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## Yeasts

### Working for biotechnology

The fermentation processes of yeasts have maintained their importance from the arts and crafts of ancient times to the commercial processes of the modern food and beverage industry. In addition, as the importance of yeasts to both fundamental and applied research became increasingly appreciated in the second half of the 20<sup>th</sup> century. Microbial geneticists began to change the focus of their work from prokaryotes to eukaryotes to such an extent that in the 1970s bakers' yeast, *Saccharomyces cerevisiae*, became known as the *Escherichia coli* of eukaryote genetics. Yeasts are also very familiar in schools and colleges because *S. cerevisiae* is probably the most widely-used organism in practical microbiology in primary and secondary education. This is because *S. cerevisiae* is safe to use, easy to grow, relatively easy to see under the microscope and has a wide range of biochemical activities that can be readily shown in the laboratory.

### Early history

The activities of yeasts were having 'merry' effects on the human race long before the existence of the microbes was known. Noah's over-indulgence in wine is noted in the Book of Genesis but there are more precise accounts of the production of alcohol in the form of beer before 6 000 B.C. by the Sumerians and the Babylonians. The leavening of bread, a process made possible by production of carbon dioxide by yeast, was used by the Egyptians in about 4 000 B.C. The well-known painted relief on the wall of a Fifth Dynasty Egyptian tomb that shows scenes of brewing and baking dates from much later, about 2 400 B.C. Production of alcoholic spirits, the 'hard stuff', by distillation was common in many parts of the world by the 14<sup>th</sup> century.

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A model of servants making bread and brewing.  
Ancient Egyptian early Xii dynasty.  
Model from Beni Hasan Tomb 366.



## Discovery of yeast

The existence of yeasts was first observed by Antonie van Leeuwenhoek, a Dutch microscope maker, in the 1680s. The proposal that microscopic forms of life are responsible for making the products of fermentation was made independently by workers in France and Germany in the first half of the 19<sup>th</sup> century, much to the disapproval of the leading chemists of the day who believed that fermentation was a purely chemical reaction. Louis Pasteur took a great interest in the controversy but it was not until the second half of the century that his rejection of the chemists' hypothesis was fully accepted. It was in the course of his work on fermentation that Pasteur demonstrated for the first time the phenomenon of 'life without air' whereby yeast could grow in the absence of oxygen ('anaerobically' by fermentation) as well as in its presence ('aerobically' by respiration).

Pasteur also attempted to show that cell-free extracts of yeast were able to convert sugar to alcohol, *i.e.*, by the action of enzymes, but was not successful despite his carefully-designed experimental method. However, the phenomenon was later observed by Eduard Buchner by accident and reported in 1897 — and biochemistry was born!

It is worth mentioning at this point that the word 'fermentation' is frequently used in two different ways: a) in the biochemical sense to describe the type of energy-generating metabolism discovered by Pasteur that takes place without the involvement of an external electron acceptor such as oxygen (also known as 'anaerobic respiration'); b) in an industrial sense for any large-scale microbial process regardless of whether fermentative or respiratory metabolism is involved.

## A role in industry

Many of Pasteur's discoveries revolutionised industrial microbiology at the time, *e.g.*, the importance of using pure cultures and the introduction of pasteurisation, and also provided the foundations for others to build on. An important example is the achievement of the Danish yeast taxonomist Christian Hansen, a contemporary of Pasteur, in developing methods for obtaining and growing a single strain of yeast in pure culture, one of which he named *S. carlsbergensis*. This advanced the work of Pasteur whose concept of a pure culture of yeast for brewing was one that was not contaminated with other microorganisms although it might include a mixture of more than one type of yeast. Yeasts were known to reproduce asexually by the characteristic process of 'budding' but Hansen made the crucial observation of their ability to reproduce sexually. This significant step led to the development of traditional breeding techniques for improving strain performance and product quality.

To be successful, a food product must satisfy a need in the market place, be attractive to the consumer, have consistently good quality and flavour and be produced in an economically efficient way. In a microbiological process the choice of a suitable microbial culture is one of the key decisions. This is where yeasts have advantages because they possess many of the features that are looked for in industrial microbiology (Table 1). The features have wide relevance — alcohol production in making beer and wine, carbon dioxide production in breadmaking, and growing yeast cells for use as inoculants for these processes or as a source of food additives and enzymes.



## Nuisance value

To maintain a sense of balance, however, it is important to note that some of the 800 species of yeasts that have been described cause problems. For example, some are a great nuisance as spoilage organisms in a range of products, *e.g.*, spoilage of beer by 'wild yeast' (*i.e.*, a yeast not deliberately used and not under full control, including strains of *S. cerevisiae*) and soft drinks by species of *Candida*, *Brettanomyces*, *Saccharomyces* and *Zygosaccharomyces*. Others cause infections in human beings, *e.g.*, *Candida* infections occur in folds of the skin (the armpit, groin, *etc.*) and the cuticle of nails, cause 'thrush' in the mouth and vagina and are a major killer of immunocompromised patients.

## Where yeasts are found

Yeasts occur naturally in a wide range of habitats including animals, plants, soil, water and air. They are particularly abundant in material that contains sugars such as nectar of flowers and the surface of fruits where they are able to grow in conditions of low water activity caused by the presence of large concentrations of dissolved sugars. Their widespread occurrence accounts for the origin of the organisms that were originally responsible for beer, wine and breadmaking in ancient times.

## Yeast physiology and metabolism

The name *Saccharomyces* is derived from the ability of yeasts to metabolise a range of carbohydrates (Greek *saccaron* = sugar + *myketes* = fungi), commonly the hexose sugar glucose. The end products of carbohydrate metabolism are influenced by the availability of oxygen. The choice of the level of aeration is dependent upon an understanding of the oxygen requirements of yeasts, *i.e.* they are facultative anaerobes. This means that they can grow in either the absence or presence of oxygen by fermenting in its absence (anaerobically) and respiring in its presence (aerobically). Therefore, anaerobic conditions are required for efficient alcohol and carbon dioxide production (alcoholic fermentation) but sterile air is forcibly added if the purpose of the process is to maximise the yield of yeast cells, *e.g.*, when making yeasts for the baking industry, in which case the sugar is fully oxidised to carbon dioxide and water. Even the waste carbon dioxide produced in industrial fermentations can be used as a resource by the specialist gas supply industry.

Another fundamental metabolic feature to consider is that ethanol is a primary metabolite, *i.e.*, product excretion parallels the logarithmic phase of growth. This is in contrast to the production of, for example, penicillin which is an example of a secondary metabolite, *i.e.*, product excretion is at its maximum near the end of the logarithmic phase of growth or in the early stages of the stationary phase. An understanding of these phenomena is crucial when deciding on the length of the fermentation period that will provide optimum product formation.

## Yeast nutrition

As with all living cells, the cell material of micro-organisms is synthesised from the nutrients that are provided in the culture medium. The nutrients must provide all the necessary *building blocks* from which the cell materials are synthesised and the *energy sources* that are necessary for enabling the various (bio)synthetic processes to take place. For some micro-organisms, the same nutrient can serve both purposes; for example, glucose can be a source of carbon and energy.

### Nutrients as building blocks

The chemical composition of cells is relatively constant throughout the living world. Hydrogen, oxygen (both obtained from water, the major essential nutrient), carbon and nitrogen are the most abundant elements in the solid material of cells, accounting for more than 90% of the cell dry weight. Therefore it is necessary to choose nutrients that provide large amounts of these elements. Other essential elements such as phosphorus, sulphur, potassium, *etc.* occur in smaller proportions in cells and consequently smaller amounts are needed. All the metallic elements can be supplied as the cations of inorganic salts; some that are needed in only very small amounts (known as 'trace elements'), *e.g.*, molybdenum and copper, are already present in sufficient amounts as contaminants in the major inorganic constituents of culture media.

Although the chemical composition of microbial cells is relatively constant, there is a great diversity among micro-organisms as regards the specific nutrients that they require. This is because micro-organisms vary in the extent to which they are able to synthesise their complex cell materials and growth factors (amino acids, purines, pyrimidines and vitamins) from simpler chemical substances. Therefore it is often convenient to put micro-organisms into various categories according to their nutritional requirements. Thus yeasts are *heterotrophs* because they need organic compounds as the principal source of carbon, and *chemotrophs* because they need a chemical source of energy. (For completeness: *autotrophs* use carbon dioxide as the principal source of carbon: *phototrophs* use light as an energy source *i.e.*, they are photosynthetic.)

At one extreme of the nutritional spectrum some cyanobacteria (blue-green bacteria) have great synthetic ability in being able to use the carbon dioxide and molecular nitrogen components of air as the sole sources of carbon and nitrogen for the biosynthesis of organic cell substances. At the other extreme, many of those micro-organisms that are pathogenic to animals have to be provided with complex organic sources of carbon and nitrogen (such as heat and enzymic extracts of meat and blood) because they have limited synthetic ability as a consequence of having evolved to a parasitic way of life. The nutritional requirements of industrial yeasts are between these extremes; a suitable culture medium contains glucose and a source of organic nitrogen and growth factors such as extracts of meat, yeast or malt.

### Nutrients as energy sources

As the cell constituents are far more complex than the nutrients from which they are synthesised, energy must be supplied before biosynthesis and growth can take place. Energy is obtained from microbial degradation of the energy-rich nutrients and is transferred to the biosynthetic reactions by means of the very reactive, high

energy compound adenosine 5'-triphosphate (ATP). However, not all of the energy that is generated is available for biosynthesis because much of it is lost as heat. ATP is generated by yeast and other micro-organisms through several different pathways. As the major pathways are well known they will be only summarised here. An important point that will emerge is that respiration is much a more efficient process than fermentation for generating ATP.

Glycolysis, the most common pathway for degradation of glucose to pyruvate, results in a net yield of two ATP molecules from each glucose molecule, *i.e.*, two ATP molecules are used in the conversion of glucose to fructose 1,6-bisphosphate and four are generated from the conversion of each of two glyceraldehyde 3-phosphates to pyruvate. This can take place in either aerobic or anaerobic conditions but it is only in aerobic conditions that the two molecules of reduced nicotinamide adenine dinucleotide (NADH) that are also produced yield six more ATP molecules during electron transport and oxidative phosphorylation.

Conversion of pyruvate to acetyl co-enzyme A (acetyl-CoA), the substrate for the TCA cycle, yields two NADHs and therefore 6 ATPs by the same route. Oxidation of each acetyl-CoA in the TCA cycle then yields a total of 24 ATPs including those produced by the oxidation in the electron transport chain of six NADHs and two reduced flavin adenine dinucleotide molecules (FADH<sub>2</sub>).

Thus the (aerobic) oxidation of each glucose molecule to 6 carbon dioxide molecules yields a total of 38 ATPs. If this is compared with the net gain of only two ATP molecules formed by the anaerobic conversion of glucose to pyruvate (see above), it is clear that respiration is much more efficient than the anaerobic process of fermentation.

To follow the energy consequences of anaerobic process to the stage of ethanol production by yeast it is necessary to consider the alcoholic fermentation of the pyruvate that is produced when glycolysis occurs in the absence of air. Pyruvate is decarboxylated to acetaldehyde which is then reduced to ethanol by alcohol dehydrogenase with NADH as electron donator.

The difference in efficiency of carbohydrate degradation in energy terms under aerobic and anaerobic conditions is of much relevance to the industrial uses of organisms such as yeasts which are grown under both conditions with the two-fold aim of producing microbial cells (aerobically) or alcohol (anaerobically).

## Yeasts in action

The industrial uses of yeasts are not limited to the traditional processes of making bread, wine and beer (Table 1). Yeasts are also a rich source of a range of industrially important enzymes such as invertase. Another enzyme converts fatty acids to lactones for use in flavourings for margarines and in fruit flavours. Yeast extract has important uses as a source of B and D vitamins, in flavour enhancement and is a common ingredient of routine microbiological culture media.

**Table 1**  
*Some uses of yeasts and yeast products*

Yeast cells	Products from yeast cells	Alcohol for drinking	Alcohols for industry
Bakers' yeast	Yeast extract	Beer, lager	Industrial alcohol
Dried food yeast	Vitamins B, D	Wine	Gasohol (motor fuel)
Single-cell protein	Enzymes	Spirits	Glycerol

## Strain improvement

The brewing industry provides a good example of how strain improvement is approached. Before embarking on time-consuming procedures for genetic improvement it is a good idea to screen the culture that is already in use to see if a strain with the desired properties is already present. The next approach to try would be mutagenesis, usually by UV irradiation. This procedure inactivates one or more genes but there is always the danger of deleting a desirable feature! The ability of yeasts to undergo sexual reproduction, and thus genetic recombination, enables the classical procedure of hybridisation to be explored by crossing haploid cultures or, more successfully, using protoplast fusion. Recombinant DNA technology is also possible but this would have to be considered in terms of legislation and public opinion.

## Some achievements

Several benefits for the brewing process have been achieved by strain improvement. Selection of strains which produce less depth of froth (or 'head') on the surface of the fermentation vessel in proportion to vessel volume were sought when open tank fermentation was replaced by the use of closed fermenters. Increased osmotic tolerance is another valuable feature of relevance in high-gravity brewing where high strength beer is brewed and then diluted for sale. Yeast strains that flocculate at the end of the fermentation are of much help in the clarification stage of production.

An important function of malt in brewing is to provide amylase from the germinated barley seeds which converts starch into sugar for fermentation to alcohol. Ordinary brewing strains of *S. cerevisiae* cannot metabolise starch but a closely related species, *S. diastaticus*, is

able to because it produces an extracellular glucoamylase. However, the beer is not suitable for drinking because it has an unpleasant phenolic flavour in the product. This problem was solved by transferring the gene for glucoamylase production from *S. diastaticus* to *S. cerevisiae* thereby producing a modified strain with a better ability to utilise the full range of carbohydrates in the wort, *i.e.*, maltose, glucose, maltotriose and particularly dextrans which constitute more than 20% of the wort. This opens the way to the more efficient manufacture of a low-carbohydrate ('light') product for which there is widespread customer demand.

Yeasts also feature in innovations in other industries. Rennin (now known as chymosin), the protein-degrading enzyme used in cheese production that was traditionally obtained from the stomach of calves, is now produced by micro-organisms including the yeast *Kluyveromyces lactis*. This was achieved by transferring the chymosin gene from calves to micro-organisms using a plasmid. In the medical field there are plans for clinical trials with human serum albumin produced by recombinant DNA yeast technology to supplement the traditional sources of human plasma and placenta. A yeast-derived vaccine for protection against hepatitis B virus has been registered in more than 100 countries.

### The Yeast Genome Project

Several years of international collaboration between more than 600 scientists from 96 laboratories on the Yeast Genome Project resulted in the publication of the complete genomic sequence of *S. cerevisiae* in 1997. The sequence was published as *The Yeast Genome Directory*, a supplement to *Nature*, Vol 387, 29 May 1997. It had been first made public on 24 April 1996 on the World Wide Web (<http://genome-www.stanford.edu/Saccharomyces>). This was a very significant event because it was the first sequence of a eukaryote to be completed and, therefore, will be used as the reference genome for all eukaryotes, including human beings. *Saccharomyces cerevisiae* was the chosen 'model' eukaryote system for many of the reasons that have made yeasts commercially important (Table 2).

Table 2

*Some key features of industrial yeasts*

Feature	Benefit
Non-pathogenic	Suitable for food use
High surface:volume ratio	High metabolic rate to give efficient product yield
Pure cultures available	Consistent product yield and quality
Genetically stable	Consistent product yield and quality
Retains viability and activity in laboratory culture and in the process	Consistent product yield and quality
Grows in large-scale liquid culture	Good product yield
Grows on inexpensive and readily-available liquid process culture media	Reduced feedstock costs
Easily removable from process culture medium by settlement, flocculation, centrifugation or filtration	Reduced downstream-processing costs
Genetic modification possible	Improved product yield and quality and process performance

However, this remarkable achievement is not an end in itself for its true significance is in the future work which is now possible. For example, there is the prospect of the more difficult task of discovering the functions of the 6 000 protein-encoding genes now identified in the sequence because the purpose of about half of them is not known. Also, the sequence is seen as a milestone in comparative biology because of the opportunity now presented of considering ways of making comparisons of whole genomes across the three domains of life (Eubacteria, Archaea and Eukarya) and of exploring fully the evolutionary relationships at the base of the phylogenetic tree. Research workers already have the work under way but 'budding' biotechnologists can be assured that there is still much research and development work to be done and progress to be made. This new branch of science known as 'genomics' that deals with the entire genome promises to provide alternative approaches to the existing time-consuming procedures for developing genetic systems thereby helping to achieve the full potential that yeasts can offer to industry and to medicine.

