Option F

18 Microorganisms and biotechnology

STARTING POINTS

- **Biodiversity** is a term for the vast numbers of living things that exist. **Classification** involves naming organisms (genus and species), and then grouping them into a hierarchical classification.
- **Prokaryotes** include the bacteria and cyanobacteria – all organisms with very small cells and **without a true nucleus**. Prokaryotes are largely unicellular.
- **Eukaryotes** – plants, animals and fungi – have cells containing many organelles, and a nucleus **contained by a nuclear membrane**. Some eukaryotes are unicellular, but many are multicellular organisms.
- There are two forms of **nucleic acid**, **DNA** and **RNA**. They differ in the 5-carbon sugar and the bases they contain, and in their roles in cells. Also, RNA is single-stranded, DNA is double-stranded.
- **Biotechnology** is the industrial application of biological processes. It has ancient origins, but today biotechnology is often linked with **genetic engineering**, particularly of microorganisms.
- This chapter extends study of aspects of cell structure begun in Chapter 1 (pages 1–36), of environmental biology begun in Chapter 6 (pages 137–77), and of genetic engineering and biotechnology begun in Chapter 5 (pages 117–36).

At one time, the living world seemed to divide naturally into two kingdoms, the **plants** and **animals**. Then, with the use of the electron microscope in biology came the discovery of the two fundamentally different types of cellular organisation, namely **prokaryotic** and **eukaryotic**. As a result, the divisions of living things were reorganised, eventually into **five kingdoms**, namely the prokaryotes (bacteria and cyanobacteria), and the four eukaryotic kingdoms of the protoctistans, fungi, plants, and animals (Figure 6.43, page 176).

More recently, studies of life under extreme environmental conditions, including extremes of temperature or salinity, and at great ocean depth, have led to discovery of a huge and growing range of previously unknown microorganisms. A systematic hunt for **extremophiles**, life forms that live in extreme places, is under way.

The biochemistry of the cells of extremophiles has proved distinctly different from existing known life forms, too. As a consequence, the study of microorganisms has fresh consequences and **applications in many fields of biology**, including ecological and environmental biology, biotechnology, and health.

In this chapter, the **diversity of microorganisms** is explored, and their **roles in the environment** discussed. Then the applications of microbiology in **biotechnology and food production** are examined.

In the Additional Higher Level extension, the **metabolism of microorganisms** is reviewed further, and finally, issues of **microbes and disease** are explored.

The diversity of microorganisms

F1.1–1.9

Vast numbers of microorganisms occur in the biosphere immediately around us, and many of them exist in environmental conditions similar to those of plants and animals. However, many more microorganisms flourish in the most unlikely places, where they survive and prosper despite the extremely challenging environmental conditions. All of these latter microorganisms are collectively referred to as the extremophiles. The numbers and different types of known extremophiles are increasing as the importance of these strange habitats becomes better understood. Extremophiles include microorganisms that:

- are salt-loving (**halophiles**), found in salt lakes and where sea water has become concentrated and salt has crystallised;

- require extremely alkaline conditions above pH 10 (**alkalinophiles**), found in soda lakes;
- survive abnormally high temperatures (**thermophiles**);
- withstand 250 atmospheres pressure (**barophiles**), as well as extremely high temperatures;
- survive extremely cold habitats (**psychrophiles**).

The microorganisms of extreme habitats have cells that we can identify as prokaryotic (page 16). That is, there is no true nucleus present, nor is the cytoplasm packed with the range of organelles so typical of eukaryotic cells.

However, the **biochemistry of the cells of extremophiles** has proved distinctly different from existing known life forms. In particular, it is the larger **RNA molecules present in the ribosomes** that have proved tell-tale molecules.

How are the RNA molecules of extremophiles analysed?

First, ribosomes are isolated from the cytoplasm of cells, and then the RNA that makes up these tiny organelles (remember, ribosomes are sites of protein synthesis) is extracted. On analysis, the sequence of nucleotides present (i.e. the sequence of the bases cytosine, guanine, adenine and uracil in this RNA) is determined.

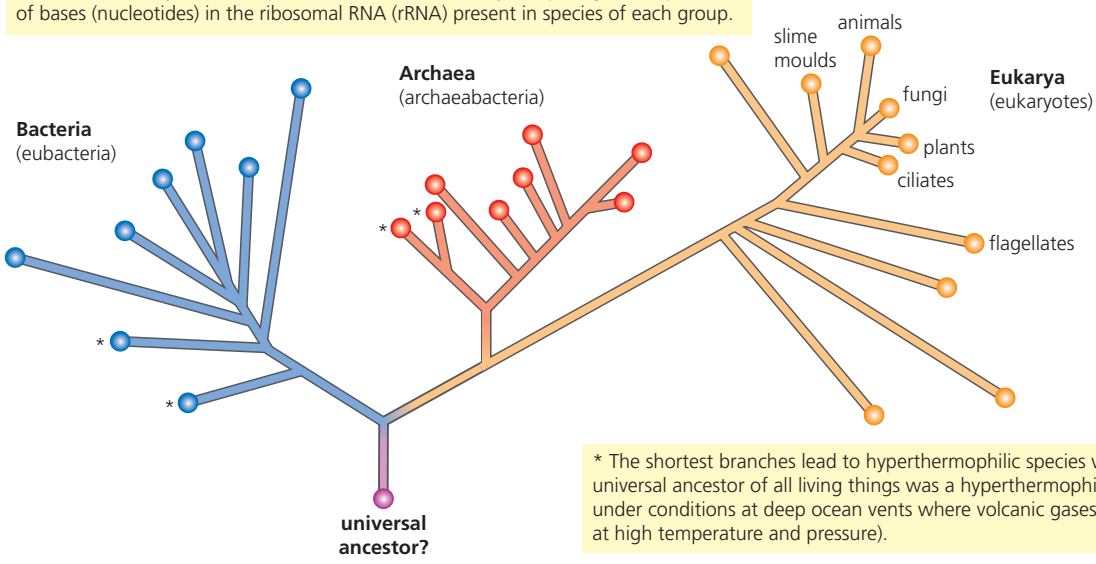
Comparisons of the base sequences in RNA molecules of the ribosomes in these cells with those of previously known bacteria and eukaryotes have led to the discovery of **new evolutionary relationships**.

The outcome has been the development of a new scheme of classification of living things. We now recognise **three major forms of life**, and these are called **domains** (Figure 18.1). The organisms of each domain share a distinctive, unique pattern of ribosomal RNA which establishes their close evolutionary relationship. These domains are:

- the **Archaea** or **Archaeabacteria** (the extremophile prokaryotes);
- the **Bacteria** or **Eubacteria** (the true bacteria – that is, the other prokaryotes);
- the **Eukarya** (all eukaryotic cells – the protoctista, fungi, plants and animals).

Figure 18.1 The classification of living organisms into domains

These evolutionary relationships have been established by comparing the sequence of bases (nucleotides) in the ribosomal RNA (rRNA) present in species of each group.



* The shortest branches lead to hyperthermophilic species which suggests that the universal ancestor of all living things was a hyperthermophile (possibly 'assembled' under conditions at deep ocean vents where volcanic gases are discharged into water at high temperature and pressure).

1 Distinguish the different forms of RNA present in cells by means of their specific roles in protein synthesis.

The characteristics of the three domains

The Archaeabacteria are organisms with which we are least familiar, so we concentrate on their unique features first. Then we can compare their structure with that of cells of organisms of the other two domains (Table 18.1).

The **Archaeabacteria** are a diverse group in both structure and physiology. Some are single cells, others are filamentous or form other types of cellular aggregate. We have noted that many are found in extreme conditions (the extremophiles), some in terrestrial habitats, others in water. For example, Archaea represent about a third of all prokaryotic biomass in polar coastal waters. However, these microorganisms occur very widely indeed in the natural world. A few live symbiotically in the gut of animal species – as do vast numbers of true bacteria, too. They probably occur everywhere.

As prokaryotes, the Archaeabacteria have **cell walls**, but the chemistry of their walls is different from the chemistry of the walls of the true bacteria. Often a variety of complex polysaccharides are present, but none contains the peptidoglycan or particular amino acids characteristic of the Eubacteria. Some have a substantial layer of protein or glycoprotein external to the polysaccharides, and some have walls of protein only.

Another distinguishing feature is the **lipids** of the cell membranes found in the Archaeabacteria. Glycolipids are present, but these have branched chain **hydrocarbons** attached to glycerol (in place of the **fatty acids** of the lipids of the Eubacteria and eukaryotes). Polar lipids are also present, and they all combine in different ways to form membranes of variable thickness and rigidity.

The Archaeabacteria, like the Eubacteria, have a **single circular chromosome** that is without **introns** (non-coding sequences of nucleotides, page 245). However, they generally possess far fewer genes than the true bacteria. Their nucleic acid is extremely variable in the proportions of the two bases guanine (G) and cytosine (C) that it contains. In species where the nucleic acid has been sequenced, the genes are unlike those found in true bacteria and eukaryotes. Also, there are relatively few **plasmids** present.

The **ribosomes** of Archaea are small (70S) like those of the Eubacteria, but they are more variable in shape and chemistry. Understanding of the evolutionary relationships of members of the three domains is based on the biochemical analysis of the ribosomes. Finally, the **physiology**, **metabolism** and **life styles** of Archaea are very variable, as described shortly. For the purposes of comparison, the structure of a typical bacterium (*Escherichia coli*) is shown in Figure 1.15 (page 16), and of a human liver cell – typical of a eukaryotic cell – in Figure 1.13 (page 15).

Look those up again now, to help you make sense of Table 18.1.

	Archaeabacterial cell (Figure 18.2)	Eubacterial cell such as *E. coli* (Figure 1.15)	Eukaryotic cell such as liver cell (Figure 1.13)
Ribosomes: size and chemical analysis	■ 70S size ■ unique biochemistry	■ 70S size ■ unique biochemistry	■ 70S size ■ unique biochemistry
Histones with nucleic acid of chromosome(s)	■ absent	■ absent	■ present, forming nucleosomes
Introns within chromosome(s)	■ absent	■ absent	■ present between exons
Plasmids in cytoplasm	■ few present	■ many present	■ typically absent
Cell walls: present or absent, and chemistry	■ present ■ chemically different from those of Eubacteria	■ present ■ chemically different from those of Archaea	■ present in green plant cells (cellulose) and in fungi (chitin) ■ absent in animal cells
Cell membranes: composition of lipids	■ glycolipids with hydrocarbon chains, in plasma membrane	■ lipids with fatty acids, in plasma membrane	■ lipids with fatty acids, in all membranes present

Table 18.1 The cellular characteristics of the three domains

Diversity within the Archaeabacteria

The wide diversity in the habitats of the Archaeabacteria may be illustrated by contrasting those of the methanogens, thermophiles and halophiles.

Methanogens occur in microhabitats where oxygen is absent – we say they are obligate anaerobes (Figure 18.2). In fact they require anaerobic environments rich in organic matter.

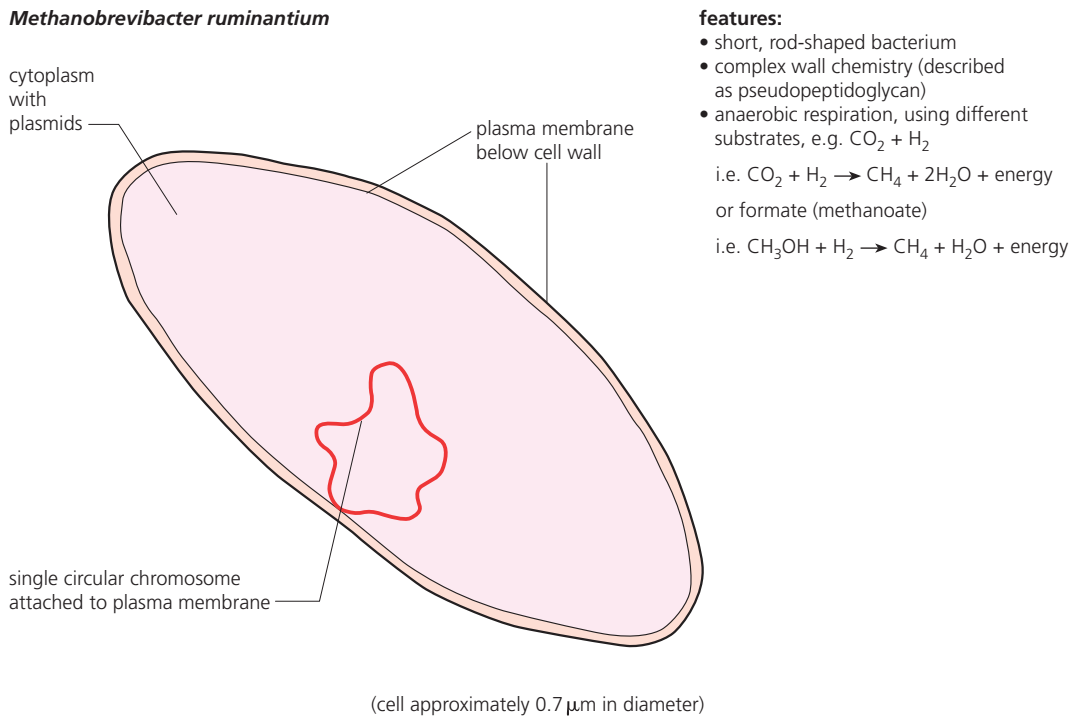


Figure 18.2 Typical methanogenic bacterium of the Archaea (drawing based on an interpretation of a TEM)

Here they produce methane as a waste product of the metabolism of a variety of respiratory substrates, including combinations of methanoate (formate), methanol, ethanoate (acetate), carbon dioxide and hydrogen.

These Archaeabacteria are found in the rumen and intestinal system of animals (typically, in ruminants such as cows, sheep and deer), in marine and fresh-water sediments rich in organic matter, and in marshes. They also occur in hot springs and in the anaerobic sludge digesters of sewage works (Figure 18.16, page 567).

A kilogram of organic matter may yield up to 600 litres of methane, so methanogens have potential in bio-fuel production in the future, when fossil fuels are scarce and more expensive. However, methane is also a powerful greenhouse gas – methanogens indirectly contribute to global warming (page 149).

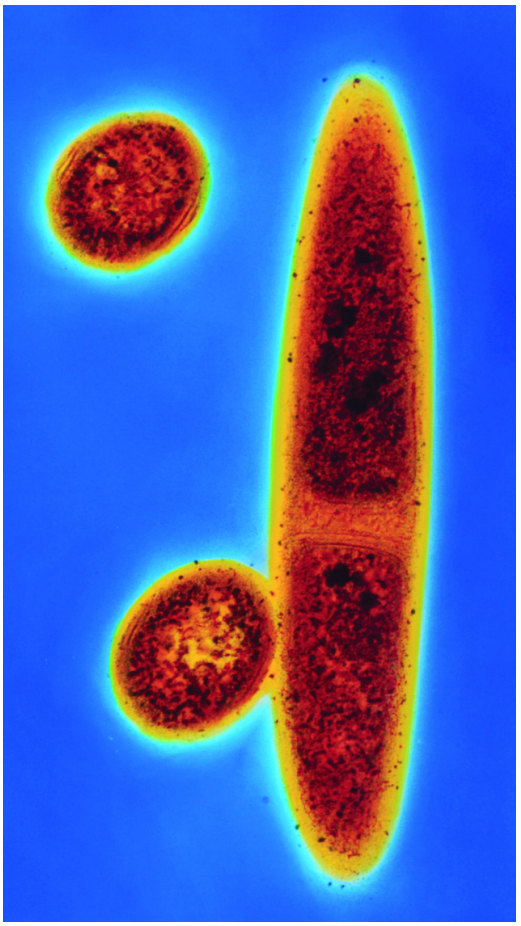
Thermophiles grow at extremely high temperatures. Prokaryotes with this property were first discovered in hot volcanic springs such as occur in Yellowstone Park in the USA. The temperature they flourished at, 70 °C, seemed exceptional at the time.

Now we know that the deep ocean-floor volcanic vents that continuously discharge hot, sulphurous volcanic gases are home to **hyperthermophiles** that require a minimum of 70 °C, and typically thrive at over 100 °C, albeit at very high pressures, so the water is not boiling (Figure 18.3).

These habitats have been suggested as sites where life itself may have originally evolved, as conditions there may have favoured the spontaneous formation of complex organic molecules, and then assisted these molecules (including polymers) to become assembled into the simplest living cells (page 457).

Halophiles grow in salt lakes and anywhere that sea water has evaporated leaving a highly concentrated solution of salts or even salt crystals. One such organism, *Halobacterium halobium*, is found among the salt crystals in salt mines.

Closely related to halophiles are members of the Archaeabacteria that require not only a high concentration of salt, but also extremely alkaline conditions (pH 10 or higher). For example, in soda lakes, *Natronococcus* occurs naturally and thrives. A high concentration of salt is maintained in the cytoplasm of this halophile, preventing dehydration of the cell.



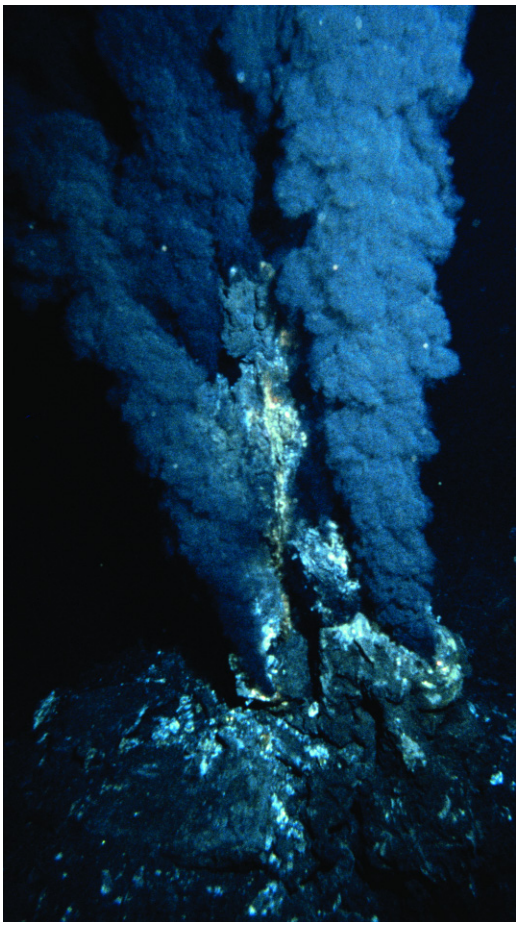


Figure 18.3 A hyperthermophile Archaeabacterium and its deep sea habitat

Left: TEM of *Methanopyrus* (false colour), size 0.5 × 2–14 μm.

Right: A deep ocean volcanic vent (upper temperature about 110 °C) from which volcanic gases bubble out, rich in sulphur and heated by geothermal energy. Extremophile Archaea found here use the sulphur compounds as a source of energy.

Diversity within the Eubacteria (true bacteria)

The structure of a bacterium, *Escherichia coli*, a very common gut commensal, is illustrated in Figure 1.15. Note that the outline shape of *E. coli* is a rod.

The very many thousands of different bacteria that have been isolated, identified and named by bacteriologists (genus and species), have been organised into the traditional hierarchical classification system used in biology:

Phylum ← Class ← Order ← Family

Alternative, commonly used ways of dividing bacteria include simple groupings based on the shape of their cells and the chemical structure of their cell walls.

Shapes of bacterial cells

Bacteria are classified into five groups on the basis of their shape; basically bacteria are rods, spheres, comma-shaped, spirals, or corkscrew-shaped. In fact, the majority are rods or spheres. The cells maintain these shapes because bacterial cells have rigid walls.

Although basically unicellular, bacteria may often appear clumped together and species showing this form of growth are named accordingly. For example, bacteria growing in pairs have the prefix diplo- attached to the generic name. Where the cells occur as a chain, the prefix strepto- is used, and where they occur in grape-like clusters the generic prefix is staphylo- (Figure 18.4).

Wall structure in bacteria

We have seen that the wall of a bacterium gives a permanent shape to the cell because of its rigidity. It also protects the cell contents against rupture due to osmosis, for example, and it helps to protect against harm by other organisms.

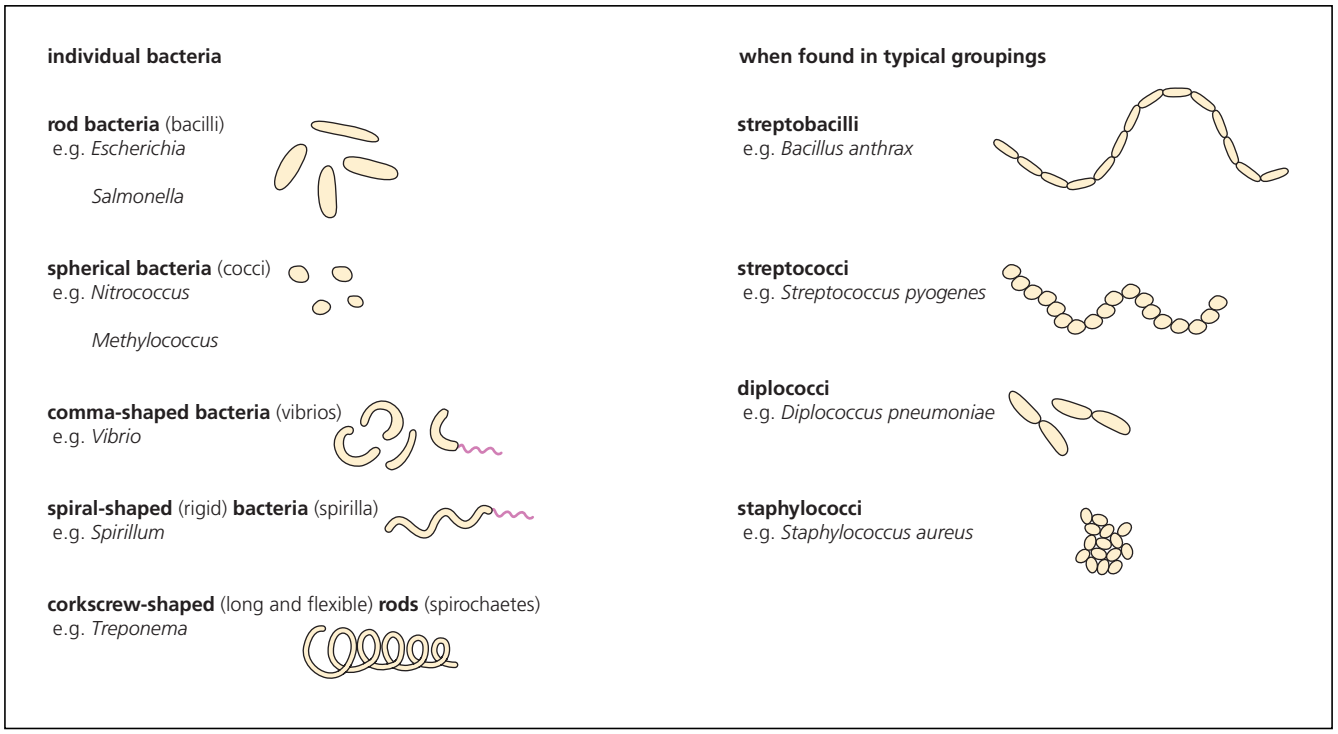


Figure 18.4 Classification of bacteria by shape

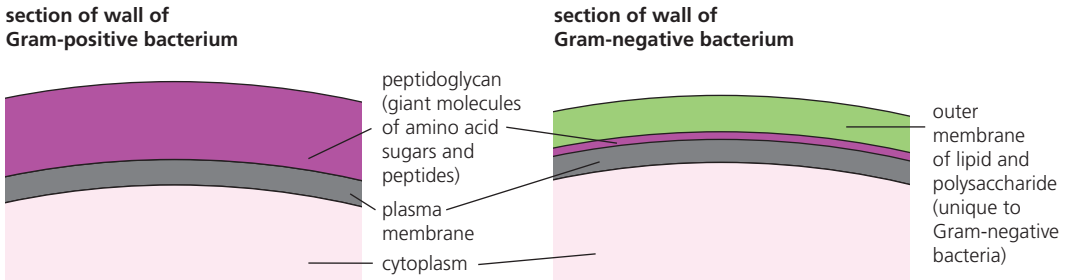
Bacterial cell walls contain giant molecules (polymers) built of amino-sugars and peptide monomers. The resulting polymer is known as **peptidoglycan**, and is a totally different substance from cellulose of plant cell walls. Bacteria have walls of this substance, but some have additional layers on the outer surface of their wall, and these additional layers change the staining property of the wall.

It was a Dane, Hans Gram (1884), who first showed that some bacteria can be stained purple with a particular dye called crystal violet. Such bacteria are now known as **Gram-positive species** (Figure 18.5). They have a wall made largely of peptidoglycan.

Other bacteria cannot retain crystal violet in their walls, and they are known as the **Gram-negative species** (Figure 18.5). They have a wall that contains peptidoglycan, but external to that layer is a shield layer largely made of lipid.

Thus, the Gram-staining effect is due to an important difference in cell-wall chemistry. It has proved to be a valuable tool for recognising bacteria and for separating them into two groups. Incidentally, the Gram-positive bacteria tend to be destroyed by penicillin and certain other drugs, whereas Gram-negative species have a wall that resists these drugs.

Figure 18.5 Gram-positive and Gram-negative bacteria walls



Some bacteria form aggregates with distinctive characteristics

Bioluminescence

The bacterium *Vibrio fischeria*, a Gram-negative, rod-shaped bacterium, is able to emit light – a phenomenon known as **bioluminescence**. The bacterium occurs in marine habitats, and large numbers are found in a mucus matrix in the light organ of a squid species and also of the

Atlantic flashlight fish. Light is generated by the action of an enzyme, luciferase, acting upon a reduced respiratory coenzyme. In effect, light is generated by deflecting electrons from the electron-transport chain and ATP synthesis (page 274). When the bacterium is in high-density populations, the organisms interact to trigger light emission.

Biofilms

When certain bacteria that are pathogens reach populations of sufficient density, they interact to produce toxins. One such is the rod-shaped bacterium *Pseudomonas aeruginosa*, which when present in large numbers in the respiratory tract of patients with cystic fibrosis (page 576), produces toxins that may overwhelm and kill the host.

Diversity within the microscopic eukaryotes

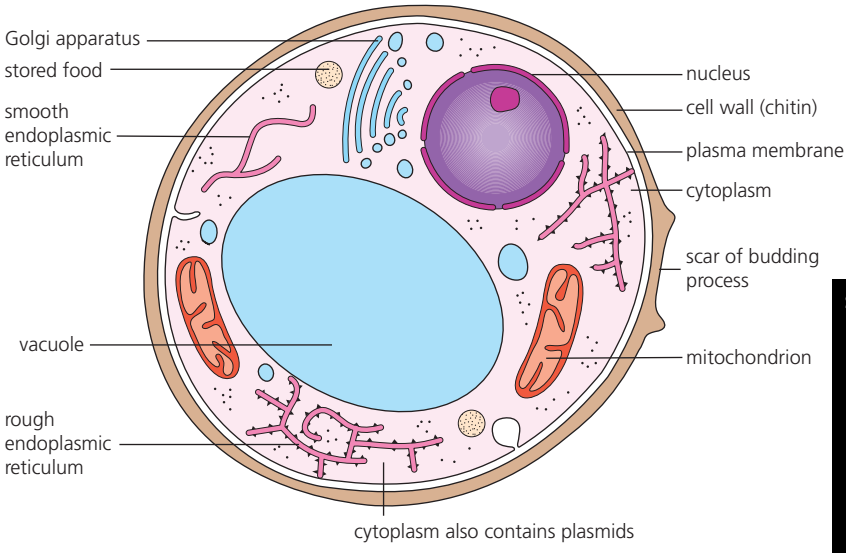
We can now consider the diversity found among microscopic eukaryotes, concentrating particularly on their nutrition, locomotion and wall structure. The eukaryotic cell is structurally distinct from that of prokaryotes; for one, eukaryotic cells are mostly very much larger, and contain an array of organelles not found in prokaryote cells.

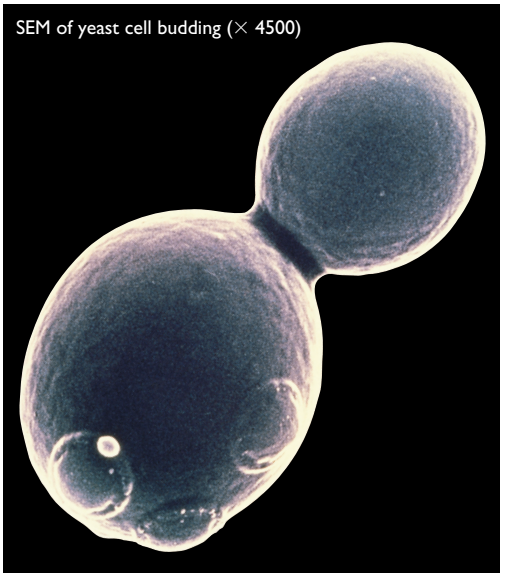
While eukaryotic microorganisms share this characteristic, they also show great diversity. This is illustrated by the six eukaryotic unicells described below. The differences are then summarised in Table 18.2.

Saccharomyces (yeast)

The name *Saccharomyces* means 'sugar fungi'. Yeasts are saprotrophic, unicellular fungi that occur everywhere sugar solution is available – in flowers, on the surface of fruits, on leaves and stems where sap is exuded. They are also found in the soil and on animal mucous membranes. Yeasts are unicellular (Figure 18.6), but under favourable growing conditions, the cells reproduce asexually and divide (called budding) so quickly they may temporarily form long branching chains of connected cells. The cell wall is composed of fungal cellulose (chitin).

Figure 18.6 The structure of *Saccharomyces*





Yeast cells are non-motile, but are readily dispersed by larger organisms visiting sites where yeasts grow. Yeast spores are carried around in air currents, too.

The yeasts respire by alcoholic fermentation and produce carbon dioxide and ethanol as waste products. The ethanol cannot be metabolised further by yeast, and it may accumulate in the growth medium. Yeast is of great economic importance because:

- waste products of fermentation are exploited in brewing, wine making, and in the baking of bread dough;
- they are easily cultured in liquid media in fermenters, and so can be useful in modern biotechnology industries, too;
- like many bacteria, they contain plasmids in the cytoplasm (a very unusual feature in eukaryote cells), and so yeast cells may be genetically modified to carry and express additional genes.

Amoeba (a protozoan)

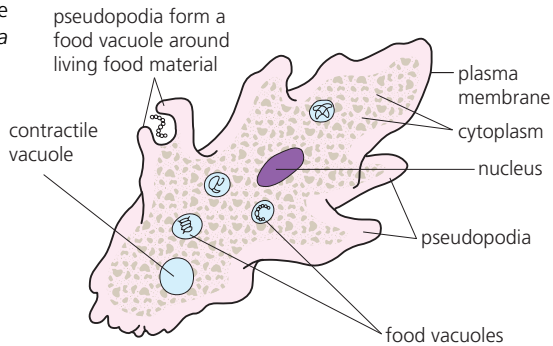


Figure 18.7 The structure of *Amoeba*

Amoeba is a protozoan, just visible to the naked eye, which occurs on the bottom of shallow lakes and ponds. Observed by light microscopy, the cell is colourless and transparent, and has an irregular and constantly changing outline (Figure 18.7). Movement occurs by flowing movements of the cytoplasm, leading to the building of almost tubular pseudopodia and the dissembling of others; this is appropriately known as amoeboid movement. No cell wall is present outside the flexible plasma membrane.

Nutrition is holozoic; *Amoeba* feeds on smaller protozoa and algae. Prey is surrounded by pseudopodia in the shape of a cup, enclosed in a food vacuole, and then digested. You are already familiar with this process from study of the activities of phagocytic white cells – part of the human body's defence against disease (page 196).

Plasmodium (malaria parasite)

Plasmodium is a parasitic protozoan that occurs in human hosts, but is unwittingly transported to new hosts by the female mosquito (*Anopheles*) when a blood meal is taken from humans. *Plasmodium* lives in liver cells at first, and then moves into red blood cells (Figure 18.8).

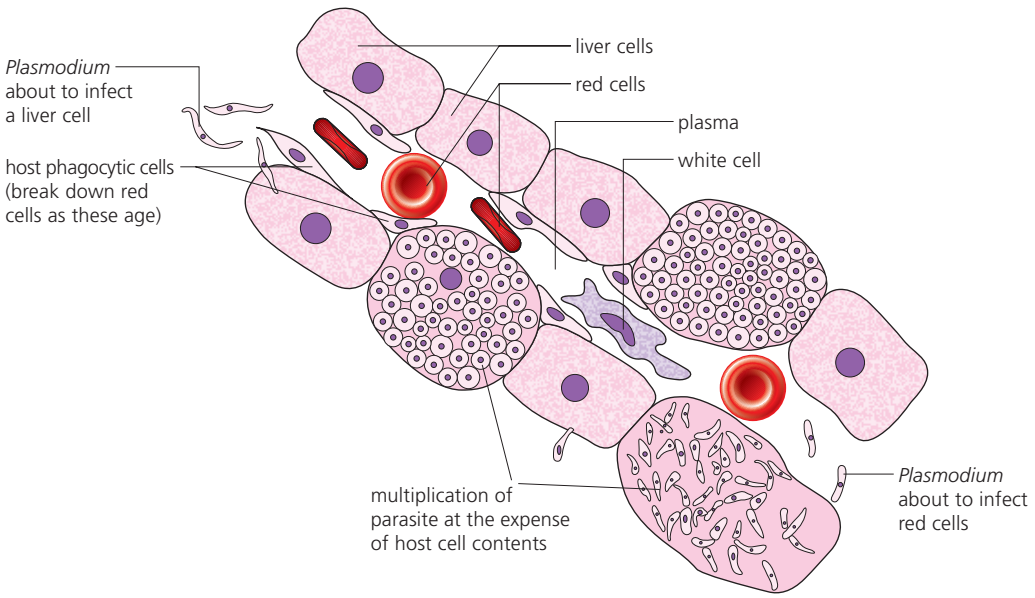


Figure 18.8 *Plasmodium*, in and out of host cells

In both tissues it feeds at the expense of the cell cytoplasm, and, at the same time, hides from the host's immune system. As the parasite escapes from host cells, toxins are released into the blood stream, and these trigger the symptoms of malarial fever, so harmful to the host.

Paramecium (slipper animalcule, a ciliate)

Paramecium is a protozoan found in ponds rich in decaying organic matter and numerous bacteria. Nutrition is largely holozoic, with bacteria the main component of the diet. This protozoan has a definite, unchanging shape because the plasma membrane is a slightly rigid pellicle through which rows of cilia project (Figure 18.9). *Paramecium* swims through the water, rounded end first, by the beating action of the cilia. Food vacuoles form at the top of a permanent gullet (cytopharynx) around acceptable microbes that have been swept up by ciliary action.

Figure 18.9 *Paramecium*

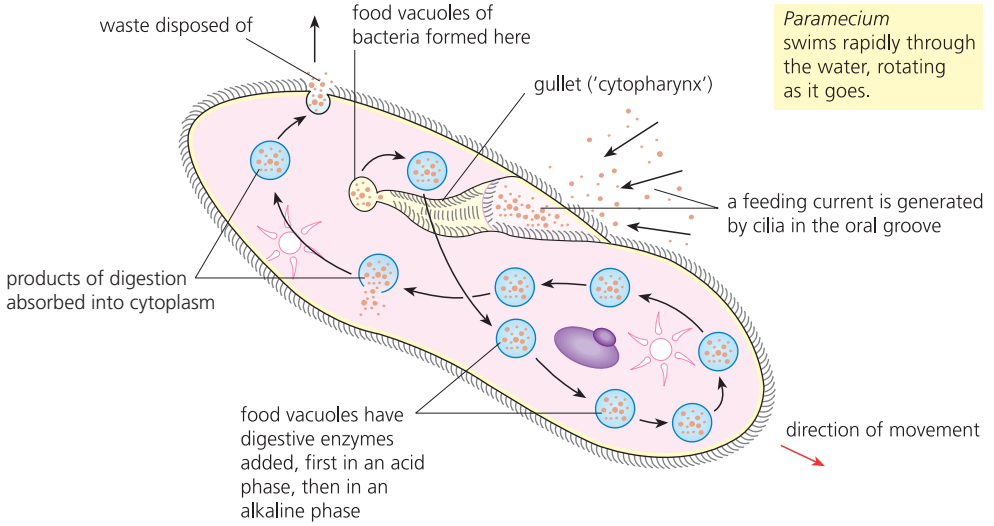
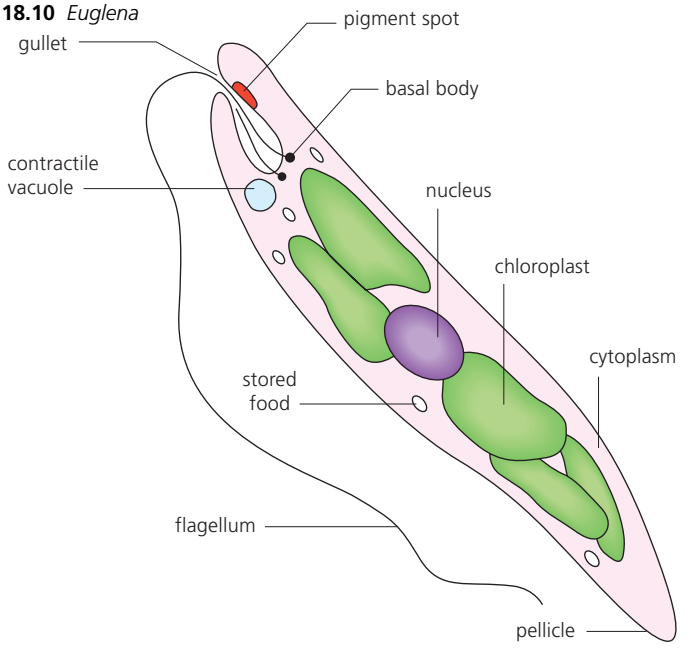


Figure 18.10 *Euglena*



Euglena (a flagellate)

Euglena is described as a plant-like protozoan. It is common in stagnant water of ponds and ditches, typically at densities that colour the water green. This tiny, spindle-shaped cell is not surrounded by a cellulose cell wall; rather the cytoplasm is bounded by a firm but flexible plasma membrane (pellicle) (Figure 18.10). In swimming movements, the long flagellum that emerges from the terminal gullet and trails beside the cell, propels the organism forwards. Movement may also occur by flowing movements of the cytoplasm. Nutrition is largely by photosynthesis and by absorption of essential ions from water for biosynthesis of essential metabolites using sugar. However, in some circumstances, *Euglena* can exist saprotrophically.

Chlorella (a green alga)

Chlorella is a unicellular alga of fresh-water ponds, present at such a density as to colour the water green. *Chlorella* is a popular organism for research because it is easily cultured in laboratories for the study of green plant metabolism. It is an immobile unicell contained within a cellulose cell

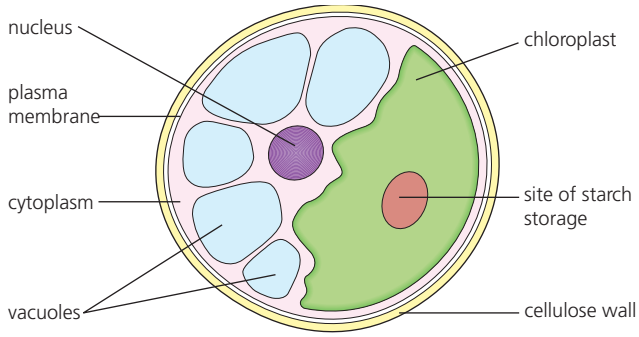


Figure 18.11 *Chlorella*

wall, and is passively transported by water currents (Figure 18.11). It has photosynthetic metabolism, and manufactures all metabolites required from the sugar formed, and from ions absorbed from the medium it lives in. Metabolically, this unicellular alga is indistinguishable from a green plant cell – hence its value as a research organism, since the supply of its nutrients and the environmental conditions it experiences are easily controlled.

Table 18.2 Diversity in microscopic eukaryotes

	Cell structure	Nutrition	Motility
Saccharomyces	■ cell wall – chitin (polymer of glucose derivative containing N)	■ saprotrophic – feeds on nectar, via fermentation	■ non-motile – chance transport by pollinators, or as spores via air currents
Amoeba	■ cell wall – absent	■ holozoic – feeds after trapping food in food vacuoles	■ motile – moves by cytoplasmic streaming
Plasmodium	■ cell wall – absent	■ parasitic – feeds on contents of liver cells and red cells	■ motile – burrows its way into host cells
Paramecium	■ cell wall – absent ■ plasma membrane – strengthened by additional protein (a pellicle)	■ holozoic – feeds after trapping food in food vacuoles	■ motile – driven by the many cilia that cover the pellicle
Euglena	■ cell wall – absent ■ plasma membrane – strengthened by additional protein (a pellicle)	■ largely photosynthetic with chloroplasts	■ motile – driven by long flagellum
Chlorella	■ cell wall – present, largely cellulose	■ photosynthetic with chloroplast	■ non-motile

2 **Compare** cell structure in eukaryotes and prokaryotes.

Introducing the viruses

Viruses are disease-causing agents, rather than microorganisms. The distinctive features of viruses are:

- they are not cellular structures, but consist of a core of nucleic acid surrounded by a protein coat called a **capsid** (Figure 18.12);
- in some viruses there is an additional **external envelope** of membrane made of lipids and proteins (e.g. in the influenza and herpes viruses, Figure 18.13);
- they are **extremely small** when compared with bacteria (most viruses are in a size range of 20–400 nm (0.02–0.4 μm); they become visible only by means of the electron microscope;
- they can reproduce only inside specific living cells, so they function as **endoparasites** in their **host** organism;
- they have to be **transported** in some way between hosts;
- viruses are **highly specific to particular host species** – some to plant species, some to animal species and some to bacteria;
- viruses are classified by the type of nucleic acid they contain, either **DNA** or **RNA**, and whether they have a **single or double strand of nucleic acid**.

Virus structures compared

A **single-strand RNA virus** is illustrated by the influenza (flu) virus (Figure 18.13). Here, eight short single strands of RNA (a segmented genome), ranging from about 800 to 2400 nucleotides long, are seen. The protein coat (the capsid) around the nucleic acid is itself surrounded by an external layer of lipid. This combined coat is flexible, so these viruses are of no fixed shape. However, a constant feature of this viral capsid are the protein 'knobs' (known as H and N), projecting through the lipid layer. These function as antigens in the human host.

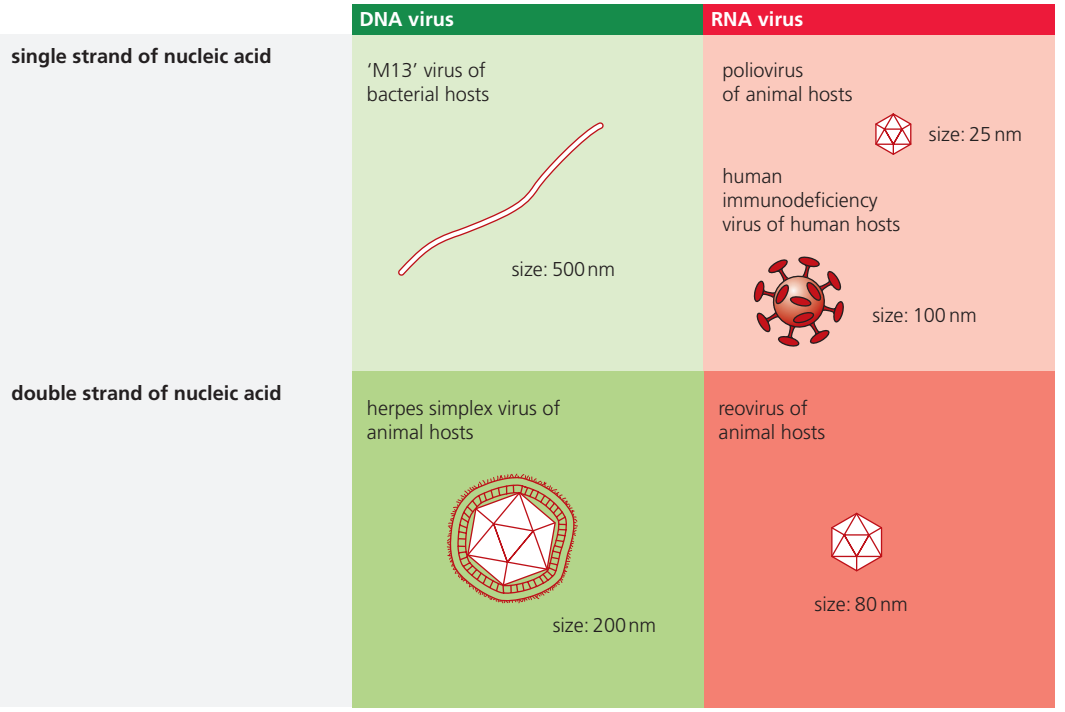


Figure 18.12 A classification of viruses

Extension: So are viruses living at any stage?

Viruses are an assembly of complex molecules, rather than a form of life. Isolated from their host cell they are inactive, and best described as crystalline. Within susceptible host cells they are highly active genetic programmes that typically take over the biochemical machinery of host cells for replication. Their component chemicals are synthesised, and then assembled to form new viruses. On breakdown (lysis) of the host cell, viruses are released, and may cause fresh infections. So, viruses are **not living organisms**, but may become **active components of host cells**.

A **double-strand DNA virus** is illustrated by the herpes virus (Figure 18.13). The protein coat (capsid) is a regular shape with twenty faces (icosahedral), surrounded by an envelope of protein and lipid, and derived (as in the case of the flu virus envelope) from the previous host cell's membrane. The envelope is acquired as the virus particle departs the host cell.

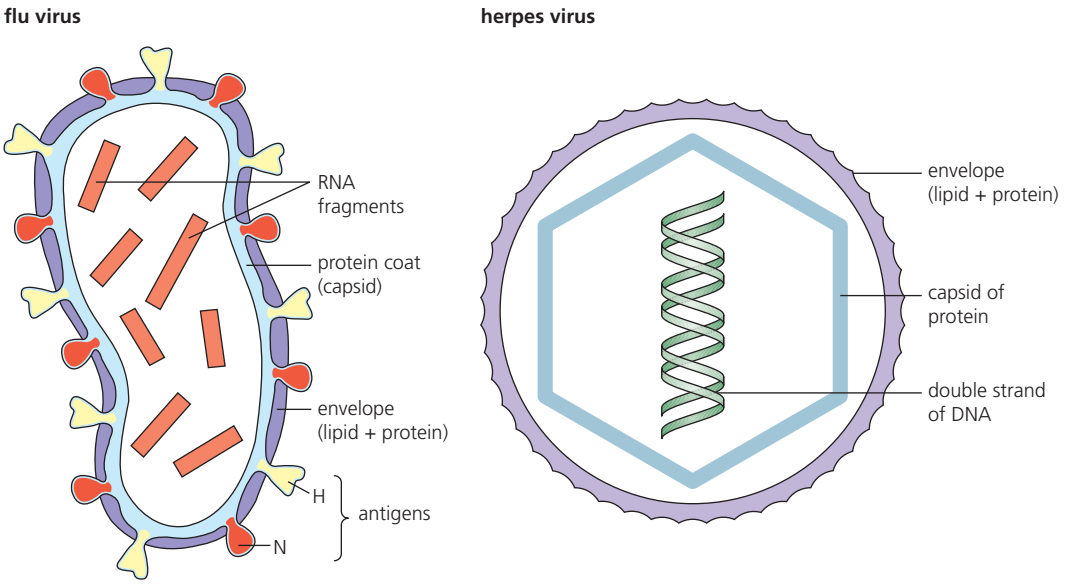


Figure 18.13 Virus structure compared

3 **Explain** why, for the replication of a virus, it is not necessarily essential for the protein capsid of the virus to enter the host cell.

Microorganisms and the environment

F2.1–2.8

Microorganisms typically have significant impacts on their environment, partly because of their numbers but also due to their forms of nutrition and metabolism. As a consequence, an alternative means of classifying them can be built on the basis of their roles within ecosystems – in effect, on their environmental impacts.

On this basis we can recognise three categories; **producers**, **nitrogen fixers** and **decomposers**. This division is based on their metabolism and nutrition, as defined in Table 18.3.

Table 18.3 Roles of microorganisms in the environment

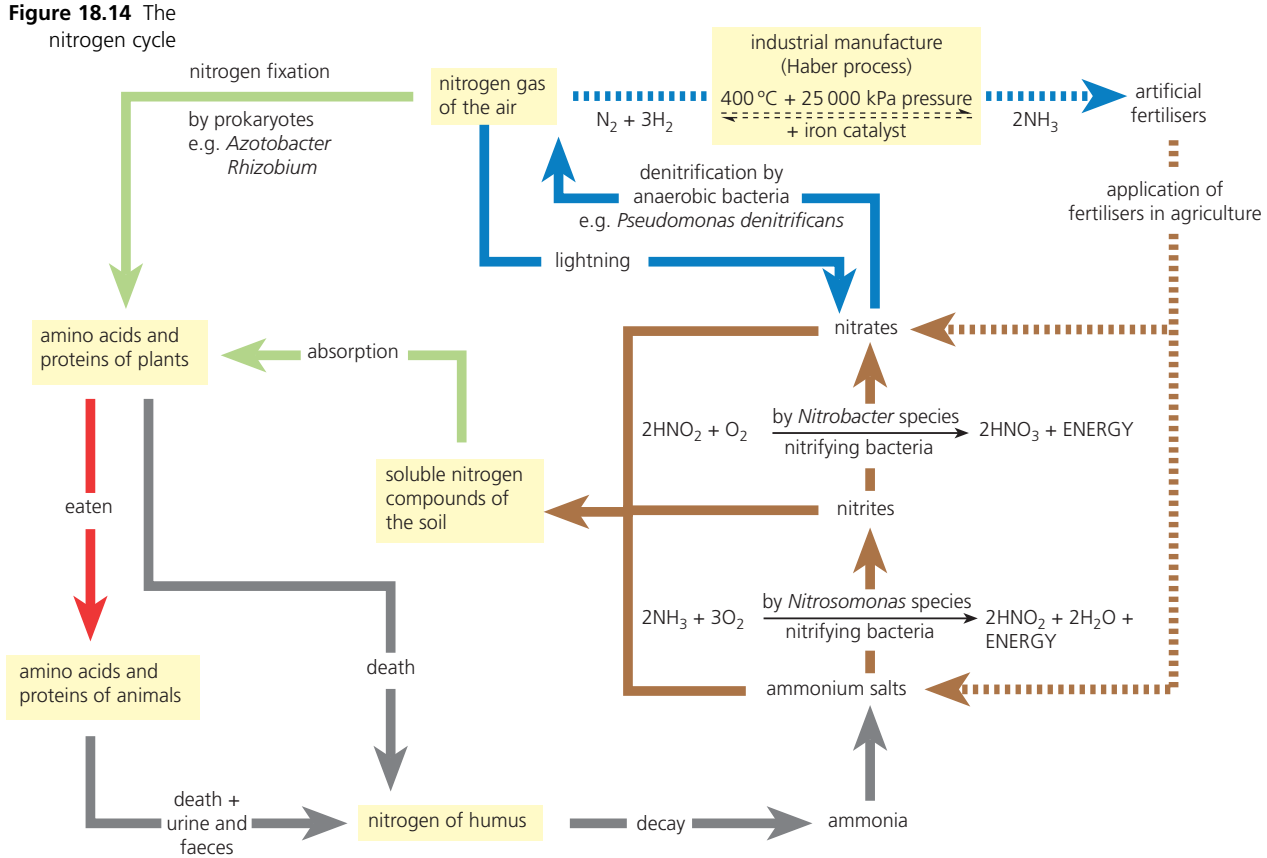
	Examples / environmental effect
Producers	■ photosynthetic bacteria such as cyanobacteria ■ produce sugar and oxygen from CO_2, using light energy
Nitrogen fixers	■ free-living and symbiotic N-fixing bacteria ■ convert nitrogen gas from the atmosphere to ammonia using energy from sunlight (free-living N-fixers) or energy from sugar obtained from host (symbiotic N-fixers)
Decomposers	■ saprotrophic bacteria (and fungi) ■ break down dead organic matter and waste matter from organisms to CO_2, H_2O and ions

So, when we examine a significant environmental process such as the nitrogen cycle, for example, we can detect microorganisms of these three categories present (Figure 18.14).

The nitrogen cycle

Although the element nitrogen in the form of di-nitrogen molecules (N_2) makes up about 80% of the Earth's atmosphere, nitrogen compounds available for use by living things are relatively scarce within the biosphere. The nitrogen cycle summarises the all-important steps by which the element nitrogen cycles between the soil, the atmosphere and living things.

Figure 18.14 The nitrogen cycle



Microorganisms of the nitrogen cycle

Nitrogen-fixing microorganisms

Some microorganisms can take in nitrogen from the air and fix this atmospheric nitrogen (they chemically reduce it) to form ammonia. The ammonia is then combined with organic acids, forming amino acids. This is known as **nitrogen fixation.**

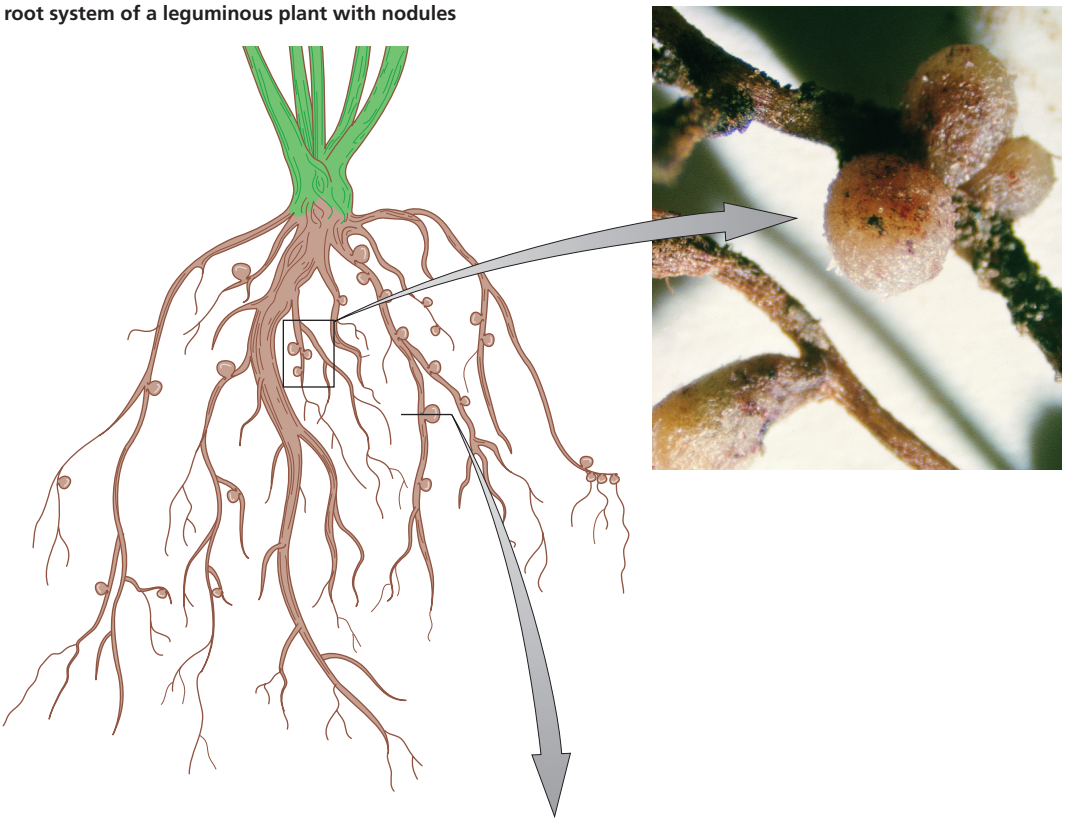
Nitrogen fixation requires energy (as ATP) and hydrogen (reducing power from $NADH_2$) from respiration; they need a great deal of both for this reduction. The enzyme involved is nitrogenase. The steps of N-fixation are summarised below.

1 nitrogen fixation **2** amino acid synthesis

$$N_2 \xrightarrow[\uparrow\ ATP + NADH_2\ \text{from respiration}]{\textit{nitrogenase}} NH_3 \xrightarrow{\text{organic acid}} \text{amino acid}$$

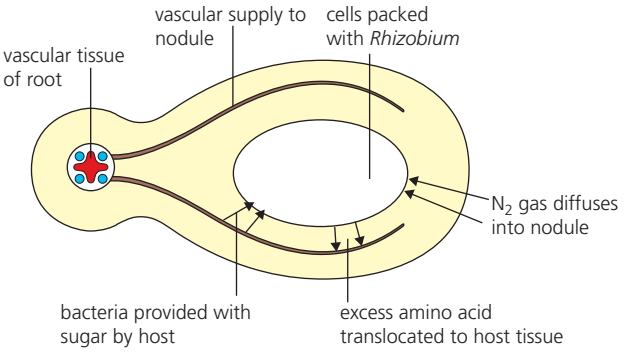
Figure 18.15 Root system with root nodules, and the functioning nodule

root system of a leguminous plant with nodules



root nodule in section

In the developing nodule, many cells become infected with *Rhizobium* and root tissue with xylem and phloem forms around the infected cells.



Some nitrogen-fixing organisms are **free-living**, meaning they exist independently in the environment. Examples include certain species of bacteria, such as *Azotobacter* spp. In *Azotobacter*, the energy (ATP) and reducing power ($NADH_2$) are supplied by aerobic respiration in the bacterium.

Some nitrogen-fixing organisms live **mutualistically in host plants**. Mutualism is the name for a close association between two organisms, in which both organisms benefit. The bacterium *Rhizobium* commonly occurs in the soil around plant roots where it feeds saprotrophically on dead organic matter. But it is also able to enter the roots of leguminous plants (clover, peas, beans, soya and many others), if growing nearby. Here it causes the host tissues to form into a nodule around the cells containing the bacterium (Figure 18.15). In this environment, the *Rhizobium* is able to reduce nitrogen gas to ammonia. From this, amino acids are immediately formed in the nodules and some, possibly most, pass out to the surrounding cells and are used by the host plant.

4 Identify the source of reducing power and energy used by *Rhizobium* in nitrogen fixation.

Because of their association with *Rhizobium*, leguminous plants can grow and flourish in soils low in nitrates. As crop plants they are found to be rich in proteins. Consequently, leguminous crops are vitally important to communities otherwise suffering from shortage of protein in their diets, such as those living by subsistence farming on very poor soils. In fact, rich and poor communities alike, all around the world, make good use of leguminous plants.

Formation of soil nitrates by nitrifying bacteria

A range of saprotrophic bacteria and fungi of the soil community break down organic matter in the soil (dead remains of organisms, waste matter excreted by animals, humus around soil particles, etc.). The final products of breakdown include carbon dioxide, water and a range of metal (e.g. Ca^{2+}, K^+) and non-metal ions (e.g. PO_4^{3-}). Vast numbers of microorganisms exhibit this type of nutrition.

Meanwhile, the combined nitrogen present is reduced to ammonia (NH_3). This is known as **ammonification**.

Ammonia occurs as the ammonium ion (NH_4^+), dissolved in the soil solution, particularly in acidic and neutral soils. Because ammonia is volatile, some loss can occur from soil, especially in basic soils (e.g. soil formed from chalk rock).

However, losses of ammonia and ammonium ions from soils is prevented when rapid **nitrification** (oxidation of NH_4^+ to NO_3^-), brought about by nitrifying bacteria, follows ammonifications.

The **first step to nitrification** is the oxidation of ammonium ions to nitrite ions. This is an aerobic process and an exothermic reaction. It occurs in the soil, carried out by enzymes of bacteria of the *Nitrosomonas* genus.

$$2NH_3 + 3O_2 \xrightarrow{\textit{Nitrosomonas}\text{ spp.}} 2HNO_2 + 2H_2O + \text{ENERGY}$$

The **second step to nitrification** is the oxidation of nitrite ions to nitrate ions. This also is an aerobic process and an exothermic reaction. It occurs in the soil, carried out by enzymes of bacteria of the *Nitrobacter* genus.

5 Identify how the combined nitrogen formed by *Azotobacter* and *Rhizobium* is made available to organisms growing in the same habitat.

$$2HNO_2 + O_2 \xrightarrow{\textit{Nitrobacter}\text{ spp.}} 2HNO_3 + \text{ENERGY}$$

Note that *Nitrobacter* and *Nitrosomonas* use the energy released from the chemical reactions they catalyse in their nutrition. The energy is coupled to the synthesis of sugar from carbon dioxide and water in a process known as chemosynthesis (chemoautotrophic nutrition, page 584).

Loss of nitrates from soil by bacteriological action – denitrification

Breakdown of soil nitrates to nitrogen gas may occur, for example, in waterlogged soils where the oxygen normally present has been driven out. The breakdown is due to the metabolism of certain anaerobic bacteria, particularly of the *Bacillus* and *Pseudomonas* genera (e.g. *Pseudomonas denitrificans*). These organisms flourish in anaerobic conditions. The effect of the anaerobic metabolism of these bacteria in soils is harmful to the growth of green plants because of this loss of nitrate from the soil.

What soil conditions favour nitrification and denitrification?

The bacteria of nitrification are strongly aerobic, and function best in soils in which air is present – the normal condition for most soils. However, if bad drainage conditions develop and water accumulates, then air is driven out of soil. We say the soil is waterlogged. In the absence of air, denitrification takes over.

Sewage treatment and the roles of microorganisms

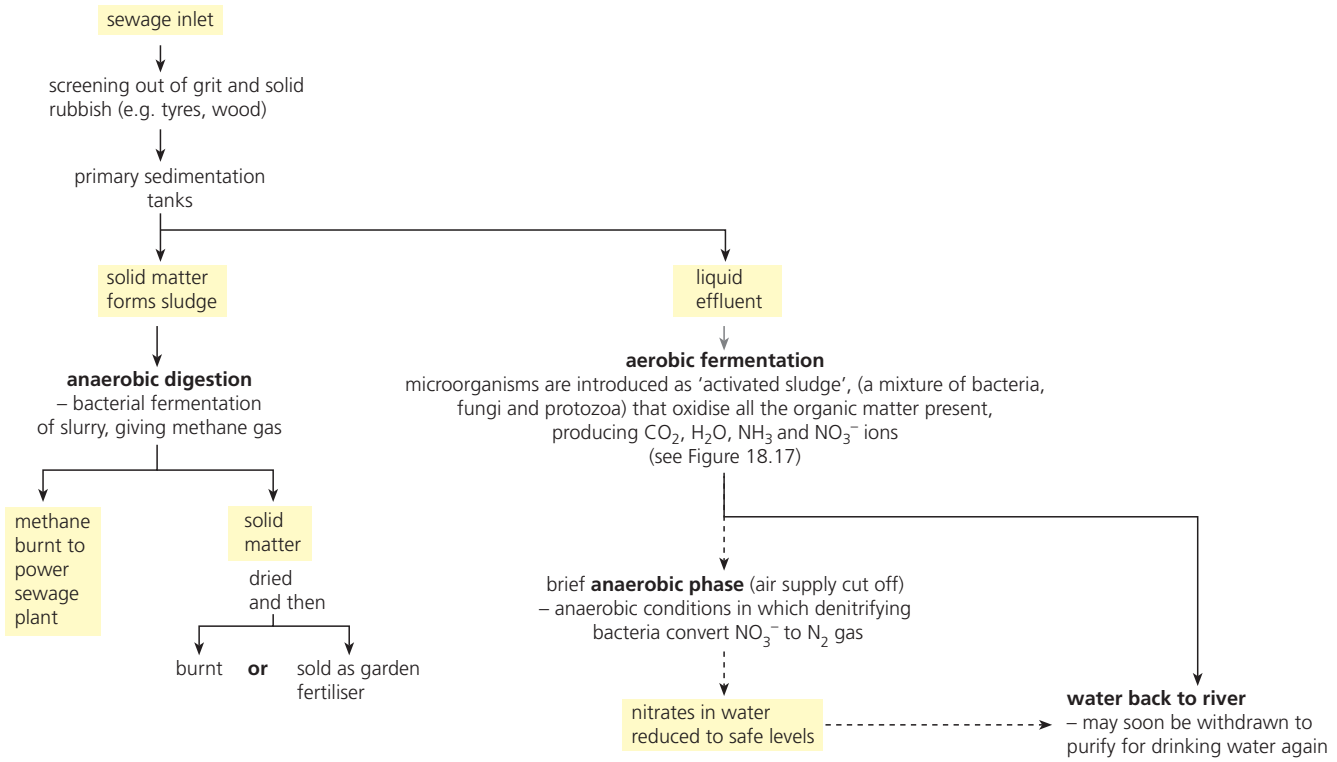
Sewage is the fluid waste of human communities, from houses and commercial properties. It is preferably piped to sewage works for processing. Sewage consists largely of used water, faeces and urine, and is rich in organic matter with ammonium, nitrate and other ions present. It contains vast numbers of microorganisms, many harmless saprotrophic ones, but also pathogenic bacteria.

Treatment of sewage to produce water safe to return to the environment involves the metabolism of vast numbers of microorganisms. During treatment (Figure 18.16), sewage is separated into **liquid effluent** and **solid matter**.

Liquids are cleaned by the aerobic metabolism of a mixture of bacteria, fungi and protozoa that is known in the industry as activated sludge. It requires vigorous efforts by water engineers to maintain the concentration of oxygen in the liquor throughout the process. There are various ways of doing this.

Solids are anaerobically digested to break down the organic matter. Liquid effluent is converted to clean water, and solid matter is converted to methane and carbon dioxide, and to solids usable as soil fertiliser (or that are disposed of by burning).

Figure 18.16 Sewage treatment



Roles of saprotrophic microorganisms in sewage

Provided aerobic conditions are maintained, saprotrophic microorganisms of the activated sludge quickly digest the entire dissolved organic matter present, producing carbon dioxide, ammonia, nitrate ions and other ions, including phosphates – and water. The water can be returned to the river largely without harm to the flora and fauna present, and may subsequently be withdrawn for re-use.

However, there is a persisting problem in the presence (and steady build up) of dissolved ions, such as nitrates and phosphates, as water resources are used again and again. We return to this issue later.

How is sufficient dissolved oxygen provided, given that oxygen gas is only slightly soluble in water, and there is a huge demand for oxygen by the saprotrophs that flourish in sewage effluent?

Dissolved oxygen is maintained in the liquid effluent by:

- **pumping air** through the liquid effluent as it passes slowly through shallow open tanks (Figure 18.17);
- **spraying the effluent** over beds of solid, porous clinker, and continually re-circulating the liquor (Figure 18.17);
- **passing the effluent through beds of reeds** selected to release air into the water around their roots (Figure 18.17).

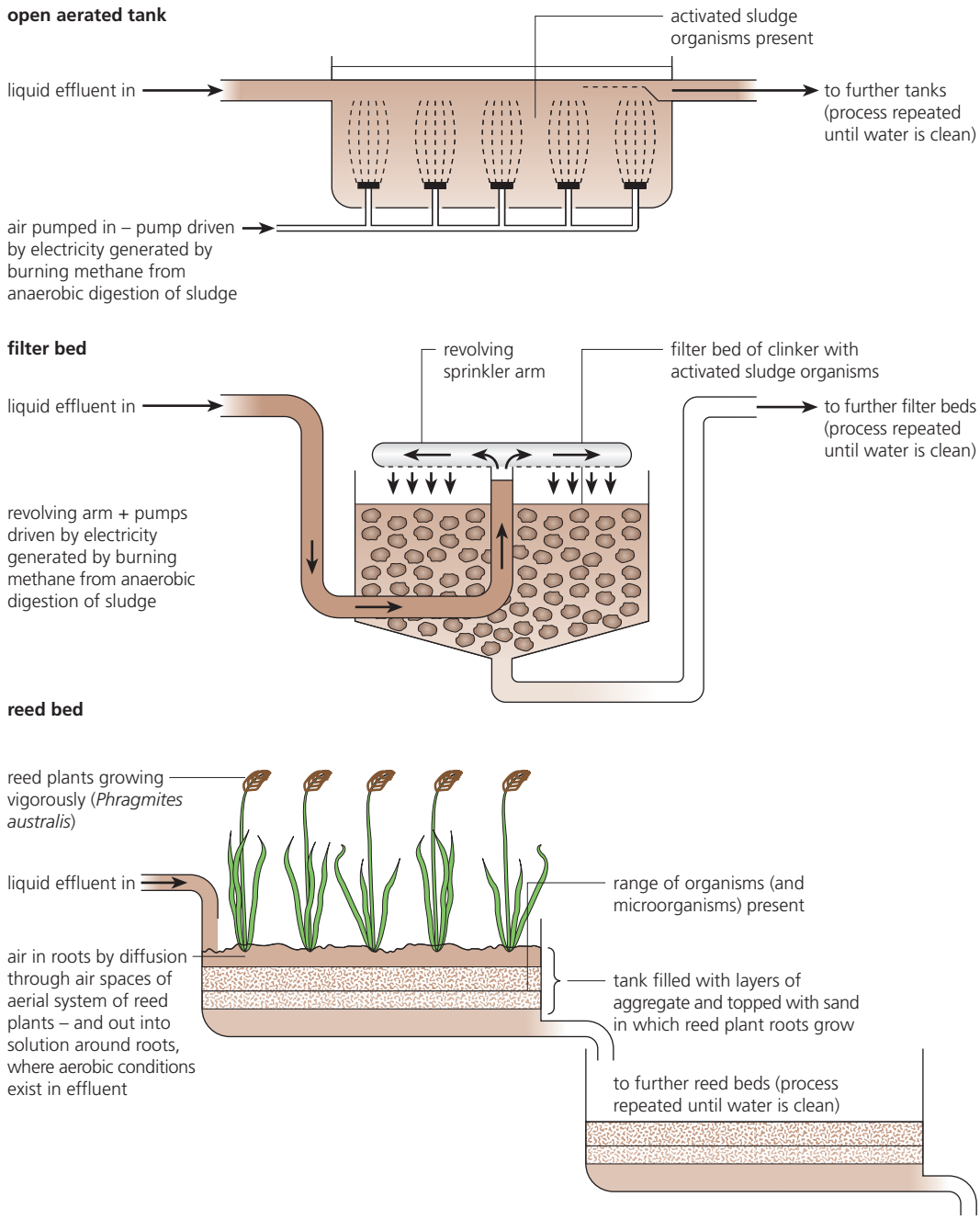


Figure 18.17 Alternative (aerobic) treatments of liquid effluent

The reed plant used in the latter case is a species of *Phragmites*, which maintains strongly aerobic conditions around the roots because air diffuses through the air spaces of leaves, stem and roots. Thus, aerobic conditions are maintained in the sand and effluent where aerobic, saprotrophic microorganisms are actively breaking down the organic matter.

Because the reed beds involve higher plants, they have the advantage of taking up ions favourable for reed growth, such as nitrates and phosphates. This reduction of ions in the water is not achieved by other methods. However, reed beds take up a huge space compared with other methods, and space is often at a premium in large cities where many people live, and where huge quantities of sewage need processing.

Sewage contamination of river water

A river that becomes contaminated with raw sewage is an environmental disaster (Figure 18.18). This is because the water is made excessively rich in organic matter at the point of contamination. Initially, saprotrophic bacteria flourish and sewage starts to break down – much as it does in a sewage works. However, in natural river conditions only limited oxygen is dissolved in the water, and there is no mechanism to pump oxygen in and maintain aerobic conditions. The explosion in the populations of aerobic bacteria that the organic matter of the sewage triggers, leads quickly to anaerobic (anoxic) conditions.

The tendency of water contaminated with organic matter to take up oxygen is called its **biochemical oxygen demand (BOD)**. BOD is defined as the amount of oxygen taken up by water over five days when incubated in the dark at 20 °C. Clean water takes up less than 5 ppm; heavily polluted water, 10 ppm or more.

The complete absence of any dissolved oxygen in river water quickly kills obligatorily aerobic organisms present in the river immediately downstream of the pollution, including the fish. As a consequence, **anaerobic bacteria flourish**. A waste product of the anaerobic decay of all the organic matter in these conditions is the unpleasant smelling gas **hydrogen sulphide**. This highly soluble gas is extremely poisonous. The remaining flora and fauna are also killed.

Slowly, the gases escape into the atmosphere, other pollutants are diluted by the river, and are metabolised by a range of organisms able to live at low oxygen concentrations. Eventually, **the river community will recover**.

Sewage is likely to have contained **pathogenic bacteria** and their spores, which may be present in the faeces. There is, therefore, an additional health hazard generated if the river is later used for bathing, or as a source of drinking water for communities downstream. Treatment with chlorine is essential in the treatment of river water for drinking purposes if public health is to be maintained.

Agricultural effluent and water-way pollution

Another common source of river water pollution is agricultural run-off from arable land that is treated with fertilisers or manure. The steps taken to maintain soil fertility under heavy cropping regimes inevitably result in a steady build up of ions such as nitrates (and phosphates) in water of the water table. Slowly, these leach into ditches, streams and rivers as rain water drains from the fields.

In water enriched with these inorganic ions, plant growth is normally luxuriant. In the growing season, water temperatures rise and then the algae of lakes and rivers undergo a population explosion, known as an **algal bloom**. The enrichment of waters with inorganic nutrients has caused an excess of aquatic plant life. This process is known as **eutrophication**. It is not restricted to rivers; the seas around estuaries are often similarly affected.

Later, after the algal bloom has died back, the organic remains of these plants are decayed by saprotrophic aerobic bacteria. As a result, the water becomes deoxygenated, exactly as when raw sewage is discharged into a river (Figure 18.18). The resulting anaerobic conditions, and the hydrogen sulphide produced, kill many aquatic organisms.

Note that in the absence of pollution, eutrophication still occurs as a natural process, but only very slowly. In rivers and lowland ponds and lakes, the amount of dissolved nutrients steadily increases. By contrast, the water of mountain lakes or tarns, and the streams that feed them, are typically low in dissolved nutrients. Here the surrounding land is often stony, with poor soil. The result is few dissolved ions are present and aquatic plants show little growth.

Figure 18.18 The effect of sewage pollution of river water

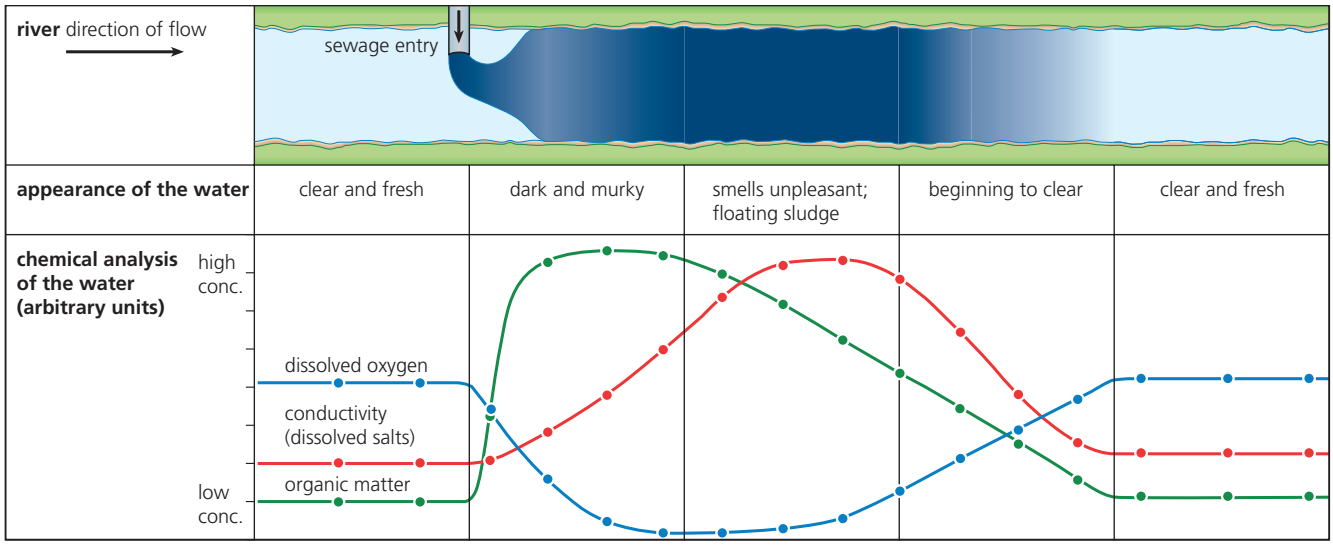
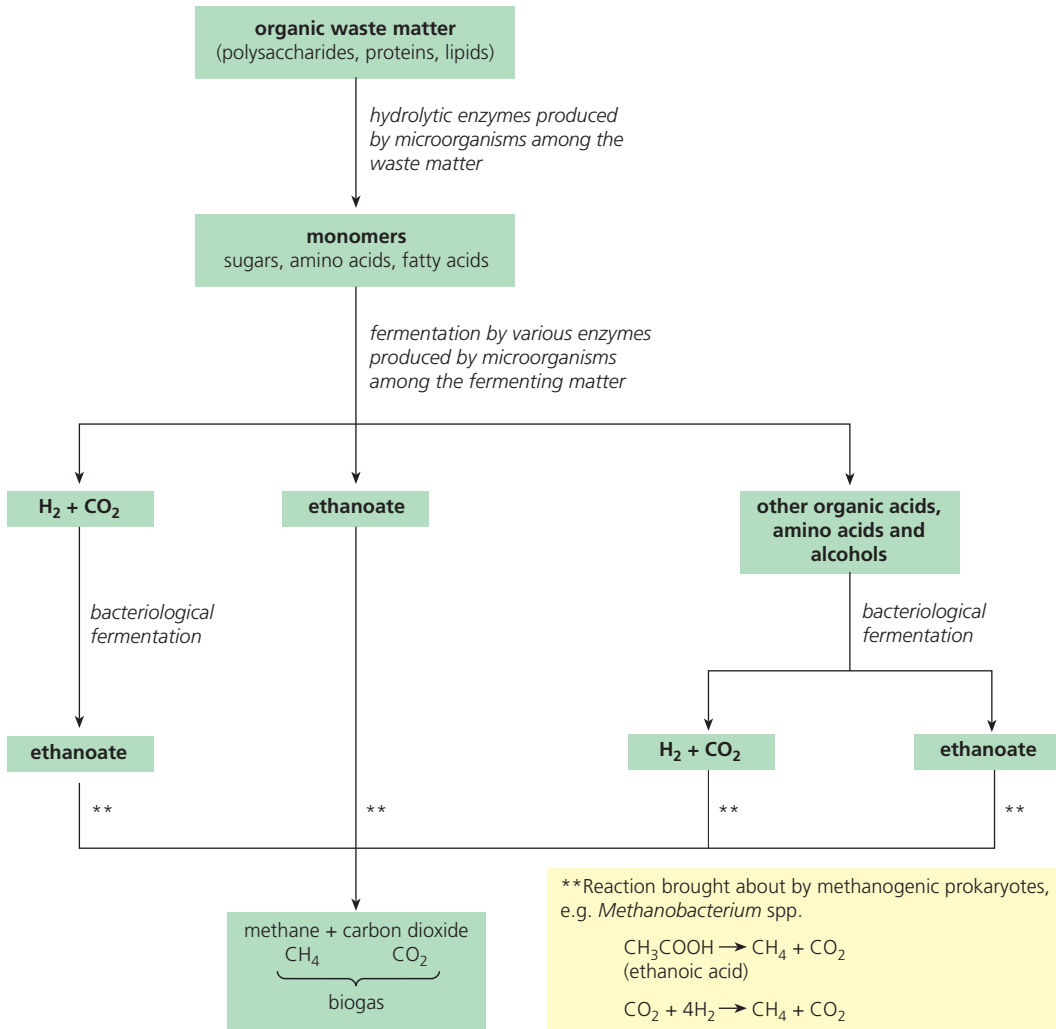


Figure 18.19 Pathways of methane synthesis from organic waste matter



Fuels from biomass

Oil, gas and coal are known as fossil fuels because these resources were laid down long ago, during the Carboniferous period of Earth's history. Dependence on fossil fuels generates problems because:

- they are non-renewable sources of energy – stocks will eventually run out;
- burning of fossil fuels releases into the atmosphere carbon dioxide gas that has been locked away for over 300 million years – consequently, the concentration of carbon dioxide (one of the greenhouse gases) in the Earth's atmosphere is rising (page 149).

As a result, there are potentially good economic and environmental reasons for developing alternative fuels from organic matter; such sources are referred to as biomass.

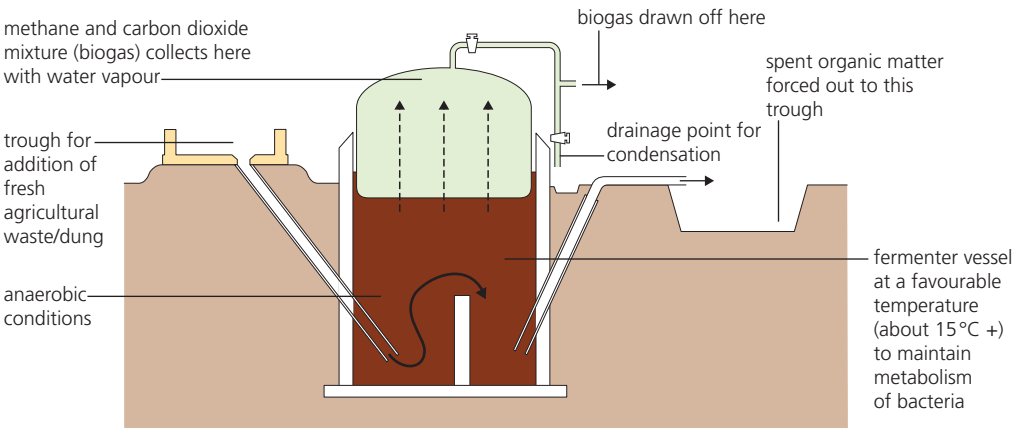
One approach involves **energy farming**, the name given to the growing of crops like sugar cane specifically for fuel production. The extracted sugar is fermented using yeast, and the resulting aqueous **ethanol** solution has to be distilled to a solution pure enough (only 4.4% water) to power cars adapted to burn ethanol. In Brazil, this fuel is called Alcool.

Alternatively, organic waste matter may be **anaerobically digested** by a combination of microorganisms to produce a **methane-rich gas**, often referred to as biogas (Figure 18.19). We have already seen that such biogas is a natural product of anaerobic digestion of sewage sludge in fermenter tanks at sewage works, where it has been used to generate electricity to power the machinery.

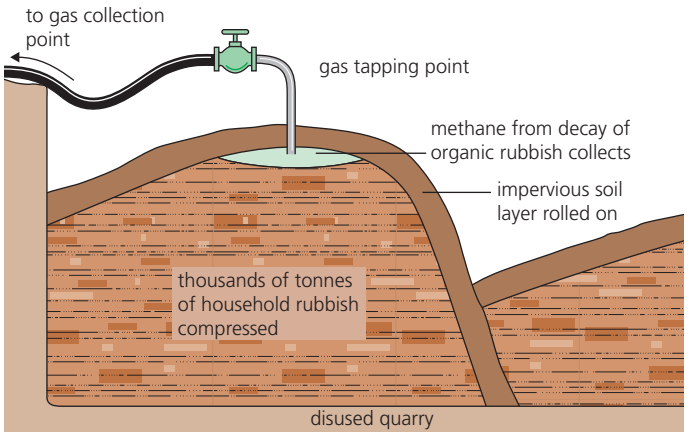
The pathway of methane synthesis from organic waste matter involves hydrolysis of the polysaccharides, proteins and lipids present to their monomers (sugars, amino acids, fatty acids and alcohols), and the fermentation of these to form organic acids such as ethanoate (acetate).

Figure 18.20 Biogas production in contrasting circumstances

a developing world biogas plant



biogas from an urban land-fill site



Then, methanogenic prokaryotes such as *Methanobacterium* spp. convert ethanoic acid and ethanoate (acetate) to methane and carbon dioxide:

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

Methane gas from waste matter in the less-developed world

In some rural communities that are under-resourced with fuel of any sort, biogas digesters are in regular use. Into the digester goes animal dung and/or agricultural waste for anaerobic fermentation. Simple digesters operate more reliably in climates where the contents of the digester will be at or above a temperature of 15 °C. The biogas formed is about 50–80% methane, 15–45% carbon dioxide and about 5% water vapour (Figure 18.20). (This compares reasonably well with natural gas from oil and gas fields, which is about 80% methane.)

6 Outline the range of microorganisms involved in the conversion of complex organic waste into molecules which methanogenic prokaryotes may exploit as substrates.

Methane gas from waste matter in the developed world

Urban communities in the developed world typically dump huge quantities of domestic waste, much of which is organic matter, into disused pits or quarries called landfill sites. The rubbish becomes compressed, anaerobic conditions quickly develop, and a cocktail of microorganisms break down the organic matter, giving off biogas. Completed landfill sites have to be vented so that these gases can safely escape. Schemes now operate to tap the biogas and pipe it to where it can be burnt to generate heat and/or power, wherever this is practical (Figure 18.20).

Microbes and biotechnology

F3.1–3.5

Biotechnology is the industrial and commercial application of biological science, particularly of microbiology, enzymology and genetics. Modern biotechnologies are enormously important to industry, medicine and research, and these technologies are often presented as a recent development. In fact, their origins go back several millennia; the earliest biotechnologies were cheese making and alcoholic fermentation such as brewing, for example. However, it is certain modern applications of biotechnology that are illustrated here.

Genetic engineering involves change in the genetic constitution of a living organism brought about by artificial means (meaning other than by conventional breeding). In genetic engineering procedures, individual genes in the form of fragments of DNA from cells of an organism may be isolated and then spliced into the genome of other organisms (page 121). Very often, but not exclusively, the gene is added to a bacterium. The result is the **genetic modification of organisms**.

This technology and the diverse techniques it has already spawned have many applications, including:

- the manufacture of drugs such as human insulin from bacteria;
- the production of genetically modified, fast-growing tree species yielding soft fibres for industrial paper production with lowered environmental harm;
- the production of genetically modified sheep that secrete human proteins required in the treatment of disease;
- the possible repair of faulty genes by the techniques of gene therapy;
- the identification of a person (or other organism) from a sample of their DNA by a process known as genetic fingerprinting.

In this revisiting of the subject of genetic engineering, there are two particular aspects to consider: the genetic engineer's use of reverse transcriptase enzyme, and the issue of gene therapy. However, we should first acknowledge that sometimes there is only a very small sample of DNA available to the genetic engineer, perhaps too little to progress with. The discovery of the **polymerase chain reaction (PCR)** has solved this. By means of the PCR, fragments of DNA may be copied repeatedly, faithfully and speedily, in a process that has been fully automated. Details of the steps of the PCR are not needed here.

Reverse transcriptase

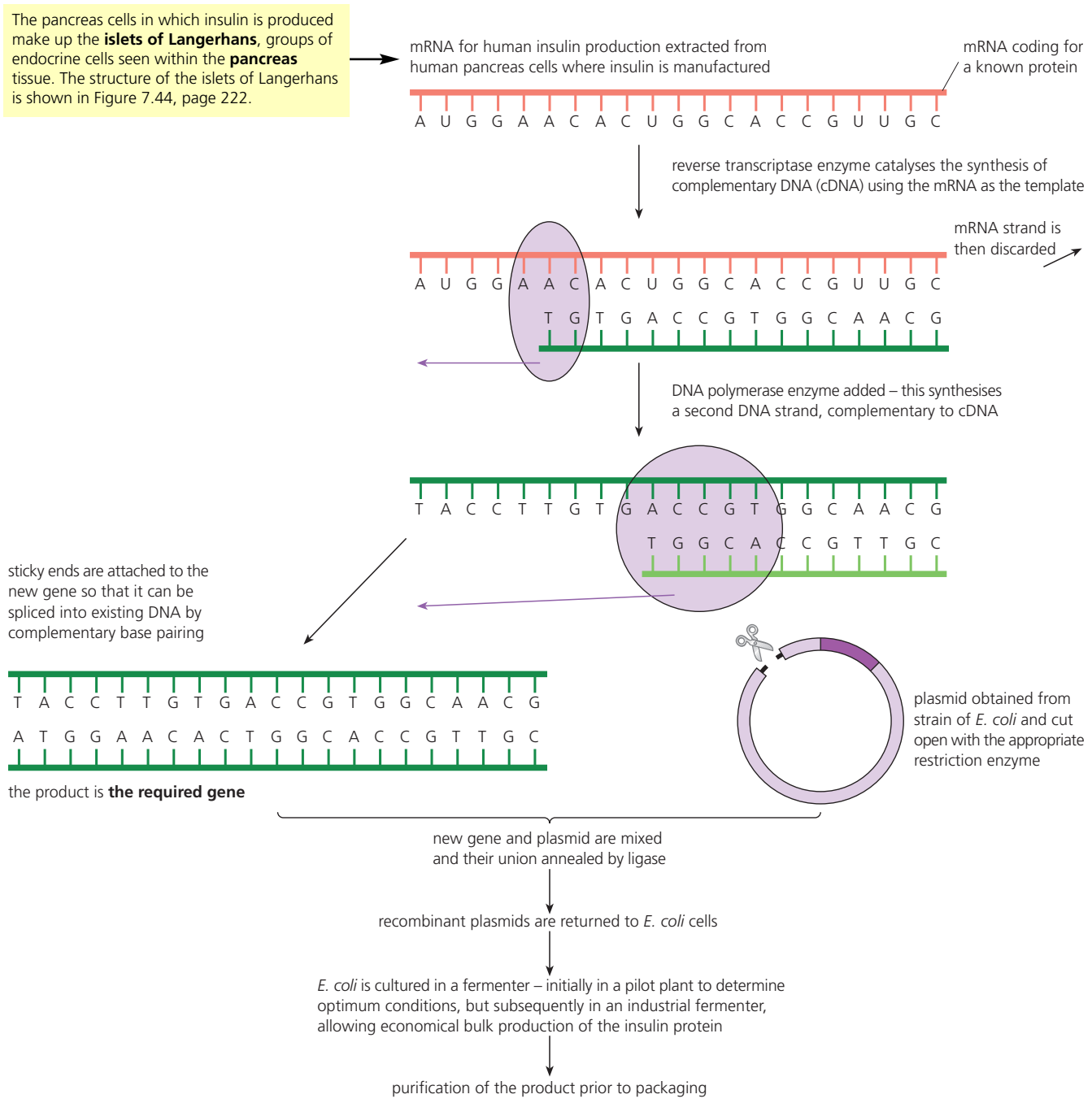
We have seen that the normal flow of genetic information is a one-way flow, from the DNA of the genes to messenger RNA (mRNA) in the cytoplasm. That is, the genetic code in DNA is transcribed into messenger RNA which passes out into the cytoplasm and is translated into the sequence of amino acids by which a protein is assembled in the ribosomes. This concept is called the **central dogma of molecular biology** (page 252).

Since Watson and Crick made this original observation, retroviruses have been discovered.

Retroviruses have RNA as their genetic material rather than DNA, yet they are able to translate the information in their RNA into double-stranded DNA. In this latter form, their genes may be added to the (eukaryotic) host's DNA, in the nucleus. The **AIDS virus is an example of a retrovirus** (page 253).

Figure 18.21 Insulin production by genetically modified (GM) bacteria

The pancreas cells in which insulin is produced make up the **islets of Langerhans**, groups of endocrine cells seen within the **pancreas** tissue. The structure of the islets of Langerhans is shown in Figure 7.44, page 222.



This conversion is possible because the virus contains an enzyme, known as **reverse transcriptase**, which it introduces into the host's cell, at the time of the initial infection. Reverse transcriptase catalyses the production of **DNA from RNA**. Today, reverse transcriptase enzyme is an essential part of the genetic engineer's tool-kit.

Why is reverse transcriptase such a useful enzyme in genetic engineering?

A eukaryotic gene, in place in a chromosome, typically consists of meaningful DNA (called **exons**) interrupted periodically along its length by apparently meaningless lengths of DNA (called **introns**). The phenomenon of exons and introns was introduced on page 247.

Apparently, most if not all eukaryotic genes have these units of non-gene DNA within their boundaries. Remember, when such a gene is transcribed into mRNA, the mRNA itself initially contains the sequence of introns, exactly as they occur in the DNA.

In the next stage of **post-transcriptional modification**, the introns are removed and disposed of. This occurs before the mRNA passes out into the cytoplasm. The resulting (shortened) length of mRNA is described as **mature**.

The presence of exons is a challenge to genetic engineers who want to isolate a eukaryotic gene because they have no easy way of cutting out the introns. Now there is an alternative approach, avoiding the problem of introns altogether. This involves **making a copy of the gene from its mRNA**, using reverse transcriptase.

For example, mRNA coding for the human hormone **insulin** can be extracted from the cells of the pancreas (page 222). Then, using reverse transcriptase, a DNA copy of the gene for insulin can be produced – a copy of the gene without introns. When this gene is then spliced into DNA that can be introduced into a bacterium, such as an appropriate strain of *E. coli*, then the bacterium can be induced to synthesise human insulin (Figure 18.21).

This is the actual mechanism by which human insulin is synthesised by the pharmaceutical industry, today. When adding genes to a bacterium, the addition is made to plasmids (page 122) which the bacterium can be induced to take up.

7 Comment on the challenge to Mendelian genetics of the existence of reverse transcriptase.

Gene therapy

Genetic diseases are heritable conditions that are caused by a specific defect in a gene. This type of disease affects about 1–2% of the human population. Common genetic diseases include cystic fibrosis, sickle cell disease (page 101), Duchenne muscular dystrophy, thalassaemia, severe combined immunodeficiency (SCID), familial hypercholesterolaemia, and haemophilia (page 114).

These conditions mostly arise from a mutation involving a single gene. The mutant allele that causes the disease is commonly recessive, so a person must be homozygous for the mutant gene for the condition to be expressed (but people with a single mutant allele are carriers). For example, cystic fibrosis is due to a recessive gene on chromosome 7. Duchenne muscular dystrophy and haemophilia are due to recessive alleles on the X chromosome, so these two conditions are sex-linked (page 113).

Somatic and germ-line therapy

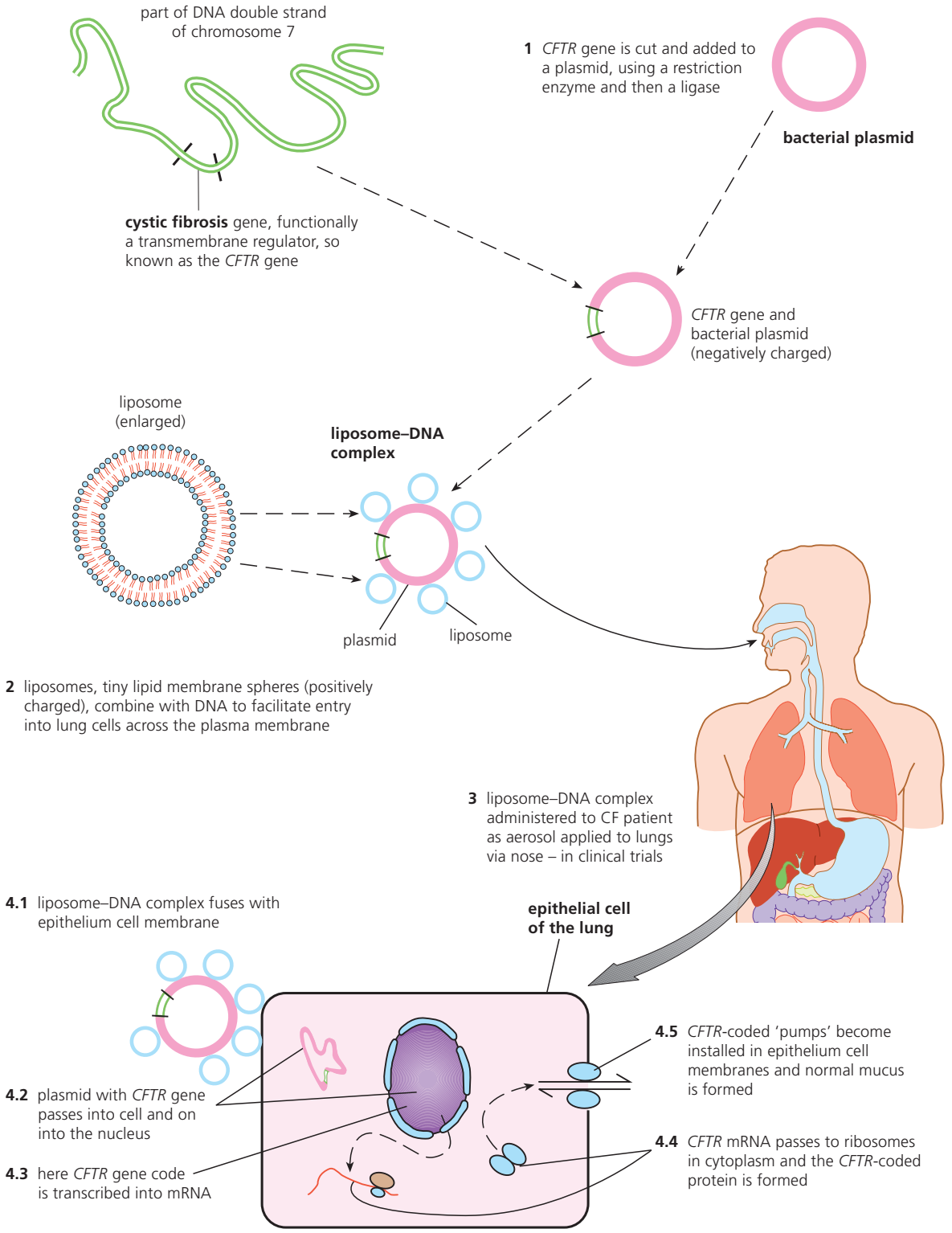
Gene therapy aspires to use recombinant DNA technology to overcome genetic disease, where this is thought safe and ethically sound. A permanent and acceptable solution is to supply the missing gene to body cells in such a way that it remains permanently functional. This solution is known as **somatic therapy** (Figure 18.22 and 18.23).

On the other hand, it is not considered safe or ethical to attempt to tamper with germ cells (cells which give rise to gametes in the testes and ovaries). This banned approach is called germ-line therapy.

8 Comment on the observation that medical treatments with expensive drugs, skilled surgery, or by gene therapy receive much attention in our media, but virtually no consideration is given to the possibility of directing funds to everyday measures to improve health and prevent disease in the first place.

The cystic fibrosis gene codes for a membrane protein that occurs widely in body cells, and pumps ions (e.g. Cl^-) across cell membranes.

Figure 18.22 Cystic fibrosis (CF); getting the healthy gene into functioning cells in the lungs



In recent clinical trials some 20% of epithelium cells of CF patients were *temporarily* modified (i.e. accepted the *CFTR* gene), but the effects were relatively short-lived. This is because our epithelium cells are continually replaced at a steady rate, and in CF patients the genetically engineered cells are replaced with cells without *CFTR*-coded pumps. Patients would require periodic treatment with the liposome–DNA complex aerosol to maintain the effect permanently.

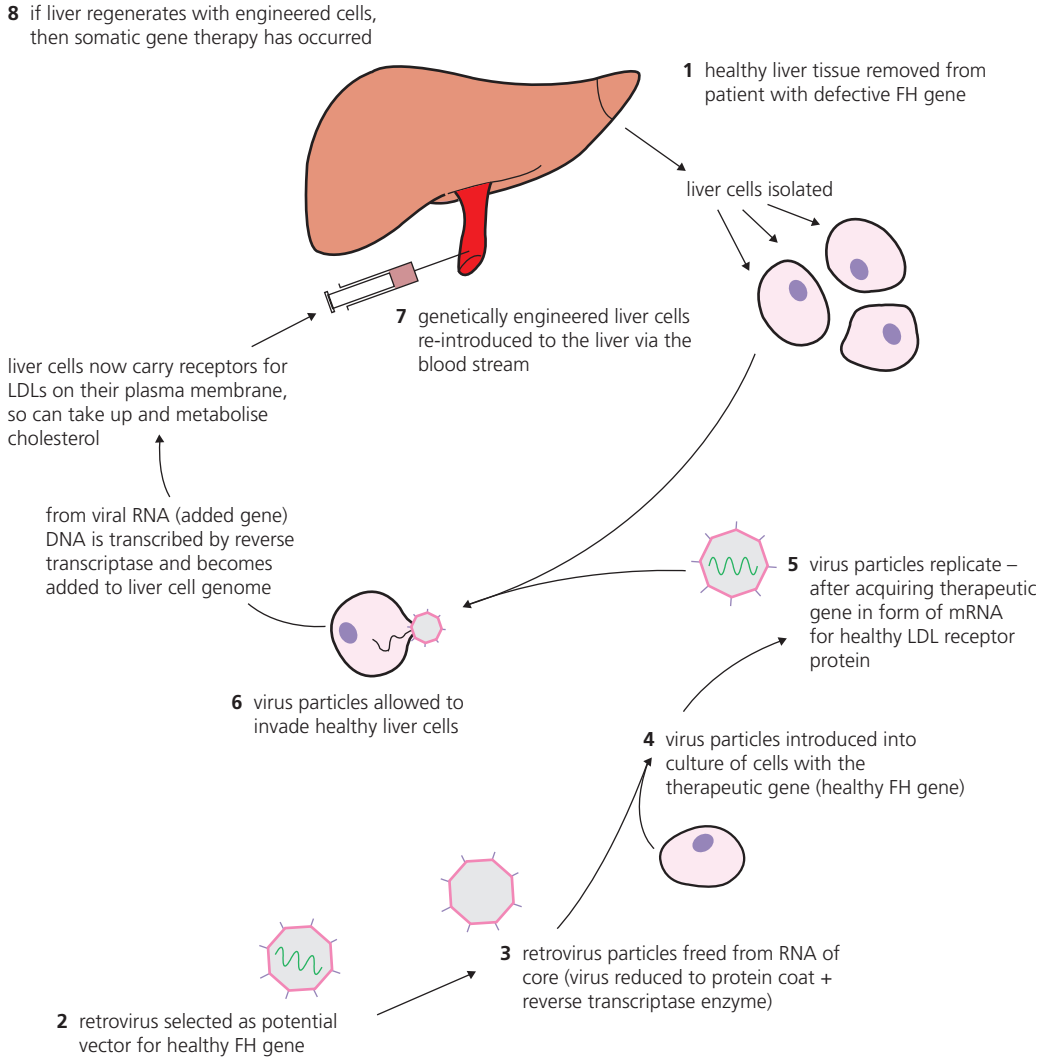
Two approaches to somatic gene therapy

Cystic fibrosis affects the epithelial cells of the body and is the most common genetic disorder in Northern Europe. The **CF** gene codes for a protein (called CFTR) that functions as an ion pump. The pump transports chloride ions across membranes and water follows. Consequently, epithelia are kept smooth and moist.

In cystic fibrosis patients, the mutant gene (**cfcf**) coding for this protein has a changed base sequence and either codes for no protein or for a faulty protein. As a result, the epithelia remain dry, and thick sticky mucus builds up. The effects are felt in the pancreas (secretion of digestive juices) and the sweat glands (salty sweat formed), for example. But for people with cystic fibrosis, a life-threatening consequence arises in the lungs which may become blocked by mucus and are prone to infection.

Figure 18.23 Familial hypercholesterolaemia (FH); correcting liver cell function by virus vector

Principle of this approach
Engineering a healthy gene into a sample of liver cells so that genetically corrected cells may be re-established in the liver and restore correct functioning.



Another possible therapy involves adding the missing genes by **injection of (harmless) retroviruses with engineered genes** directly into the patient's blood stream.

Gene therapy here involves getting copies of the healthy gene to the cells of the lung epithelia, delivered in an aerosol spray (Figure 18.22). The spray contains tiny lipid bilayer droplets called liposomes to which copies of the healthy gene are attached. Liposomes fuse with cell membrane lipid and deliver the gene to the epithelial cell. In trials, the treatment is effective, but only until the epithelial cells are routinely replaced. The treatment has to be regularly repeated. The cure can only be more permanent when it is targeted on the cells that make epithelial cells.

Familial hypercholesterolaemia (FH) is a failure in cholesterol metabolism in the body. Cholesterol travels in the blood in particles called low-density lipoproteins (LDLs), each containing about 1500 cholesterol molecules. A person with FH has defective receptors for LDLs on the membranes of cells that normally take in cholesterol and metabolise it (e.g. use it in membrane synthesis). As a result, people with FH have abnormally high concentrations of cholesterol in their blood – they may have yellow deposits of cholesterol under their skin as a result. FH causes early cardiovascular disease by causing the depositing of cholesterol in the walls of blood vessels, leading to atherosclerosis (page 420).

Gene therapy for FH involves engineering a healthy gene into a sample of liver cells so that the genetically corrected cells may be re-established in the liver and restore correct functioning. Here gene therapy has been attempted using a disabled **virus as the vector** (transporter) of the healthy gene (Figure 18.23). This is chosen because a virus may attach its nucleic acid to the host cell chromosome. If a healthy FH gene has been added to the viral genome, then the healthy FH gene may also become part of the cell's genome.

The risks of gene therapy

In 1990, two children who had SCID and were living in a sterile bubble in order to survive, were given a working copy of the gene they lacked which had been inserted into a sample of their own white cells. These people now lead normal healthy lives.

This success established that somatic gene therapy can be made to work. However, there are many failures, too, and the process carries risks for the patient. Risks include:

- the use of liposomes as the vector is inefficient because the gene cannot (as yet) be made to insert itself into chromosomes in the nucleus in cells; therefore, the treatment must be repeated at regular intervals and all medical treatments carry risk;
- disabled viruses are more efficient vectors of a healthy, working gene, but the virus coat, on entry, often triggers an immune response, causing tissue damage;
- retroviruses are the most efficient vectors so far attempted, but when they integrate a new gene into the patient's genome it may be at a position that inactivates other genes – for example, the effect of insertion may be to trigger the activation of proto-oncogenes with the result that a fatal cancer infection is caused at the same time that the inherited genetic disease is cured.

There will probably be other problems and disappointments on the way to the development of safe, reliable and effective treatments for inherited diseases.

9 The human disease SCID is also treated by genetic modification of extracted body cells, using a virus vector. When the corrected cells are returned to the body the patient may be cured for life (body cells are now able to produce the missing enzyme). **Explain** why this genetic modification (GM) treatment of SCID in a child patient, for example, is described as a case of somatic therapy only.

Microorganisms and food production

F4.1–4.4

The exploitation of microorganisms first occurred several millennia ago, in the processes of bread production, wine production and beer brewing, for example. These processes exploit the unicellular fungus *Saccharomyces* (yeast) (Figure 18.6, page 559).

Yeasts respire by alcoholic fermentation (page 80) and produce carbon dioxide and ethanol as waste products (waste in the sense that the *Saccharomyces* cells cannot use them further). The ethanol is exploited in brewing and wine making; the carbon dioxide is required to make leavened (raised) bread.

Brewing

Beer is made from barley grains (or rice or maize) which are first allowed to germinate. In this first step, the enzymes of the grains are used to convert the stored starch and proteins to sugars and amino acids. To the resulting sugar solution, yeast is added and produces alcohol (Figure 18.24).

Figure 18.24 Brewing of beer and lager

harvesting of barley

↓

malting of barley by inducing germination of grains (moisture + warmth); starch within the grains is converted to sugar, proteins to amino acids

↓

cracking – germination stopped by heating of grains to a light roasting; passed between rollers to open the germinated grains

↓

mashing with hot water to remove sugar and amino acids; remainder forms 'brewers grains', sold for animal feed

↓

boiling of the wort: additional sugar may be added – hops are added to boiling wort, adding flavour (bitterness) and anti-microbial substances

↓

cooling follows

↓

fermentation – yeast is added to convert the sugar to ethanol and CO_2

- *Saccharomyces cerevisiae* – a top-fermenting yeast (distributed in wort as fermentation proceeds, but carried to surface of the wort by CO_2 given off); used to produce **beers and ales** at temperatures of 14–23 °C over a period of 5–7 days
- *S. carlsbergensis* – a bottom-fermenting yeast (settles to bottom of tank); used to produce **lager** at a temperature of 6–12 °C over a period of 8–14 days

↓

(by-products are CO_2, sold as dry ice, and yeast for yeast extract manufacture)

↓

conditioning of the beer, stored in casks; yeast settles out, beer cleared by filtration; standardisation of colour and flavour

↓

bottled or sold in barrels – a solution of ethanol at 3.5–8.0% ethanol (10% in barley wine); some beers are pasteurised

the fermentation stage in the brewing of beer, using a top-fermenting yeast

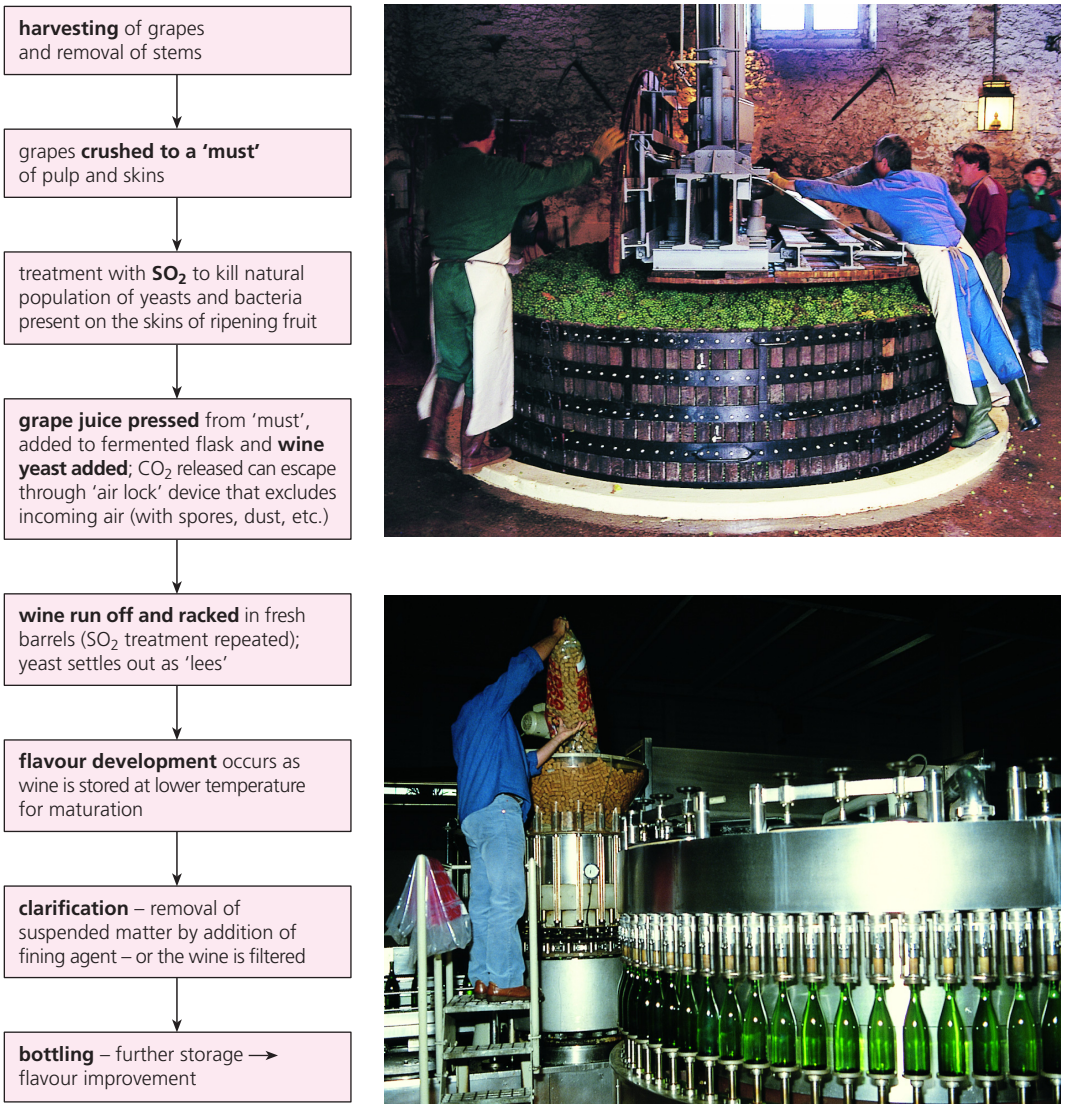
10 **Explain** why grain reserves of starch and protein must be hydrolysed by the grain's enzymes, prior to the addition of yeast.

Flavours of particular brews are due to the ingredients selected (e.g. the malt, water, hops and yeast), the proportions used, and the final cask conditioning of the live beer allowed by different brewers. The difference between lagers and beers is due to the yeasts used. Market variety is also due to the industrial development of keg beers – processed, pasteurised beers, served using nitrogen and carbon dioxide gas mixtures. These keg beers are viewed unfavourably by real ale enthusiasts.

Wine making

Wine production begins with the harvesting of ripe grapes, followed by crushing of the fruit to produce a must (a slurry of pulp, skins and pips). Today, the must is treated with sulphur dioxide to kill the flora of wild yeasts and bacteria that lived on the skins of the ripening fruit. Then a chosen strain of yeast is added to ferment the sugar. There are many variations to the basic process of wine production (Figure 18.25) by which the distinctive flavours and aromas (known as bouquet) of different wines are produced.

Figure 18.25 Red and white wine production



White wine is made from white grapes (or juice only from red grapes).
Red wine is made from red grapes – the 'must' (with skins) is fermented.

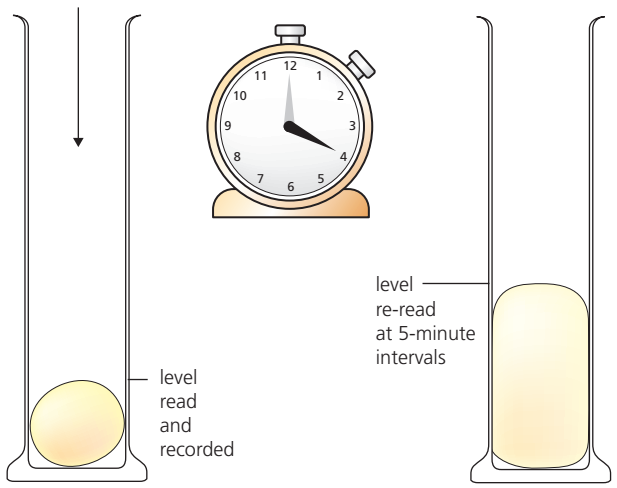
Bread making

Figure 18.26 Yeast and the rising of dough

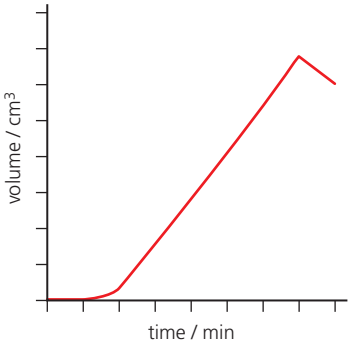
Dough is a mixture of flour, sugar, water and bakers' yeast. Worked into a ball and then kept in a warm place, the dough expands. This is due to the CO_2 released from the yeast respiration and trapped in the dough.

Dough mixture:
- 1 part bakers' yeast
- 1 part sucrose
- 6 parts flour
- 4 parts water.

Work into a dough.

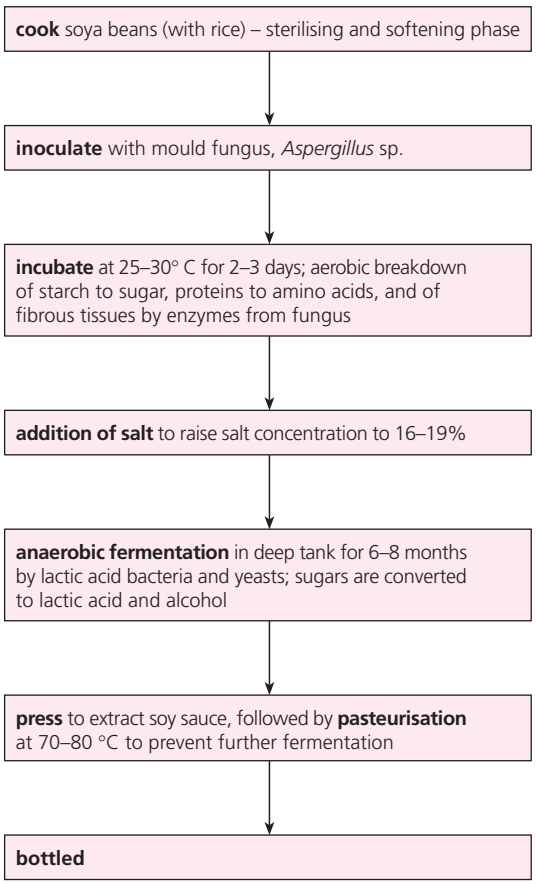


change in volume of dough against time



What would be the effect of:
- using different sugars?
- varying the temperature (measuring cylinder clamped in water bath)?
- using yeast killed by heating?

Figure 18.27 Production of soy sauce



Bread is made from flour and water, and smaller proportions of sugar, salt, yeast, vegetable fat, emulsifier and vitamin C. By baking, bread becomes a solid foam, having many tiny pockets of carbon dioxide distributed throughout its structure. First, ingredients are mixed or kneaded. Then they are left to ferment, during which process, starch in the flour is largely turned to sugars, and fermentation of sugars is begun. Also, the proteins of flour, known as gluten, become hydrated and form silky, elastic fibrils. Bubbles of carbon dioxide are retained in the dough, due to properties imparted by the hydrated gluten.

The dough is cut into loaf-sized pieces and allowed to ferment further. Subsequently the loaves are baked, during which all ethanol in the dough is driven out into the atmosphere. The effect of fermentation on the dough can be demonstrated and investigated experimentally (Figure 18.26).

Soy sauce production

Soya bean (*Glycine max*) is one of the oldest cultivated crops, originating in South East Asia. Soya is an example of a leguminous plant, housing *Rhizobium*

bacteria in root nodules. This has important consequences for the plant (and for those who feed on its seeds). Today it is grown very widely, and the beans are used mostly for oil, animal feed, and soya flour preparations for humans.

Various fermented foods have been derived from soya beans. Soy sauce is a food flavouring and colouring agent derived from soya beans by fermentation brought about by the common mould fungus *Aspergillus* sp. (Figure 18.27).

Food preservation

Microorganisms are ubiquitous, so their presence on food is inevitable. Consequently, if food items are not consumed as soon as they have been prepared, decay by microorganisms must be prevented in some way if the food is not to deteriorate. Figure 18.28 summarises possible approaches to food processing for preservation. For any particular type of food, the method of preservation chosen must maintain the original character (flavour and appearance), as well as nutritive value, as far as possible.

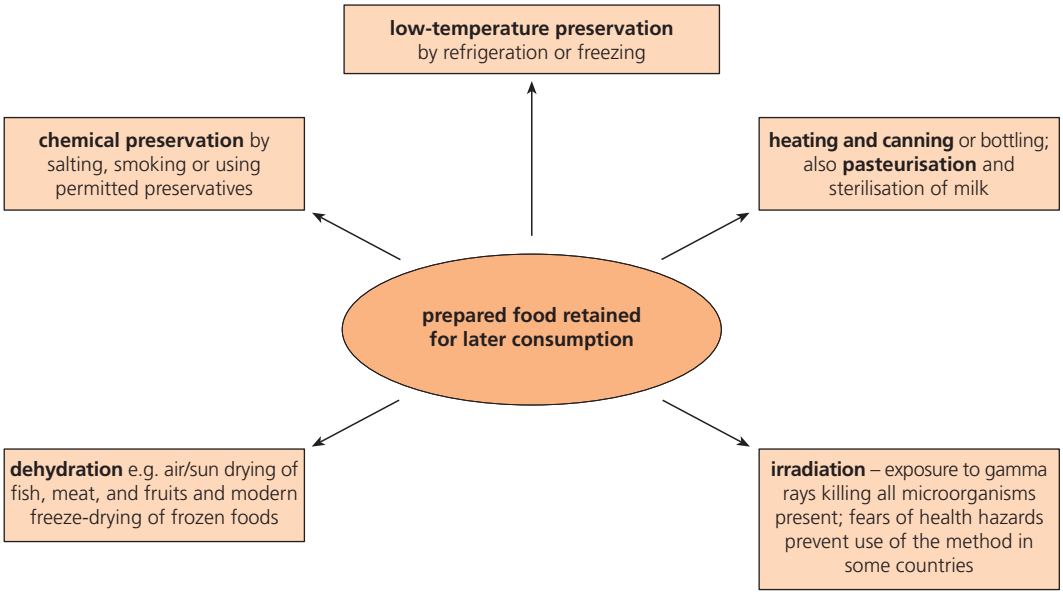


Figure 18.28 Techniques of food preservation

We can illustrate the process of food preservation by examining the uses of **salt**, **sugars** and **acids**.

Common salt, **sodium chloride**, is probably the oldest preservative, used especially with meat and fish. Traditionally, foods are soaked in a concentrated solution of rock salt. Sodium chloride (the major ingredient of the rock salt) dissociates to form sodium ions and chloride ions, and these ions strongly attract water molecules (page 27). Much more water diffuses from the cells and tissues than diffuses in and the food material is dehydrated. Any bacterium present (and any entering subsequently) is destroyed by dehydration, because the salt penetrates deep into the food.

Rock salt contains small amounts of nitrates. Bacteria in meat turn nitrates to nitrites, and nitrite ions combine with haemoglobin, forming nitrohaemoglobin. This substance gives the meat a strong pink colour, even when cooked. The permanent pink colour of meat tissue preserved in this way shows that the salts have reached all tissues, and the meat is safe to eat. Nitrates and nitrites are frequently used in the preservation of meat products.

Sugars are used as a preservative of fruits, especially in jams. A high concentration of sugar causes dehydration by osmosis and thus destroys any microorganisms that land on the jam and attempt to grow.

Pickling in **vinegar (ethanoic acid solution)** is another traditional method of preserving food. Here it is the very low pH that inhibits the growth of microorganisms, particularly bacteria. Most food-spoilage bacteria do not grow at pH values below 5. Foods commonly pickled include vegetables such as cabbage (sauerkraut), and some meats and fruits.

An alternative to adding acid is to allow acidity to develop in the food by the action of bacteria, such as the lactic acid forming bacteria, ethanoic acid bacteria, and proprionic acid bacteria. However, these organisms cannot lower the pH below pH 4, so the shelf-life of food preserved in this way is limited. Yoghurts, for example, are kept under refrigeration.

11 **Identify** the possible human health risks of specifically using sugar, salt and an organic acid as food preservatives.

Food poisoning (enteritis) caused by a bacterium

Enteritis is an **acute disease** (i.e. arising suddenly, but of short duration) that results from the ingestion of food containing certain microorganisms (usually bacteria) or microbial toxins. The organisms involved may reproduce themselves in food (e.g. when prepared food is not refrigerated) or in the host (the human patient). The linings of the small and large intestine are where the bacteria or its toxins have their effects. Typically, the patient develops abdominal pain and diarrhoea, with or without vomiting and fever.

The rise and rise of food poisoning

The number of reported cases of food poisoning has been rising at an accelerating rate in many developed countries (Figure 18.29). Possible reasons include:

- fewer meals are prepared and cooked at home, and more ready-meals are bought in;
- more fast-foods are eaten while on the move, with little opportunity to wash hands;
- demand for cheaper food has lead to intensive farming of livestock, followed by slaughter at centralised abattoirs with larger throughputs of carcasses;
- changes (mutations) in the microorganisms themselves;
- greater public awareness of food poisoning symptoms, improved diagnosis by doctors, and better detection at hospital pathology labs, may mean more cases are now reported and recorded.

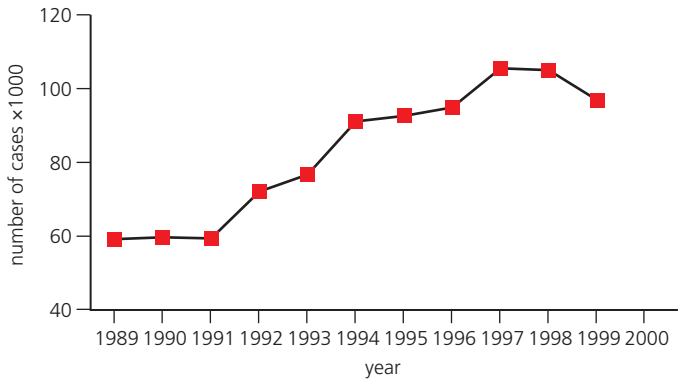


Figure 18.29 Food poisoning cases in a Western European country in recent years

Staphylococcus aureus – a food poisoning organism

Staphylococcus aureus is a Gram-positive coccus, capable of producing toxins in contaminated foods. People who handle cooked foods (e.g. cream-filled pastries, chicken and other meat products, puddings and creamy salad dressings) with contaminated hands may introduce the bacteria. However, if this food is kept refrigerated after preparation, the bacteria will fail to multiply. But if the food is left in a warm place (such as a kitchen or out of doors at a picnic), then *Staphylococcus* will grow at an alarming rate, and will secrete toxins into the food. If the contaminated food is eaten, nausea, vomiting and diarrhoea occur in 1–6 hours (Figures 18.30 and 18.31). Note that re-cooking the contaminated food does not remove these toxins – they are heat stable.

Figure 18.30 How *Staphylococcus aureus* causes food poisoning

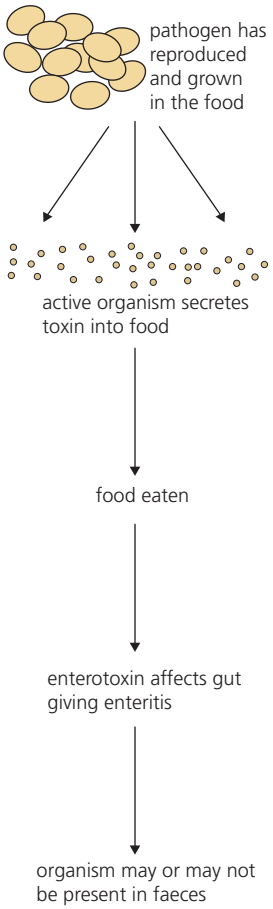
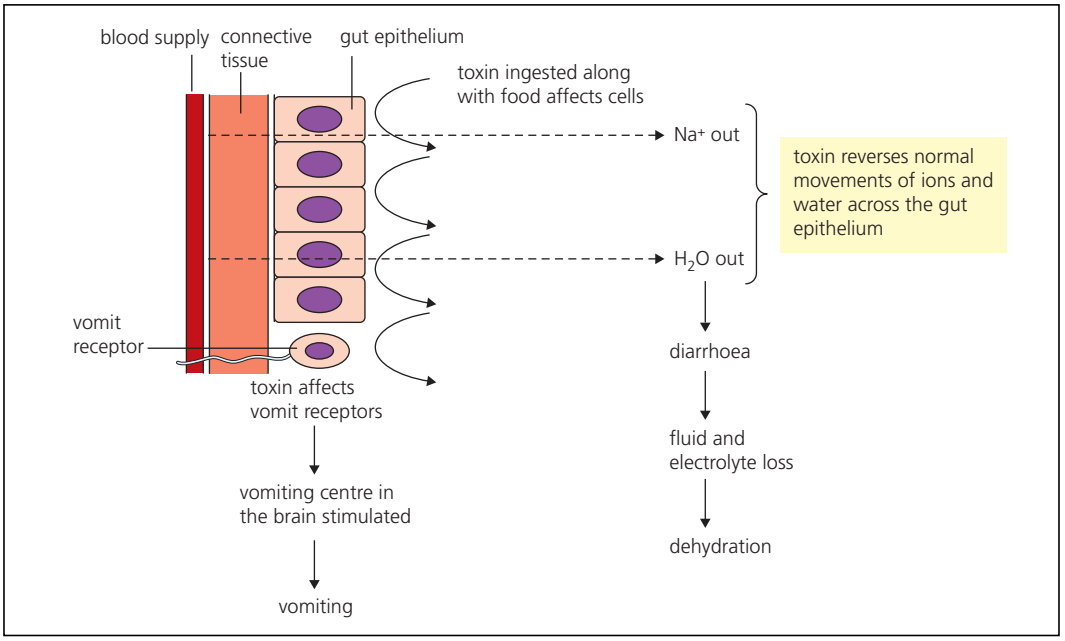


Figure 18.31 What happens when *Staphylococcus aureus* toxin is ingested



Treatment of food poisoning

Patients with food poisoning suffer from mild (or possibly severe) dehydration if vomiting and diarrhoea continue for some time. The need is to minimise discomfort from the fever experienced, and to maintain body fluids as and when possible.

In extreme cases, providing clean water may not be enough and oral administration of a dilute solution of electrolytes may be needed to make good the fluid and ions lost from the body. Any additional treatment with antibiotics is normally inappropriate – the bacterium has had its effects through the action of its toxins, which will progressively subside. Fluid (and electrolyte) replacement alone should restore normal body functions, with time.

12 Explain why significant losses in ions and salts in cases of enteritis pose a serious short-term threat to health.

TOK Link

A huge number of microorganisms inhabit the human body, together with occasional visitors, including unpleasant pathogens. Given the plethora of microorganisms about our bodies, how may a particular disease be correlated with the presence of a causative pathogenic bacterium?

Extension: Prevention is better than a cure!

In the issue of food poisoning, prevention requires high standards of hygiene during storage of food and during the preparation of meals to ensure that when consumed, food is free from pathogenic bacteria. The importance of using hygienic methods in food handling arises from the ubiquitous presence of dangerous microorganisms and in the speed with which infected food can become a source of toxins. The practical steps concern the avoidance of initial contamination, the prevention of multiplication of microorganisms, and the thorough cooking of food to be eaten hot (or later, when cool).

Metabolism of microorganisms

F5.1–5.6

Nutrition is the means by which organisms obtain the **energy** they require, and a source of **carbon** for the organic compounds they need. In biology of the eukaryotes, our focus has largely been restricted to the contrasting modes of nutrition of green plants (photosynthesis), and of animals and fungi (various forms of heterotrophic nutrition). Diversity in metabolism is a major feature of prokaryotes (both the Eubacteria and Archaeabacteria); indeed, they show greater variety in nutrition than the eukaryotes do.

Depending on how they obtain nutrients, prokaryotes can be divided into four major categories: photoautotrophs, photoheterotrophs, chemoautotrophs and chemoheterotrophs.

Photoautotrophs are organisms that use light energy to generate ATP and to produce organic compounds from inorganic substances.

An example is the cyanobacterium *Anabaena* (Figure 18.32). This cyanobacterium is found in fresh-water habitats. (*Anabaena* is also an example of a free-living nitrogen-fixing organism.)

Photoheterotrophs are organisms that use light energy to generate ATP, but obtain organic compounds from other organisms.

An example is the purple non-sulphur bacterium *Rhodospirillum*, an organism found in mud, lake water and sewage.

Chemoautotrophs are organisms that use energy from chemical reactions to generate ATP and produce organic compounds from inorganic substances.

An example is *Nitrobacter*, a soil bacterium that releases energy from the oxidation of nitrites to nitrates, and so plays a role in the nitrogen cycle (page 564).

Chemoheterotrophs are organisms that use energy from chemical reactions to generate ATP and obtain organic compounds from other organisms.

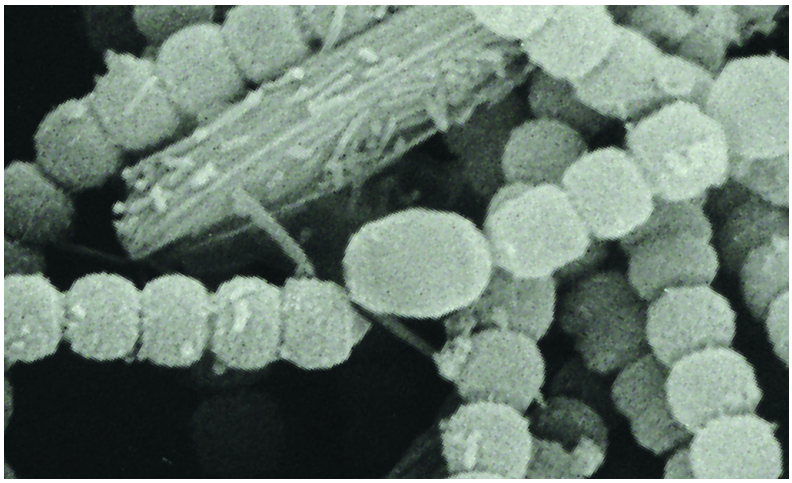


Figure 18.32 SEM of an *Anabaena* filament showing photosynthetic cells and occasional, larger heterocysts (site of nitrogen-fixation)

Very many of the bacteria belong in this huge group of microorganisms that decompose organic matter as and when it becomes available to them, ultimately enabling the recycling of nutrients for the benefit of the remainder of the food chain. *Escherichia coli* is an example – just one of the very many bacteria found in the human gut. Here, the metabolism of *E. coli* involves the fermentation of sugars.

13 Draw and **label** a diagram of the filamentous cyanobacterium, *Anabaena*, as shown in the SEM in Figure 18.32. (The guidelines for drawing scientific illustrations on page 10 may be helpful.)

Comparing photoautotrophy and photoheterotrophy

Photoautotrophs, such as the cyanobacterium *Anaebena*, and photoheterotrophs, such as the purple non-sulphur bacterium *Rhodospirillum*, are both prokaryotes and so do not contain chloroplasts. Their photosynthetic pigments (mainly chlorophyll *a* in the cyanobacteria, and bacteriochlorophyll in the non-sulphur bacteria) are located in simple membrane systems called mesosomes that are intuckings from the plasma membrane.

The steps of photosynthesis in these prokaryotes differ, too (Table 18.4).

In the **cyanobacteria**, carbon dioxide is the source of carbon, and this molecule is reduced to carbohydrate, using electrons obtained by the splitting of water. Oxygen is evolved as a waste product. The cyanobacteria are aerobic organisms.

Photosynthesis in the cyanobacteria (in summary):

$H_2O + CO_2 \rightarrow [CH_2O] + O_2$ where $[CH_2O]$ represents carbohydrate

In the **purple non-sulphur bacteria**, light energy is used to synthesise ATP (cyclic photophosphorylation, page 290), but **organic carbon is the carbon source**. The purple non-sulphur bacteria are anaerobic organisms, typically found in habitats where oxygen is absent or at very low concentrations. They also cannot survive in high concentrations of hydrogen sulphide, hence their name.

Table 18.4 A comparison of photoautotrophic and photoheterotrophic metabolism

	Photoautotroph (e.g. the cyanobacterium *Anabaena*)	Photoheterotroph (e.g. purple non-sulphur bacteria *Rhodospirillum*)
Energy source	■ light energy is used to obtain electrons from H_2O ■ light energy is also used to generate ATP from ADP + P_i ■ respiration is aerobic	■ light energy to remove electrons from organic molecules ■ light energy is also used to generate ATP from ADP + P_i, by cyclic photophosphorylation ■ respiration is anaerobic
Carbon source	■ CO_2 is reduced to carbohydrate	■ a variety of sources (e.g. organic or amino acids) are reduced to carbohydrate

Comparing chemoautotrophy and chemoheterotrophy

The **chemoautotrophs**, like all autotrophs, use an external energy source to synthesise carbohydrates from carbon dioxide (Table 18.5). They are sometimes referred to as the chemosynthetic bacteria. They differ from photosynthetic bacteria in that they take energy from inorganic chemical reactions (*), not light energy, in order to manufacture carbohydrate from carbon dioxide and water:

$CO_2 + H_2O + ENERGY^* \rightarrow [CH_2O] + O_2$ where $[CH_2O]$ represents carbohydrate

As a consequence, they are commonly found growing in the absence of light.

It is some particular exergonic chemical reaction that each species of chemoautotroph catalyses. Examples of economically important chemoautotrophs include the iron bacteria that live deep in iron ore deposits, and the nitrifying bacteria found in soils, which are illustrated here:

$$2NH_3 + 3O_2 \xrightarrow{\textit{Nitrosomonas}\text{ spp.}} 2HNO_2 + 2H_2O + ENERGY^*$$

$$2HNO_2 + O_2 \xrightarrow{\textit{Nitrobacter}\text{ spp.}} 2HNO_3 + ENERGY^*$$

Chemoheterotrophs, like all heterotrophs, obtain complex nutrients from food molecules taken in from their environment (Table 18.5). These complex organic molecules are their source of carbon (and energy) – they metabolise these molecules into all the other metabolites the cell requires. (Chemoheterotrophs may be further classified on the basis of the particular range of organic molecules on which they are dependent.)

Energy for metabolism is obtained by respiration of glucose and other metabolites absorbed. Very many chemoheterotrophs are aerobic, obtaining the energy required (as ATP) by aerobic respiration. Others are anaerobes, and obtain ATP by fermentation of various substrates.

Table 18.5 A comparison of chemoautotrophic and chemoheterotrophic metabolism

	Chemoautotrophs (e.g. *Nitrosomonas*)	Chemoheterotrophs (e.g. *Acetobacter* – aerobic; *Clostridium* – anaerobic)
Energy source	■ inorganic chemical reactions catalysed as the source of energy for synthesis of carbohydrate ■ respiration is the source of ATP for metabolism	■ complex organic molecules taken into the cell ■ respiration is the source of ATP for metabolism
Carbon source	■ CO_2 is reduced to carbohydrate	■ complex organic molecules taken into the cell

Bacteria and the bioremediation of the environment

Bioremediation is the process of exploiting microorganisms in the removal of pollutants from the environment. For example, we see this activity in the spontaneous removal of engine oils that drip from machines and engines, cars and other vehicles. These commonly seen deposits accumulate on nearby surfaces, but are normally removed (almost unnoticed by us), due to their degradation by naturally occurring (chemoheterotrophic) bacteria, provided the deposits are kept moist by occasional rain. A wide range of naturally occurring, ubiquitous prokaryotes and unicellular eukaryotes are able to hydrolyse and oxidise mineral oil to carbon dioxide (Figure 18.33).

When a major spill from an oil tanker occurs, leading to massive oil slicks that eventually reach seashore habitats, it has been found that about 80% of the non-volatile components of the oil may be oxidised and removed within a year of the pollution event. While vast numbers of marine and littoral species of non-vertebrates are destroyed by the hydrocarbon pollutants in the initial disaster, removal of oil slicks may be speeded by spraying the oil with essential inorganic nutrients, chiefly phosphates and nitrates, which aid the saprotrophic bacteria, and thus speed up bacteriological oxidation.

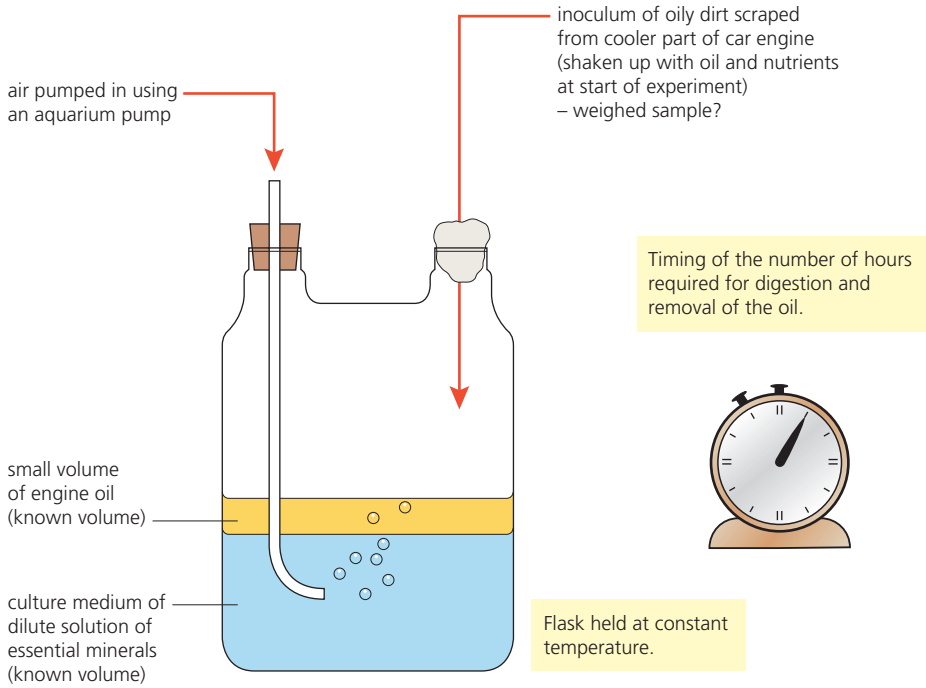


Figure 18.33 Investigating the degradation of mineral oil spills

Bioremediation of soils

Pesticides are products of the agrochemical industry that are very widely used to control harmful organisms that are a danger to crops or herds. Their use extends to the horticultural industries that are not organically orientated, of course, and the use of pesticides has been enthusiastically adopted by many people who maintain gardens in developed countries.

Modern pesticides are substances designed to kill specific types of pest, including plant weeds (herbicides), insects (insecticides), fungi (fungicides), and slugs and snails (molluscicides). Pesticides have enormously improved productivity in agriculture, but their use has generated problems in the environment. Herbicides are the most widely used group of pesticides, but insecticides are also widely used and they may cause the greater problems for humans.

Pesticides are a wide range of different compounds. Many of them are organic molecules suitable as carbon sources and electron donors for various soil microorganisms, but others are not. Those that can be biodegraded are eventually removed from soils (Table 18.6), but their breakdown may not be entirely due to the activity of microorganisms. Some substances leach away into underground water, and others may spontaneously degrade with time.

Substance	Time for disappearance from soil
Herbicide: 2,4-D	4 weeks
Herbicide: 2,4-T	20 weeks
Insecticide (chlorinated compound): DDT	4 years
Insecticide (chlorinated compound): Aldrin	3 years
Insecticide (organophosphate compound): Malathion	1 week
Insecticide (organophosphate compound): Parathion	1 week

Table 18.6 Persistence of a selection of pesticides

Industrial solvents, now widely used in developed countries at least, are organic compounds such as dichloroethylene, trichloroethylene, chloroform, and some brominated and fluorinated related compounds, to name but a few. These toxic substances (some are also suspected of being carcinogens) are frequently detected as persistent contaminants of ground waters. This is a well documented problem in parts of the USA, for example.

A variety of different bacteria are able to break down these molecules, including the removal of the chlorine component. For example, the bacterium *Dehalobacterium* uses the compound dichloromethane in its metabolism, producing essential respiratory substrates, formate and acetate, as follows (in summary):

$$3CH_2Cl_2 + CO_2 + 4H_2O \rightarrow 2CHOOH + CH_3COOH + 6HCl$$

Substrate-level phosphorylation ($ADP + P_i \rightarrow ATP$, page 272) occurs in the steps of both formate and acetate formation.

In areas where the underground waters are seriously polluted, the activities of natural populations of bioremedial microorganisms may be enhanced at the site by drilling wells and vents so that micronutrients and sources of oxygen (as compressed air or peroxide solutions) can be added for the benefit of the microorganisms. The natural clean-up reactions are accelerated in this way, although the process may take months and years to complete. No doubt the dispersal of the pollutants and the depth of the geological strata they occupy is also a factor.

Selenium and bacteriological action

Reactions between microorganisms and certain inorganic elements and compounds, are not always advantageous to humans. For example, some microorganisms may alter the physical characteristics of metals and metalloid elements like arsenic and selenium, in the course of their metabolism.

The metalloid element selenium is not itself toxic to microorganisms. However, some may inadvertently convert inorganic selenium to a methylated form in the processes of their metabolism. In this form, selenium is easily taken up by organisms of local food chains. When methylated selenium is absorbed by mammals and other vertebrates, it is transported in the blood stream and is able to cross the blood–brain barrier. In the brain, this compound causes neurological damage that can be fatal.

Microbes and disease

F6.1–6.10

Disease is generally defined as an unhealthy condition of the body. Many diseases are due to parasitic organisms that invade the body, and are referred to as **pathogens**. The diseases caused by them are called **communicable diseases** because the pathogen may be passed from host to host.

Microorganisms cause the majority of communicable diseases, but of course, relatively few of the vast numbers of microorganisms are pathogens (most are free-living saprotrophs). Despite this, pathogenic microorganisms generally appear to have earned microorganisms a bad name; people often equate bacteria with disease, despite the many ways our quality of life and environment are maintained by activities of microorganisms.

The infectious disease cycle and the transmission of pathogens

The steps of an infectious disease event are:

source of pathogen → transmission → establishment in host → exit from host/fresh infection.

A major feature of this cycle of events is the transmission between hosts and the process of infection. Very few pathogens are able to move between host organisms by their own means; most rely on some method of transport to a new host. The methods of disease transmission are listed in Table 18.7.

Method of transmission	Examples
contact	■ clothing–skin contacts or direct skin contact (e.g. **ringworm**, caused by a fungus, *Microsporum*) ■ sexual contact (e.g. **chlamydia**, caused by a bacterium, *Chlamydia trachomatis*) ■ contact with bathing water with aquatic larvae of parasite (e.g. **schistosomiasis**, caused by blood fluke, *Schistosoma*)
droplet – in air currents	■ sneezes from infected people (e.g. **influenza**, caused by influenza virus)
dust – in air currents	■ sneezes by infected people creating contaminated dust that persists in ill-ventilated enclosures (e.g. **tuberculosis** (**TB**), caused by a bacterium, *Mycobacterium tuberculosis*)
food and drink	■ prepared, contaminated food left in warm conditions (e.g. **food poisoning**, caused by a bacterium *Staphylococcus aureus*) ■ drinking water contaminated by sewage (e.g. **cholera**, caused by a bacterium *Vibrio cholerae*)
via other vehicles	■ contaminated surgical instruments such as hypodermic needles (e.g. **hepatitis B**, caused by a virus of same name) ■ contaminated blood transfusion (e.g. **AIDS**, caused by human immunodeficiency virus)
vector	■ external transmission (e.g. **salmonella food poisoning**, by a housefly transferring a bacterium such as *Salmonella enteridis*) ■ internal transmission (e.g. **malaria**, by a female mosquito taking blood meal and transferring protozoan parasite, *Plasmodium vivax*)

Table 18.7 How pathogens are transmitted between hosts

Profiles of infections

Some pathogens cause disease only after successful penetration of host cells (intracellular pathogen). Some diseases are caused by toxins from pathogens, so the pathogen merely has to be present in an area of the body, such as the gut (extracellular pathogen).

Chlamydia, an intracellular pathogen

Chlamydia trachomatis is an intracellular bacterial parasite of cells, initially cells of the urinogenital tract, and is transmitted during sexual intercourse. It causes a condition known as chlamydia. This has become the commonest sexually transmitted disease of the developed world. Symptoms vary, depending on the precise sites of infection, but typically infection with this organism makes urination painful, and if the conjunctiva of the eyes are infected, results in painful red eyes. Infection of the ovarian tubes causes abdominal pain; infection of the testes causes swelling and pain.

Streptococcus, an extracellular pathogen

Streptococcus pyrogens is an extracellular bacterial parasite widely distributed in humans. Most infected individuals are merely carriers who show no symptoms of disease. When patients succumb to a 'strep' infection it may be an infection of the skin (such as impetigo or other skin infection), or scarlet fever, or a sore throat, or a form of pneumonia, for example.

The types of toxin that may cause disease

Many disease-causing microorganisms have their adverse effects via the toxins they produce. Disease-causing toxins may originate within the pathogen and be secreted, or exist on its outer surface. Either way, toxins are molecules that interfere with normal host body cell functioning, sometimes doing this at the site of infection, and sometimes more widely in the body.

Toxins are classified as **exotoxins** and **endotoxins** (Figure 18.34).

Exotoxins are soluble proteins (often enzymes) secreted by the pathogen and causing specific symptoms. Exotoxins may travel from the site of infection to other tissues where they have their effects. Some of the most lethal substances known are exotoxins. For example, **diseases caused by exotoxins** include **botulism** and **tetanus**.

- The neurotoxin **botulin** is secreted by *Clostridium botulinum*, an anaerobic bacterium of soil and pond mud. When consumed in contaminated food that has been inadequately cooked, botulin binds to the synapses of motor neurones and prevents release of neurotransmitter substances. As a result, muscles cease to contract and paralysis results.
- Tetanus is caused by *Clostridium tetani*, an anaerobic bacterium of soil and animal faeces. When it contaminates deep anaerobic wounds, it releases a neurotoxin that interferes with synapses in the spinal cord and motor nerves. The result is uncontrolled muscle contraction leading to muscular spasms that affect the whole body, but which in particular cause lock jaw and breathing difficulties.

Endotoxins are lipopolysaccharides on the **outer surface of the bacterium wall** of Gram-negative bacteria (page 558). Endotoxins are heat stable, but generally toxic only at high doses. They have their effects in several ways. Typically they may cause fever, shock, blood coagulation, diarrhoea, or inflammation. **Diseases caused by endotoxins** include **Salmonella food poisoning**:

- *Salmonella enteritidis*, a pathogen that invades the gut and introduces a toxin there, causes a food-based infection. The symptoms are a sudden onset of headaches, chills, vomiting and diarrhoea. At this stage, the toxin is mostly still attached to the bacterial walls. This is followed by a fever that lasts a few days, and is caused when the dislodged toxin reaches the blood circulation. Even at this stage, the bacterium itself remains in the lumen of the gut, without invading cells.

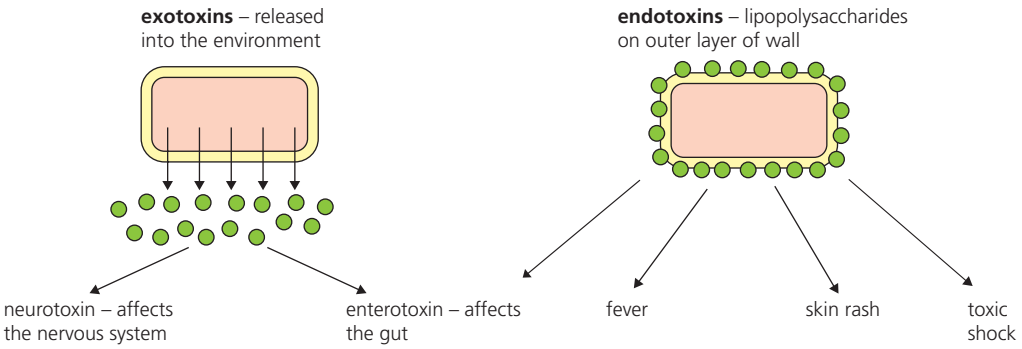


Figure 18.34 Exotoxins *versus* endotoxins

Disease prevention by avoidance of contamination

The prevention of infection by pathogenic microorganisms requires their inhibition and removal from the environments of people (or animals) vulnerable to the diseases they cause. Similarly, in laboratories where microorganisms are cultured, and places where food is manufactured and packaged, bacterial contamination has to be prevented. In premises where drugs, surgical equipment and related items are prepared and packaged, the same issue is uppermost. There are a number of ways this is tackled.

Disinfectants

Disinfectants are chemical agents used to check and prevent growth of microorganisms, typically on the surface of objects and equipment likely to be contaminated. Ideally, a disinfectant is effective against a wide variety of microorganisms, Gram-positive and Gram-negative bacteria and others, for example, together with dormant spores, fungi and viruses.

At the same time, disinfectants should not be toxic to humans and should not react with or corrode surfaces or equipment.

A wide spectrum of substances have been used. **Phenol** was the first disinfectant (and antiseptic), used by Joseph Lister in 1867, when he introduced the idea of preventing contamination of wounds during surgery (antiseptic surgery). Today, Lysol, a mixture of phenolics, is available as a commercial disinfectant. Phenolics have the advantage of remaining active on surfaces for a long time after application, but they leave a strong unpleasant odour and cause skin irritation.

Alcohols are some of the most widely used disinfectants today, often ethanol or isopropanol at 70–80% concentration. The five **halogen elements** (particularly, chlorine, bromine and iodine) are important antimicrobials, too. **Chlorine** is used in public water supplies, and has applications in food industries, for example. **Iodine**, complexed with an organic carrier to form iodophor, is widely used in hospitals and laboratories.

Antiseptics

Antiseptics are chemical substances applied to living tissues to prevent infection; they kill microorganisms present and inhibit growth of any pathogen. An antiseptic should not irritate the patient's skin, nor kill tissues, so necessarily, antiseptics are generally less toxic substances than disinfectants (or used at lower concentration). Most antiseptics are used for hand washing, and for treating surface wounds. Common antiseptics are alcohols at 60% or higher concentration, hydrogen peroxide at 3% solution, some phenol-containing compounds and iodine preparations.

Pasteurisation

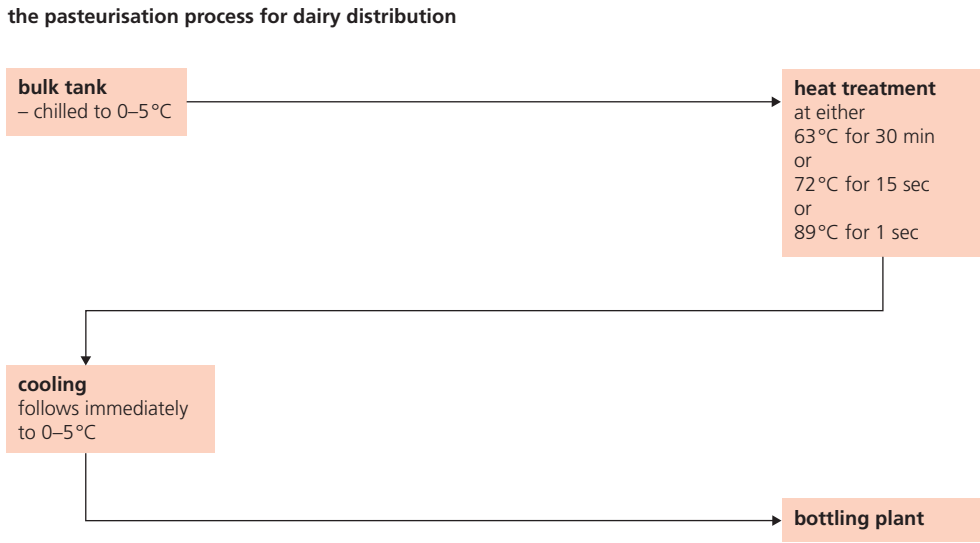
Pasteurisation is the process of reducing the microbial population of fresh milk and other heat-sensitive liquid foods. The name of this procedure acknowledges the genesis of this technique – first devised by Louis Pasteur to kill microorganisms in wine.

Heat treatment of milk is necessary since it is an ideal medium for the growth of microorganisms, and it is a difficult product to keep free from contamination from the air. Occasionally, an unhealthy cow may be the source of harmful bacteria, too, including *Mycobacterium tuberculosis* (causing TB), *Brucella abortus* (causes brucellosis), or *Streptococcus pyogenes* (causing sore throats and scarlet fever).

Pasteurisation of milk (treatment at 72 °C for 15 seconds, then rapidly cooled to below 10 °C) kills over 99% of the bacteria present. Pasteurised milk keeps longer, too, because bacteria that turn lactose (milk sugar) into lactic acid (which causes milk to sour) are also killed off (Figure 18.35).

Alternatively, milk can be rendered bacteriologically sterile by ultra-high temperature (UHT) treatment (homogenised, then heated to 132 °C for 3 seconds, then cooled). However, this treatment alters the protein content of the milk.

Figure 18.35 Pasteurisation of milk



Irradiation

Some forms of electromagnetic radiation are harmful to microorganisms, including two forms of ionising radiation:

- **X-rays**, produced by an X-ray source;
- **gamma rays**, emitted during certain forms of radioactive decay.

High levels of ionising radiation kill microorganisms outright. This form of energy brings about many disabling chemical changes in cells, but the destruction of DNA is the most damaging. Its usefulness as a sterilisation agent is its penetration deep into objects. Gamma radiation emitted from a cobalt-60 source is used to sterilise drug solutions and equipment, including disposable supplies such as syringes, following their manufacture. It can be used to sterilise food, too, but this is not widely applied to date.

Ultra-violet radiation, particularly at 260 nm wavelength, also kills all forms of microorganisms, also by inactivating their nucleic acids. Actually, little UV light below 300 nm wavelength reaches the Earth's surface because of the action of the upper ozone layer. However, damage to this ozone shield has followed on certain forms of atmospheric pollution in recent times (page 627). UV lamps are used in bacteriological laboratories, particularly in inoculation cabinets, but care is taken to ensure humans are not exposed, particularly since the radiation will damage skin and eyes. UV light does not penetrate through glass or far into water, but it is effective in the destruction of microorganisms in water when a thin film is passed under an appropriately powered source.

14 Design an experiment to demonstrate that microbes, freely circulating in the air, are able to contaminate exposed matter.

Antibiotics – their mechanisms of action

Many bacterial infections can be treated with antibiotics because antibiotics in low concentration inhibit the growth of microorganisms. Most antibiotics are naturally occurring chemical substances obtained mainly from certain fungi and bacteria commonly found in the soil.

The first antibiotic to be discovered, isolated and developed (not an easy task – it took from 1929 to 1944) was penicillin. Over 400 different antibiotics have since been isolated, but only about 50 have proved not toxic to patients (they show **selective toxicity**), and so have achieved wide usage.

Antibiotics effective against a wide range of pathogenic bacteria are called **broad-spectrum antibiotics**, and these include chloramphenicol and tetracyclines. Others, including penicillin and streptomycin, are **effective against a limited range of bacteria**.

How do useful antibiotics terminate a bacterial infection without harming the mammalian host?

Antibiotics specifically damage bacterial pathogens by disrupting one of three major aspects of the growth or metabolism of bacteria that occur in ways that are more or less specific to prokaryotes. Since the particular components, metabolites or enzymes concerned are not found in eukaryotic cells in the same form, the antibiotic is not toxic to the mammalian host tissues.

Cell wall synthesis inhibition

The most effective antibiotics work by interfering with the synthesis of bacterial cell walls (Figure 7.15, page 194). Once the cell wall is destroyed, the delicate plasma membrane of the bacterium is exposed to the destructive force generated by excess uptake of water by osmosis, and possibly also to attack by antibodies and phagocytic macrophages.

Several antibiotics, including penicillin, ampicillin, and bacitracin, bind to and inactivate specific wall-building enzymes. These are the enzymes required to make essential cross-links between the linear polymers of the walls, in particular species. In the presence of the antibiotic, wall polymers continue to be synthesised by the pathogens, but the individual strands are not linked and bound together. The walls fall apart.

Protein synthesis inhibition

Other antibiotics inhibit protein synthesis by binding with ribosomal RNA. The ribosomes of prokaryotes (known as 70S) are made of particular RNA subunits, together with many polypeptides (which mainly function as enzymes). The ribosomes of eukaryotic cells are larger (80S), and are built with different RNA molecules and polypeptides. Antibiotics like streptomycin, chloramphenicol, tetracyclines, and erythromycin all bind to prokaryotic ribosomal RNA subunits that are unique to bacteria. Here their presence causes protein synthesis to be terminated.

Nucleic acid synthesis inhibition

A few antibiotics interfere with DNA replication or transcription, or they block mRNA synthesis. These antibiotics – for example, the quinolones – are not as selectively toxic as other antibodies. This is because the processes of replication and transcription do not differ so greatly between prokaryotes and eukaryotes as wall synthesis and protein synthesis do.

The life cycle of the influenza virus

We have seen that the influenza (flu) virus contains eight short strands of RNA (a segmented genome), surrounded by a protein capsid, and external to this is a layer of lipid (Figure 18.13, page 563). Protein 'knobs' (antigens with specific names, traditionally shortened to H and N) project through the lipid layer.

The virus enters the human host's lungs via droplets inhaled through the mouth and nose. The epithelial cells lining the bronchus and bronchioles are the cells that the virus parasitises. Entry into epithelial cells occurs by endocytosis (Figure 18.36).

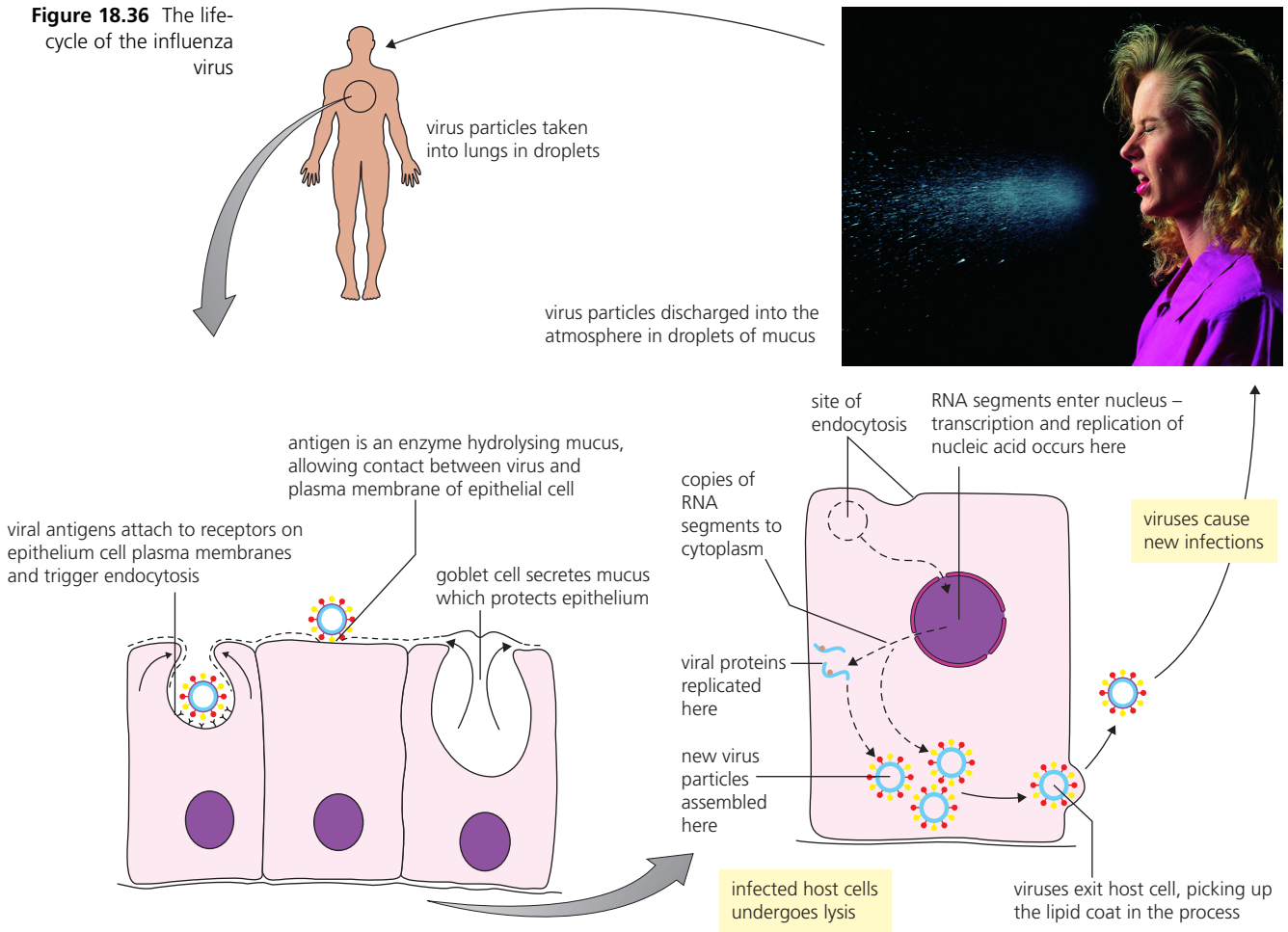


Figure 18.36 The life-cycle of the influenza virus

Then, replication of the virus occurs – new RNA segments are produced in the nucleus and the capsid proteins are produced in the cytoplasm of the host cell. New viral particles are assembled, and the lipid capsule is added as the virus exits the dying host cells.

The host cells now break down (lysis) and toxins are released at this stage. Destruction of the epithelial cells paves the way for serious secondary bacterial infections of the host lung tissues, typically bacterial pneumonia.

The flu life cycle (*if we can talk about a life cycle in a disease-causing agent that is not strictly described as living*) is described as lytic.

Influenza viruses are classified into antigenic groups according to the H and N proteins of their capsids. The chemistry of these antigenic proteins is controlled by the genome of the virus, which is potentially very variable. Alterations of the virus genome frequently arise, and they lead to new antigenic types and therefore to new strains of flu in humans. Sometimes, humans (or other hosts) have little or no resistance to the new strain. The immune system will not have previously met the new antigens. So, no memory cells (page 354) exist in the lymph nodes.

Because of this, flu is frequently a major epidemic disease; epidemics occur almost every year.

An epidemic disease is one of widespread occurrence in a community at a particular time.

However, strains arise from time to time with exceptional genes for virulence and transmissibility. A strain with just the right mix of virulence and transmissibility leads to a pandemic in which millions of humans die.

A pandemic disease is one that is prevalent over a whole country, continent, or the world.

During the last century there were three pandemics (Table 18.8), and it is inevitable that flu pandemics will occur again, from time to time.

Pandemic	Profile
Spanish flu 1918–9	■ Killed about 40 million people (compare the 10 million victims of the First World War – at the time called The Great War). ■ Nations struggled to cope; the end of the war was a time when resources were exceptionally stretched, and viruses were not understood. ■ Vaccines were targeted at bacteria. ■ Civilians were put in quarantine (but troop movements continued between continents). ■ High levels of personal hygiene were advocated.
Asian flu 1957–8	■ Killed about 1 million people. ■ Medical knowledge was more advanced than in 1918, but also, the strain was substantially less virulent. ■ Strain was identified and vaccines were produced, but it was not possible to produce sufficient. ■ Quarantine measures were used, again to little effect.
Hong Kong flu 1968–9	■ Killed about 1 million people. ■ By this date the WHO existed, and its global flu surveillance network gave early warning of an imminent pandemic as the disease spread from its origins in South East Asia. ■ Vaccines were developed quickly, but not enough could be produced in time to meet the full demand.

Table 18.8 The most recent past flu pandemics

The epidemiology of a flu pandemic

Epidemiology is the study of the occurrence, distribution and control of diseases.

There are three pre-requisites for a flu pandemic:

- **a novel virus subtype must reach human hosts** from its point of origin, and human immune systems must be unfamiliar with it;
- the virus must **replicate in humans** and cause disease there;
- the virus must be **efficiently transmitted between humans**.

You may remember the public discussions and anxiety about the bird flu epidemic that threatened us in 2005–06. That strain of flu was identified as H5N1. The interlocking flight paths of wild birds, migrating between seasonal feeding grounds in different parts of the globe, transmitted the infection among wild populations of birds, and sometimes passed it to farmed bird stocks. (Clearly there are many adverse features that underpin the onset of a pandemic which cannot be controlled.)

Some unfortunate people in countries spread as widely apart as the Far East and Eastern Europe, who had direct close contact with infected birds (and sometimes indirect contact), contracted the disease. For some, this exposure proved fatal.

The H5N1 strain of 2005, although extremely virulent, failed the third pre-requisite listed above – it was not a strain that was quickly and easily transmitted between individual humans, and so no pandemic ensued (at that time). Had the virus adapted in that way too, then it is highly likely that the next flu pandemic would have occurred at that point.

What measures are required in the face of a possible flu pandemic?

The international success experienced in controlling the SARS (severe acute respiratory syndrome) epidemic of 2003 confirmed the value of a **supranational health initiative**. Here, the role is to maintain oversight of the whole world scene, exchange updated information, warn countries most likely to next be in the path of the infection wave, and extol best practice. Currently, the World Heath Organization (WHO) and the technical agencies it liaises with fill this role. We have the essential early warning and response system that allows timely exchange of essential information.

15 Distinguish between:

a antibiotics and vaccines
b inflammation and immunity
c vector and host.

The technical difficulties in the **production of influenza vaccine** to the new antigens, as soon as these can be identified, at the required speed and in volumes that would be required, are the subject of continuing research. So, too, is the development of newer and more effective prophylactic drugs to maintain the resistance to infection of key medical and pharmaceutical staff, at the very least.

Equally important is the planning by governments concerning the **maintenance of vital national services** such as food and fuel supplies, and public safety, given that 25% or more of the adult population may be incapacitated at the height of a pandemic.

Malaria – caused by a protozoan, transmitted by mosquitoes

Malaria is the most important of all insect-borne diseases. About 80% of the world's malaria cases are found in Africa south of the Sahara, and here some 90% of the fatalities due to the disease occur (Figure 18.37). It is estimated that about 400 million people are infected, of whom 1.5 million (mostly children under 5 years old) die each year.

Malaria is caused by *Plasmodium*, a protozoan, which is transmitted from an infected person to another person by the blood-sucking mosquito *Anopheles*.

Transmission of *Plasmodium* by the mosquito and its effects on the host

Only the female mosquito is the vector (the male mosquito feeds on plant juices). *Anopheles* is a fluid-feeding heterotroph (not an ectoparasite, as sometimes stated). It detects its human host, lands, and inserts mouthparts (which are formed into a long thin proboscis) into a blood vessel below the skin surface. A meal of blood is taken quickly – there is a danger that an active, alert human will swat the insect. Mosquitoes tend to feed at night, on sleeping victims, but those that alight on patients already ill with malaria are likely to be able to feed unhindered (Figure 18.38).

For any blood-sucking insect, the mammalian blood-clotting mechanism presents a problem, and has to be overcome. As the mosquito's proboscis penetrates the vein, a secretion from its salivary glands passes down the hypopharynx and inhibits clotting. At this point *Plasmodium* may enter its human host (if the *Anopheles* carries an infective stage). Meanwhile, the *Anopheles* loads up with a blood meal. This is essentially 80% water, and excess water has to be lost from the body as a priority.

The effects of *Plasmodium* on the human host depend partly on the species of *Plasmodium* involved, and partly on how many previous bouts of malaria the patient has had and hence the degree of immunity that has developed.

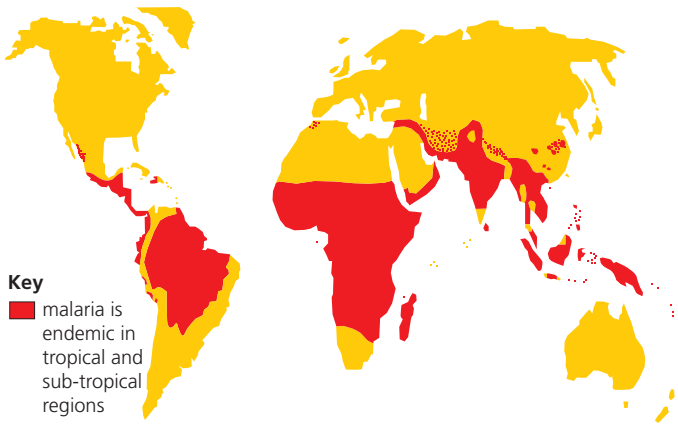


Figure 18.37 World distribution of malaria

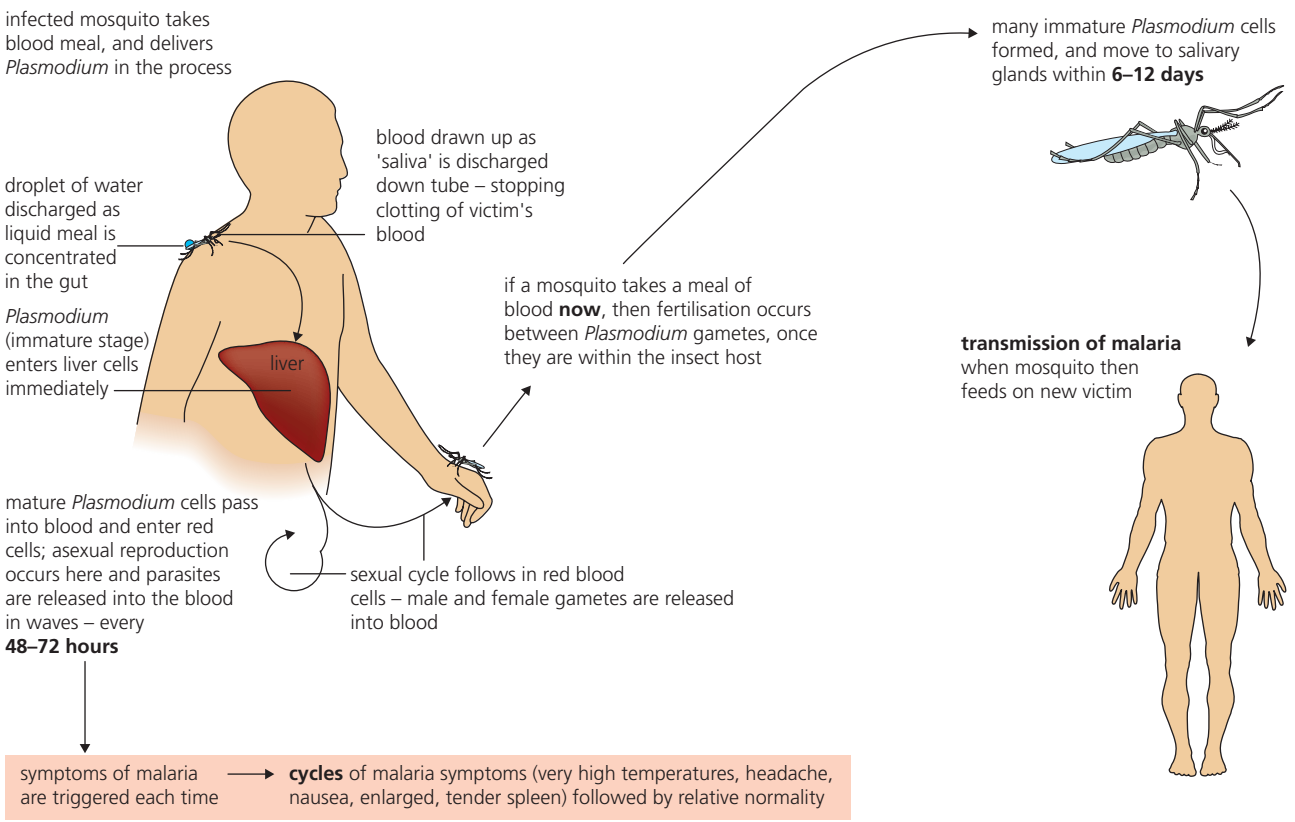


Figure 18.38 Anopheles feeding and the transmission of malaria

During the peak of infection, parasites are released from red cells into the plasma of the blood stream every two to three days, together with toxins. The body temperature rises to 40–40.5 °C, with intense fever symptoms. The spleen becomes enlarged and painful. Afterwards, the body temperature falls below normal, and there is profuse sweating.

Prions and the cause of encephalopathies

Proteins called **prions** are believed to be the agents that cause diseases known as encephalopathies in which the brain becomes spongy and forms holes where once there were neurones. The term 'prion' is a contraction, derived from **pr**ote**i**nace**o**us i**n**fectious particle.

The affected organisms, which may be human (with Creutzfeldt–Jacob disease – CJD), sheep (with scrapie), or cattle (with bovine spongiform encephalopathy – BSE), lose physical condition and eventually become totally unco-ordinated. The cow in Figure 18.39 had difficulty in standing. In humans, the memory is lost, as well as body control, prior to death.

Figure 18.39 A cow with BSE

What are prion proteins and how may they cause disease?

Prion proteins are natural components of cells of mammals and also of yeasts, but they probably occur more widely. Prions are, therefore, assumed to have some important role, but this is not yet understood. What has been established is that the normal tertiary structure of prion protein (**PrP^c**) consists of multiple α helices, but that this large molecule

can unfold and form into a different molecule (**PrP^{sc}**) where two of the helices become β pleated sheets (Figure 8.20, page 256). The prion is said to have flipped (Figure 18.40).

Once this change in shape has occurred, **PrP^{sc}** can trigger the same change in shape in normal **PrP^{c}** with which it is in contact. Then the mass of **PrP^{sc}** molecules apparently coalesce into insoluble fibrils. When this happens in neurones of the brain, neurone destruction occurs and the encephalopathy condition follows.

In 1997, Stanley Prusiner of the University of California at San Francisco was awarded a Nobel Prize for his work on his prion theory of encephalopathies. Other workers held that an as-yet undiscovered virus was involved. However, the infectious agent for encephalopathies remains infectious even after bombardment with radiation sufficient to destroy all DNA or RNA present (essential to a virus). Since proteins are not destroyed by radiation, prions are most likely to be responsible.

Encephalopathies are cannibalistic in origin

The first known encephalopathy disease was *kuru*, observed in people of Papua, New Guinea, whose custom it was to honour their dead by eating them. Men ate muscle tissue, but women and children received brain tissue. Only the latter eventually died of *kuru* – when the ancestor had also died of that disease.

Another prion disease with a long history is scrapie. Until recently, encephalopathies had not been known to jump the species barrier, say from sheep to cattle. Recent farming practices of using offal from sheep or cows in manufactured animal feed are likely to have spread encephalopathies among cattle. Also, pituitary extract prepared by vets for injection into other cattle may have been a source.

What allows the prions of one species to infect some but not all other susceptible species?

It may depend on how similar the primary structures of different prion proteins are. If the size and amino acid sequence of prions of two species are sufficiently similar, then they may infect both.

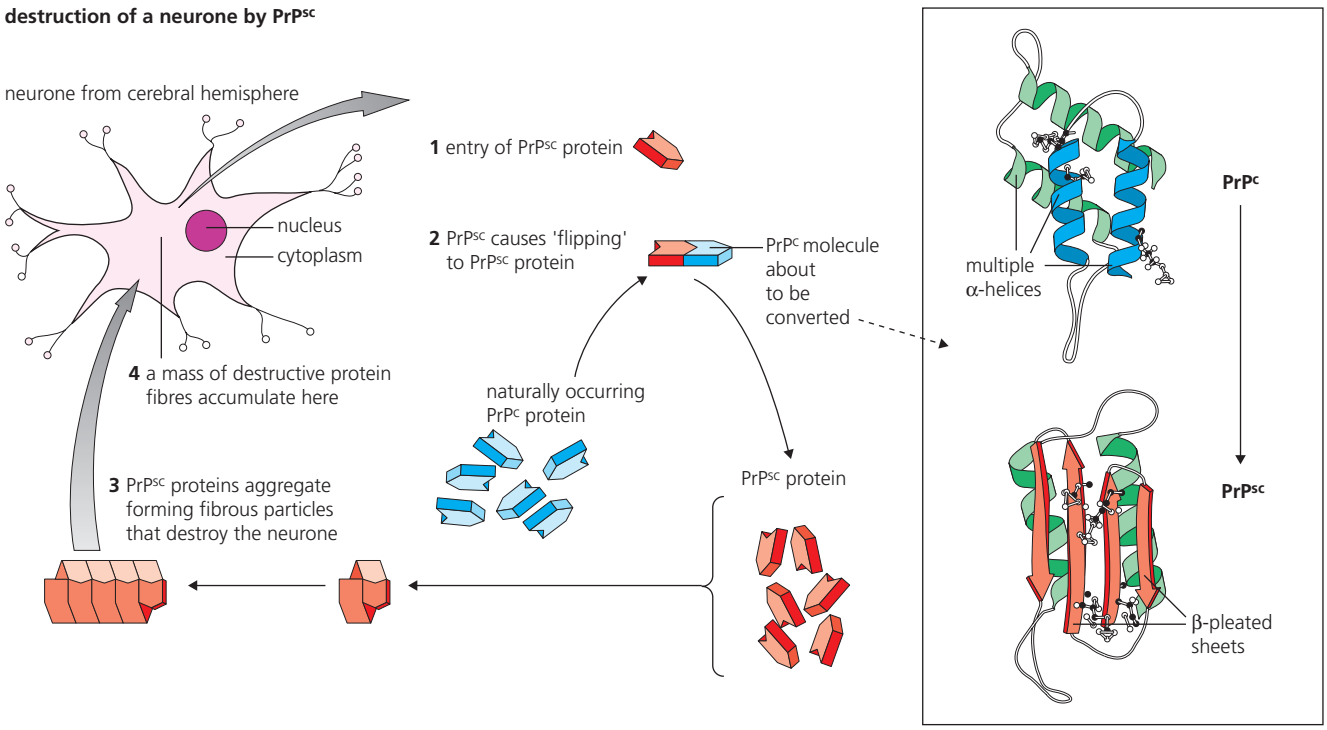
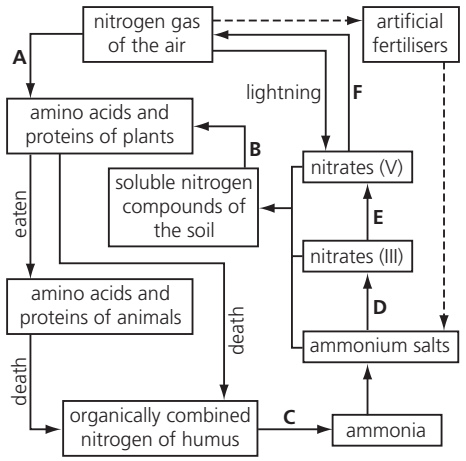


Figure 18.40 Prion protein flipping and the triggering of an encephalopathy

Examination questions – a selection

Questions 1–6 cover syllabus issues in this chapter (a new Option)**.**

Q1 The flow chart below represents the main events of the nitrogen cycle, in several stages of which microorganisms play a central role.



a State the different types of organism that may be involved in **step A** and where they occur within the environment. (6)

b Outline how the events of **step B** are brought about. (6)

c Identify the types of organism involved in **step C** and state where they may be found within the environment. (4)

d Describe how ammonium salts may be converted to nitrites (**step D**) and nitrites to nitrates (**step E**) in the soil. (6)

e Suggest typical soil conditions that bring about **step F**. (1)

f Part of the nitrogen cycle flow diagram is represented by dotted lines. Explain how these steps differ fundamentally from the remainder of the cycle. (2)

Q2 **a** Distinguish between *division* and *domain* in the classification system of living things. (4)

b Compare the characteristic features of cell structure and biochemistry by which the organisms of the three domains are differentiated. (6)

c Outline the distinguishing features of methanogens as archaebacteria. (4)

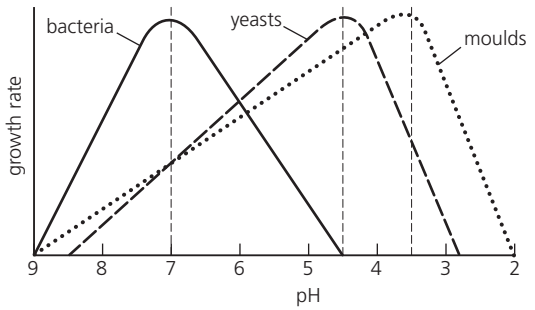
d Suggest why viruses are **not** classified within any domain. (2)

Q3 **a** Describe **two** ways in which the metabolism of saprotrophic microorganisms is exploited in the conversion of human sewage to safely disposable products. (8)

b Outline **one** method by which biomass may provide a source of fuel. (4)

Q4 The steps to genetic engineering are brought about with the aid of several naturally occurring enzymes – sometimes known as the genetic engineer's 'tool-kit'. Identify **four** of these enzymes, explaining their roles *in vivo* and in genetic modification processes. Present your answer in the form of a table. (12)

Q5 The graph below shows the effect of pH on the growth rate of three types of microorganism.



a Discuss what is meant by *growth rate* of a microorganism. (2)

b State the pH range in which yeasts grow and what their optimum pH is. (2)

c Calculate the range of pH tolerance of the group of microorganisms whose growth is least inhibited by differing conditions of acidity and alkalinity. (1)

d In acid soils, state which microorganisms are more likely to be responsible for the decay of dead organic matter, and which microorganisms are likely to be least active in decay processes. (2)

e In the preservation of food using vinegar, explain which of these groups of microorganisms will be least inhibited. (1)

f State one alternative technique of food preservation to vinegar and explain how this substance inactivates the growth of microorganisms that may contaminate. (3)

Q6 **a** Explain why carbon and nitrogen are defined as macronutrients but calcium and magnesium are micronutrients. (3)

b Define *photoautotroph* and *photoheterotroph* by reference to the way that energy is transferred and the source of carbon used. (4)

c Construct a labelled diagram of the carbon cycle and annotate it to emphasise the ecological significance of the metabolism of chemotrophic prokaryotes. (6)