Second Edition

Food, Fermentation, and Micro-organisms

Charles W. Bamforth | David J. Cook

WILEY Blackwell

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Dedicated to our wives, Diane Bamforth and Sarah Cook, for their love and forbearance.

> *God made yeast, as well as dough, and loves fermentation just as dearly as he loves vegetation.*
>
> *Ralph Waldo Emerson (1803–1882)*

> *Fermentation and civilization are inseparable.*
>
> *John Ciardi (1916–1986)*

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Preface

Fermentation and civilisation are inseparable, according to the American poet John Ciardi. His observation aptly conveys the significance of our relationship with micro-organisms, dating back thousands of years to the earliest records. It is as if the ability to master control of micro-organisms, to harness their benefits in food and beverage production, is in some way a rite of passage; a seal of approval that civilisation has evolved.

There is ample evidence amongst artefacts from early civilisations that preservation and fermentation using micro-organisms were valued, even sacred, practices used to enhance the safety, storage and palatability of foodstuffs. Long before Anton van Leeuwenhoek first set eyes on microbes under a microscope in the seventeenth century we had learned to control to good effect the biochemistry of organisms that we could neither see nor countenance the existence of. How clever is that? Furthermore, the ability to pass on and perfect such practices through many generations is a hallmark of civilisation.

Moving to present times, what could be regarded as more civilised than enjoying a pint of beer and sustenance at your local public house? Picture the scene … the beautifully balanced, low carbonation, best bitter ale in a jugged glass. Ploughman's lunches of ham, salami, cheese, pickled onions and freshly baked crusty bread. The delights of the curry, with naan and poppadom, yoghurty dips. Glasses of cider or the finest wine. And the rich chocolate pud. Perhaps a postprandial port, or Armagnac or Southern Comfort.

Just look at that list. Ralph Waldo Emerson hit the nail on the head: what a gift we have in fermentation, the common denominator between all these foodstuffs and many more besides. In this book we endeavour to capture the essence of these very aged and honourable biotechnologies for the serious student of the topic. It would be impossible in a book of this size to do full justice to any of the individual food products – those seeking a fuller treatment for each are referred to the bibliography at the end of each chapter. Rather we seek to demonstrate the clear overlaps and similarities that sweep across all fermented foods, stressing the essential basics in each instance.

Introduction

Campbell-Platt defined fermented foods as '*those foods that have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification in the food*'. The processes may make the foods more nutritious or digestible, or may make them safer or tastier, or some or all of these.

Most fermentation processes are extremely old. Of course nobody had any idea of what was actually happening when they were preparing these products – it was artisan stuff. However experience, and trial and error, showed which were the best techniques to be handed on to the next generation, so as to achieve the best end results. Even today, some producers of fermented products – even in the most sophisticated of areas such as beer brewing – rely very much on 'art' and received wisdom.

Several of the products described in this book originate from the Middle East (the Fertile Crescent – nowadays the region embracing Iraq) some 10 000–15 000 years ago. As a technique, fermentation was developed as a low energy way in which to preserve foods, featuring alongside drying and salting in days before the advent of refrigeration, freezing and canning. Perhaps the most widespread examples have been the use of lactic acid bacteria to lower the pH and the employment of yeast to effect alcoholic fermentation. Preservation occurs by the conversion of carbohydrates and related components to end products such as acids, alcohols and carbon dioxide. There is both the removal of a prime food source for spoilage organisms and also the development of conditions that are not conducive to spoiler growth, e.g. low pH, high alcohol and anaerobiosis. The food retains ample nutritional value, as degradation is incomplete. Indeed changes occurring during the processes may actually increase the nutritional value of the raw materials, for example the accumulation of vitamins and antioxidants or the conversion of relatively indigestible polymers to more assimilable degradation products.

The crafts were handed on within the home and within feudal estates or monasteries. For the most part batch sizes were relatively small, the production being for local or in-home consumption. However the Industrial Revolution of the late eighteenth century led to the concentration of people in towns and cities. The working classes now devoted their labours to work in increasingly heavy industry rather than domestic food production. As a consequence the fermentation-based industries were focused in fewer larger companies in each sector. Nowadays there continues to be an interest in commercial products produced on the very small scale, with some convinced that such products are superior to those generated by mass production, e.g. boutique beers from the brewpub and breads baked in the street corner bakery. More often than not, for beer

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if not necessarily for bread, this owes more to hype and passion rather than true superiority. Often the converse is true, but it is nonetheless a charming area.

Advances in the understanding of microbiology and of the composition of foods and their raw materials (e.g. cereals, milk), as well as the development of tools such as artificial refrigeration and the steam engine allowed more consistent processing, whilst simultaneously vastly expanding the hinterland for each production facility. The advances in microbiology spawned starter cultures, such that the fermentation was able to pursue a predictable course and no longer one at the whim or fancy of indigenous and adventitious microflora.

Thus we arrive at the modern day food fermentation processes. Some of them are still quaint – for instance the operations surrounding cocoa fermentation. But in some cases, notably brewing, the technology in larger companies is as sophisticated and highly controlled as in any industry. Indeed latter day fermentation processes such as those devoted to the production of pharmaceuticals were very much informed by the techniques established in brewing.

Fermentation in the strictest sense of the word is anaerobic, but most people extend the use of the term to embrace aerobic processes and indeed related non-microbial processes, such as those effected by isolated enzymes.

In this book we will address a diversity of foodstuffs that are produced according to the broadest definitions of fermentation. We start in Chapter 1 by considering the underpinning science and technology that is common to all of the processes. Then, in Chapter 2, we give particularly detailed attention to the brewing of beer. The reader will forgive the authors any perceived prejudice in this. The main reason is that by consideration of this product (from a fermentation industry that is arguably the most sophisticated and advanced of all of the ones considered in this book) we address a range of issues and challenges that are generally relevant for the other products. For instance the

Table I.1 The relationship between feedstock, primary fermentation products and derived distillation products.

Raw material	Non-distilled fermentation product	Distilled fermentation derivative
Apple	Cider	Apple brandy, Calvados
Barley	Beer[a]	Whisk(e)y
Cacti/succulents	Pulque	Tequila
Grape	Wine	Armagnac, Brandy, Cognac
Palmyra sap	Toddy	Arak
Pear	Perry	Pear brandy
Honey	Mead	Honey jack
Rice	Sake	Shochu
Sorghum	Sorghum beer	
Sugar cane/ molasses		Rum
Wheat	Wheat beer	

[a] Whisky is not strictly produced by distillation of beer, but rather from the very closely related fermented unhopped wash from the mashing of malted barley.

consideration of starch is relevant to the other cereal-based foods, such as bread, sake and, of course, distilled grain-based beverages. The discussion of Saccharomyces and the impact of its metabolism on flavour is pertinent for wine, cider and other alcoholic beverages (Table I.1 gives a summary of the main alcoholic beverages and their relationship to the chief sources of carbohydrate that represent fermentation feedstock). We can go further: one of the finest examples of vinegar (malt) is fundamentally soured unhopped beer.

The metabolic issues that are started in Chapter 1 and developed in Chapter 2 will inform all other chapters where microbes are considered. Thus, from these two chapters, we should have a well-informed grasp of the generalities that will enable consideration of the remaining foods and beverages addressed in the ensuing chapters.

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1

The Science Underpinning Food Fermentations

Use the word 'biotechnology' nowadays and the vast majority of people will register an image of genetic alteration of organisms in the pursuit of new applications and products, many of them pharmaceutically relevant. Even the *Merriam-Webster Dictionary* tells us that biotechnology is 'biological science when applied especially in genetic engineering and recombinant DNA technology'. Fortunately the *Oxford English Dictionary* gives the rather more accurate definition as 'the branch of technology concerned with modern forms of industrial production utilising living organisms, especially microorganisms, and their biological processes'.

Accepting the truth of the second of these, then we can realise that biotechnology is far from being a modern concept. It harks back historically vastly longer than the traditional milepost for biotechnology, namely Watson and Crick's announcement in the Eagle pub in Cambridge (and later, more formally, in *Nature*) that they had found 'the secret of life'.

Eight thousand years ago our ancient forebears may have been, in their own way, no less convinced that they had hit upon the essence of existence when they made the first beers and breads. The first micro-organism was not seen until draper Anton van Leeuwenhoek peered through his microscope in 1676 and neither were such agents firmly causally implicated in food production and spoilage until the pioneering work of Needham, Spallanzani and Pasteur and Bassi de Lodi in the eighteenth and nineteenth centuries.

Without knowing the whys and wherefores, the dwellers in the Fertile Crescent were the first to make use of living organisms in fermentation processes. They truly were the first biotechnologists. And so beer, bread, cheese, wine and most of the other foodstuffs being considered in this book come from the oldest of processes. In some cases these have not changed very much in the ensuing aeons.

Unlike the output from modern biotechnologies, for the most part we are considering high volume, low value commodities. However for products such as beer, there is now a tremendous scientific understanding of the science that underpins the product, science that is none the less tempered with the pressures of tradition, art and emotion. For all of these food fermentation products, the customer *expects*. As has been realised by those who would apply molecular biological transformations to the organisms involved in the manufacture of foodstuffs, there is vastly more resistance to this than for applications in, say, the pharmaceutical area. You don't mess with a person's meal!

Historically, of course, the micro-organisms employed in these fermentation processes were adventitious. Even then, however, it was realised that the addition of a part

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of the previous process stream to the new batch could serve to 'kick off' the process. In some businesses this was called 'back slopping'. We now know that what the ancients were doing was seeding the process with a hefty dose of the preferred organism(s). Only relatively recently have the relevant microbes been added in a purified and enriched form to knowingly trigger fermentation processes.

The two key components of a fermentation system are the organism and its feedstock. For some products, such as beer, there is a radical modification of the properties of the feedstock, rendering them more palatable (in the case of beer, the grain extracts pre-fermentation are most unpleasant in flavour; by contrast, grape juice is much more acceptable). For other products the organism is less central, albeit still important. One thinks for instance of bread, where not all styles involve yeast in their production.

For some products, such as cheese, the end product is quite distinct from the raw materials as a result of a series of unit operations. For products such as beer, wine and vinegar the product is actually the spent growth medium – the excreta of living organisms if one was to put it crudely. Only occasionally is the product the actual micro-organism itself – for example the surplus yeast generated in a brewery fermentation or that generated in a microbial biomass ('single cell protein') operation such as the production of mycoprotein.

The organisms employed in food fermentations are many and diverse. Key players are the lactic acid bacteria, in dairy products for instance, and yeast, in the production of alcoholic beverages and bread. The lactic acid bacteria, to illustrate, may also have a positive role to play in the production of certain types of wines and beers, but equally they represent major spoilage organisms for many such products. It truly is a case of the organism being in the right niche for the product in question.

In this chapter we will focus on the generalities of science and technology that underpin fermentations and the organisms that are involved. We will look at commonalities in terms of quality – for example the Maillard reaction that is of widespread significance as a source of colour and aroma in many of the foods that we are considering. The reader will discover (and this betrays the primary expertise of the authors) that many of the examples given are from beer making. It must be said, however, that the scientific understanding of the brewing of beer is somewhat more advanced than that for most if not all of the other foodstuffs described in this book. Many of the observations made in a brewing context very much translate to what must occur in the less well-studied foods and beverages.

1.1 Micro-Organisms

Microbes can essentially be divided into two categories: the prokaryotes and the eukaryotes.

The former, which embrace the bacteria, are substantially the simpler, in that essentially they comprise a protective cell wall, surrounding a plasma membrane, within which is a nuclear region immersed in cytoplasm (Figure 1.1). This is a somewhat simplistic description, but sufficient for our needs. The nuclear material (deoxyribonucleic acid, DNA) of course figures as the genetic blueprint of the cell. The cytoplasm contains the enzymes that catalyse the reactions necessary to the growth, survival and reproduction of the organisms (the sum total of reactions of course being referred to as *metabolism*). The membrane regulates the entry and exit of materials into and from the cell.

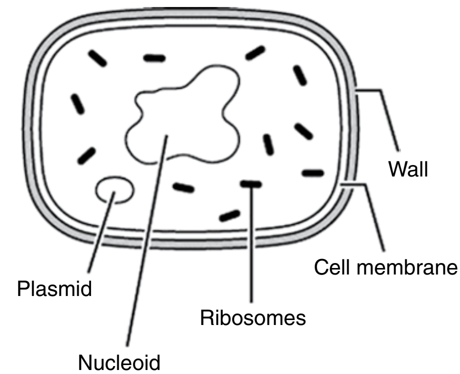

Figure 1.1 A simple representation of a prokaryotic cell. The major differences between Gram positive and Gram negative cells concerns their outer layers, with the latter having an additional membrane outwith the wall in addition to a different composition of the wall itself.

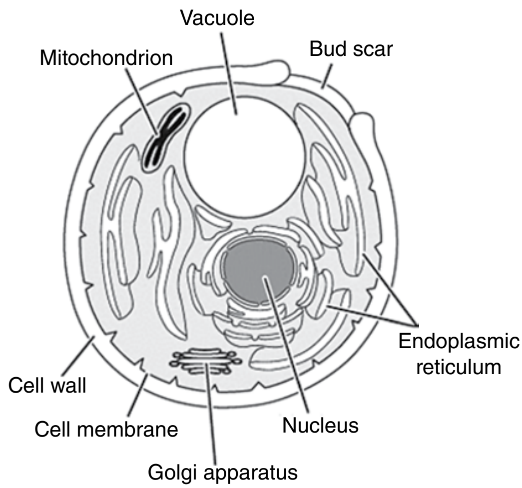

Figure 1.2 A simple representation of a eukaryotic cell.

The eukaryotic cell (of which bakers or brewers yeast, *Saccharomyces cerevisiae*, a unicellular fungus, is the model organism) is substantially more complex (Figure 1.2). It is divided into organelles, the intracellular equivalent of our bodily organs. Each has its function. Thus the DNA is located in the nucleus which, like all the organelles, is bounded by a membrane. All the membranes in eukaryotes (and prokaryotes) comprise lipid and protein. Other major organelles in eukaryotes are the mitochondria, wherein energy is generated, and the endoplasmic reticulum. The latter is an interconnected network of tubules, vesicles and sacs with various functions including protein and sterol synthesis, sequestration of calcium, production of the storage polysaccharide glycogen and insertion of proteins into membranes. Both prokaryotes and eukaryotes have polymeric storage materials located in their cytoplasm.

Table 1.1 lists some of the organisms that are mentioned in this book. Some of the relevant fungi are unicellular, for example Saccharomyces. However the major class of fungi, namely the filamentous fungi with their hyphae (moulds), are of significance for a number of the foodstuffs, notably those Asian products involving solid state fermentations, e.g. sake and miso, as well as the only successful and sustained single cell protein operation (Chapter 17).

Table 1.1 Some micro-organisms involved in food fermentation processes.

Bacteria		Fungi	
Gram negative[a]	**Gram positive**[a]	**Filamentous**	**Yeasts and non-filamentous fungi**
Acetobacter	Arthrobacter	Aspergillus	Brettanomyces
Acinetobacter	Bacillus	Aureobasidium	Candida
Alcaligenes	Bifidobacterium	Fusarium	Cryptococcus
Escherichia	Cellulomonas	Mucor	Debaromyces
Flavobacterium	Corynebacter	Neurospora	Endomycopsis
	Lactobacillus	Penicillium	Geotrichum
Gluconobacter	Lactococcus	Rhizomucor	Hanseniaspora (Kloeckera)
Klebsiella	Leuconostoc	Rhizopus	Hansenula
Methylococcus	Micrococcus	Trichoderma	Kluyveromyces
Methylomonas	Mycoderma		Monascus
Propionibacter	Staphylococcus		Pichia
Pseudomonas	Streptococcus		Rhodotorula
Thermoanaerobium	Streptomyces		Saccharomyces
Xanthomonas			Saccharomycopsis
Zymomonas			Schizosaccharomyces
			Torulopsis
			Trichosporon
			Yarrowia
			Zygosaccharomyces

[a] Danish microbiologist Hans Christian Gram (1853–1928) developed a staining technique used to classify bacteria. A basic dye (crystal violet or gentian violet) is taken up by both Gram-positive and Gram-negative bacteria. However the dye can be washed out of Gram-negative organisms by alcohol, such organisms being counterstained by safranin or fuchsin. The latter stain is taken up by both Gram-positive and Gram-negative organisms, but does not change the colour of Gram-positive organisms, which retain their violet hue.

1.2 Microbial Metabolism

In order to grow, any living organism needs a supply of nutrients that will feature as, or go on to form, the building blocks from which that organism is made. These nutrients must also provide the energy that will be needed by the organism to perform the functions of accumulating and assimilating those nutrients, to facilitate moving around, etc.

The microbial kingdom comprises a huge diversity of organisms that are quite different in their nutritional demands. Some organisms (*phototrophs*) can grow using light as a source of energy and carbon dioxide as a source of the carbon, the latter being the key element in organic systems. Others can get their energy solely from the oxidation of inorganic materials (*lithotrophs*).

All of the organisms considered in this book are *chemotrophs*, insofar as their energy is obtained by the oxidation of chemical species. Furthermore, unlike the *autotrophs*, which can obtain all (or nearly all) their carbon from carbon dioxide, the organisms that are at the heart of fermentation processes for making foodstuffs are *organotrophs* (or *heterotrophs*) in that they oxidise organic molecules, of which the most common class is the sugars.

1.2.1 Nutritional Needs

The four elements required by organisms in the largest quantity are carbon, hydrogen, oxygen and nitrogen. This is because these are the elemental constituents of the key cellular components of carbohydrates (Figure 1.3), lipids (Figure 1.4), proteins (Figure 1.5) and nucleic acids (Figure 1.6). Phosphorus and sulphur are also important in this regard. Calcium, magnesium, potassium, sodium and iron are demanded at the milligram level, whilst microgram amounts of copper, cobalt, silicon, manganese, molybdenum, selenium and nickel are needed. Finally, organisms need a pre-formed supply of any material that is essential to their well-being, but that they cannot themselves synthesise, namely the vitamins (Table 1.2). Micro-organisms differ greatly in their ability to make these complex molecules. In all instances the vitamins form a part of coenzymes and prosthetic groups that are involved in the functioning of the enzymes catalysing the metabolism of the organism.

As the skeleton of all the major cellular molecules (other than water) comprises carbon atoms, there is a major demand for carbon.

Hydrogen and oxygen originate from substrates such as sugars, but of course also come from water.

The oxygen molecule, O_2, is essential for organisms growing by aerobic respiration. Although fermentation is a term that has been most widely applied to an anaerobic process in which organisms do not use molecular oxygen in respiration, even those organisms that perform metabolism in this way generally do require a source of this element. To illustrate, a little oxygen is introduced into a brewer's fermentation so that the yeast can use it in reactions that are involved in the synthesis of the unsaturated fatty acids and sterols that are essential for it to have healthy membranes. Aerobic metabolism, too, is necessary for the production of some of the foodstuffs mentioned in this book, for example in the production of vinegar.

All growth media for micro-organisms must incorporate a source of nitrogen, typically at $1–2\,g\,l^{-1}$. Most cells are about 15% protein by weight, and nitrogen is a fundamental component of protein (and nucleic acids).

As well as being physically present in the growth medium, it is equally essential that the nutrient should be able to enter into the cell. This transport is frequently the rate-limiting step. Few nutrients enter the cell by passive diffusion and those that do tend to be lipid-soluble. Passive diffusion is not an efficient strategy for a cell to employ as it is very concentration dependent. The rate and extent of transfer depend on the relative concentrations of the substance inside and outside the cell. For this reason, facilitated transportation is the major mechanism for transporting materials (especially the water-soluble ones) into the cell, with proteins known as permeases selectively and specifically catalysing the movement. These permeases are only synthesised as and when the cell requires them. In some instances energy is expended in driving a substance into the cell if a thermodynamic hurdle has to be overcome – e.g. a higher concentration of the molecule inside than outside. This is known as 'active transport'.

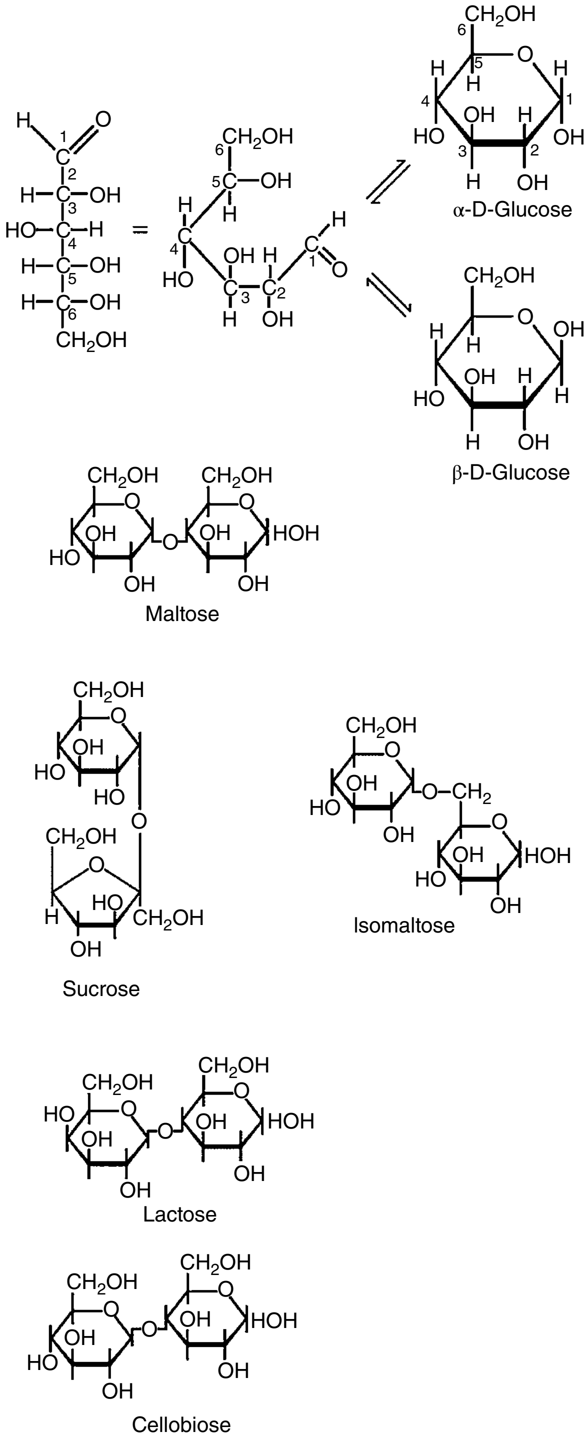

Figure 1.3 *(Continued)*

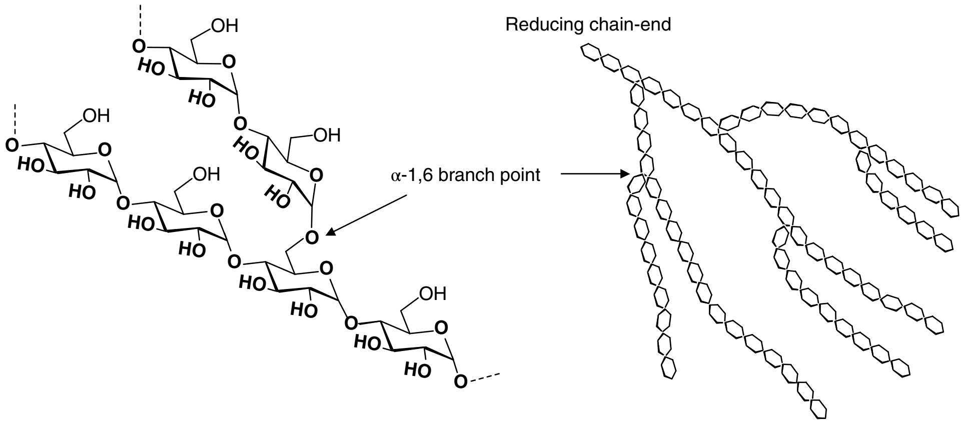

Figure 1.3 (Cont'd) carbohydrates. (a) Hexoses (sugars with six carbons) such as glucose exist in linear and cyclic forms in equilibria (top). the numbering of the carbon atoms is indicated. In the cyclic form if the OH at C_1 is lowermost the configuration is α. If the OH is uppermost then the configuration is β. At C_1 in the linear form is an aldehyde grouping, which is a reducing group. adjacent monomeric sugars (monosaccharides, in this case glucose) can link (condense) by the elimination of water to form disaccharides. Thus maltose comprises two glucose moieties linked between C_1 and C_4, with the OH contributed by the C_1 of the first glucosyl residue being in the α configuration. Thus the bond is $\alpha 1 \rightarrow 4$. For isomaltose the link is $\alpha 1 \rightarrow 6$. For cellobiose the link is $\beta 1 \rightarrow 4$. Sucrose is a disaccharide in which glucose is linked $\beta 1 \rightarrow 4$ to a different hexose sugar, fructose. similarly lactose is a disaccharide in which galactose (note the different conformation at its C_4) is linked $\beta 1 \rightarrow 4$ to glucose. (b) Successive condensation of sugar units yields oligosaccharides. This is a depiction of part of the amylopectin fraction of starch, which includes chains of $\alpha 1 \rightarrow 4$ glucosyl units linked by $\alpha 1 \rightarrow 6$ bonds. The second illustration depicts the amylopectin fraction of starch. Note that there is only one reducing chain-end, all the others being bound up in glycosidic linkages.

An additional challenge is encountered with high molecular weight nutrients. Whereas some organisms, e.g. the protozoa, can assimilate these materials by engulfing them (*phagocytosis*), micro-organisms secrete extracellular enzymes to hydrolyse the macromolecule outside the organism, with the resultant lower molecular weight products then being assimilated. These extracellular enzymes are nowadays produced commercially in fermentation processes that involve subsequent recovery of the spent growth medium containing the enzyme and various degrees of ensuing purification. A list of such enzymes and their current applications is given in Table 1.3.

1.2.2 Environmental Impacts

A range of physical, chemical and physicochemical parameters impact the growth of micro-organisms, of which we shall consider temperature, pH, water activity, oxygen, radiation, pressure and 'static' agents.

1.2.2.1 Temperature

The rate of a chemical reaction was shown by Svante Arrhenius (1859–1927) to increase twofold to threefold for every 10 °C rise in temperature. However cellular macromolecules, especially the enzymes, are prone to denaturation by heat, and this accordingly limits the temperatures that can be tolerated. Although there are organisms that can