from fuller accounts given by Barnett (1998, 2000) and Barnett & Lichtenthaler (2001).

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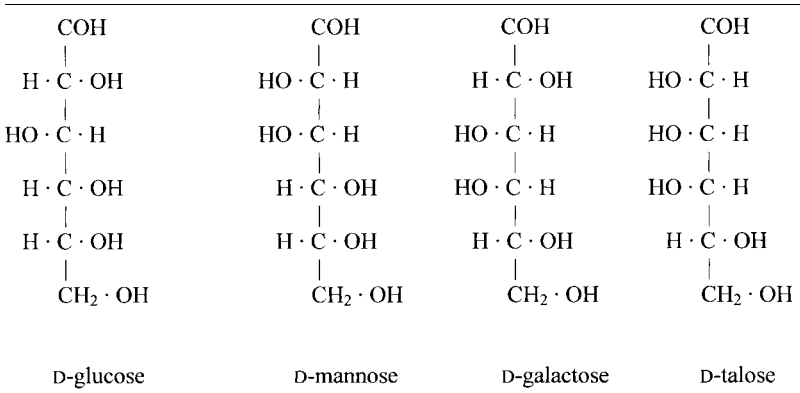

Fig. 6. Straight chain formulae of four sugars tested for fermentability by Fischer & Thierfelder (1894).

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