V
Microorganisms in the Environment

13
Microbial Associations

During the course of this book we have stressed on more than one occasion that microorganisms exist in nature not on their own as pure cultures but in, on or alongside numerous other organisms, microbial or otherwise, with which they may have to compete in the never-ending struggle for survival. In a number of cases, this coexistence may extend beyond merely sharing the same environmental niche; some microorganisms form a close physical association with another type of organism, from which special benefits may accrue for one or both parties. Such an association is termed *symbiosis* ('living together': Table 13.1). Three general forms of symbiotic relationship may be defined:

> *Symbiosis* is sometimes taken to mean a relationship between different organisms from which both participants derive benefit. We use the term in its broader sense, as described in the text.

- *Parasitism*: an association from which one partner derives some or all of its nutritional requirements by living either in or on the other partner (the *host*), which usually suffers some harm as a result.
- *Mutualism*: an association from which both participants derive benefit. The relationship is frequently obligatory, that is, both are dependent upon the other for survival. Mutualism that is not obligatory is sometimes called *protocooperation*.
- *Commensalism*: an association from which one participant (the commensal) derives benefit, and the other is neither benefited nor harmed. The relationship is not usually obligatory.

Microorganisms may be associated with plants, animals or other types of microorganism in any of these types of symbiosis (Tables 13.2, 13.3 and 13.4).

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Table 13.1 Types of symbiotic association. Participants in symbiosis may derive benefit, harm or neither from the association. + denotes benefit, x denotes harm and – denotes neither

Association	Species A	Species B
Mutualism	+	+
Protocooperation	+	+
Commensalism	–	+
Parasitism	x	+

Table 13.2 Microorganism–animal associations

Microorganism	Animal	Type of relationship
Anaerobic bacteria	Ruminants	Mutualism
Flagellated protozoans	Termites	Mutualism
Sulphur-oxidising bacteria	*Riftia* (marine tube worm)	Mutualism
Luminescent bacteria	Fish, molluscs	Mutualism
Bacteria, yeasts	Honey guide (bird)	Mutualism
Fungus	Leaf-cutter ants	Protocooperation
Resident bacteria of skin, large intestine, etc.	Humans	Commensalism

Table 13.3 Microorganism–plant associations

Microorganism	Plant	Type of relationship
N_2-fixing bacteria	Legumes	Mutualism
Mycorrhizal fungi	Various	Mutualism
Agrobacterium tumefaciens	Various	Parasitism (crown gall disease)
Acremonium (fungus)	Grass	Mutualism

Table 13.4 Microorganism–microorganism associations

Microorganism	Microorganism	Type of relationship
Fungi	Alga/blue-green	Mutualism (lichen)
Amoebas, flagellates	Methanogenic archaea	Mutualism

13.1 Microbial associations with animals

Termites are insects belonging to the order Isoptera that are found particularly in tropical regions. Their well-known ability to destroy trees and wooden structures such as buildings and furniture is due to a resident population of flagellated protozoans in their hindgut, which are able to break down cellulose. Termites lack the enzymes necessary to do this, and would thus starve to death if the protozoans were not present. In return, however, they are able to provide the protozoans with the anaerobic conditions they require to ferment the cellulose to acetate, carbon dioxide and hydrogen. The acetate is then utilised as a carbon source by the termites themselves.

In addition to the protozoans, anaerobic bacteria resident in the hindgut also play an important role in the metabolism of the termites. Acetogenic and methanogenic species compete for the carbon dioxide and hydrogen produced by the protozoans. The acetogenic bacteria contribute more acetate for the termite to use, whilst methanogens produce significant amounts of methane. Some methanogens exist as endosymbionts within the protozoans. In other types of termite, no resident population of cellulose digesters is present. Instead, the termite ingests a fungus, which provides the necessary cellulolytic enzymes.

> The total global amount of methane production by termites is comparable to that generated by ruminants.

Another example of a host's staple diet being indigestible without the assistance of resident microorganisms is provided by the brightly coloured African bird known as the honey guide. The honey guide eats beeswax, and relies on a two-stage digestion process by bacteria (*Micrococcus cerolyticus*) and yeast (*Candida albicans*) to render it in a usable form.

At the bottom of the deepest oceans, around geothermal vents, live enormous (2 m or more) tube worms belonging to the genus *Riftia*. These lack any sort of digestive system, but instead contain in their body cavity a tissue known as the *trophosome*. This comprises vascular tissue plus cells packed with endosymbiotic bacteria. These are able to generate ATP and NADPH by the oxidation of hydrogen sulphide generated by volcanic activity, and fix carbon dioxide via the Calvin cycle, providing the worm with a supply of organic nutrients. Hydrogen sulphide is transported to the trophosome from the worm's gill plume by a form of haemoglobin present in its blood (Figure 13.1).

Warm-blooded animals including humans play host in their lower intestinal tract to vast populations of bacteria. Although some of these are capable of producing useful metabolites such as vitamin K, most of them live as *commensals*, neither benefiting nor harming their host. It could be argued, however, that the very presence of the resident intestinal microflora acts as an important defence against colonisation by other, pathogenic, microorganisms, thus making the association more one of mutualism.

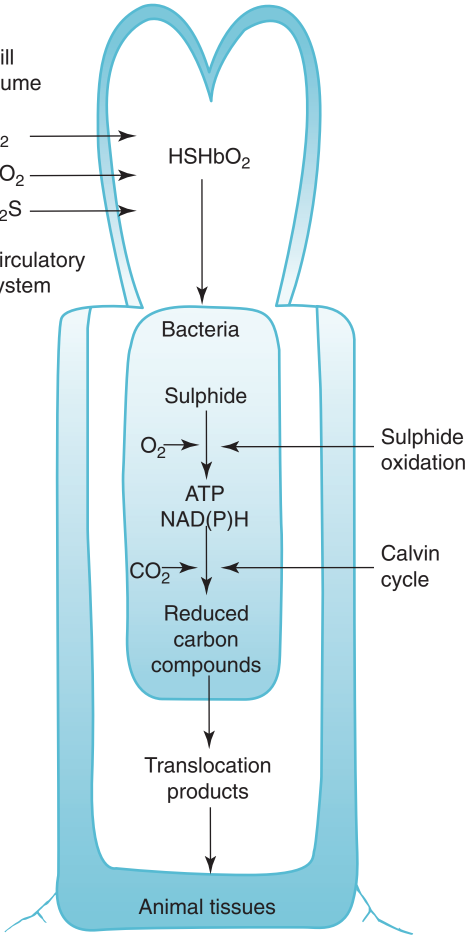

Figure 13.1 Symbiosis in *Riftia*, the giant tube worm. Found in deep-sea hydrothermal vents, *Riftia* acts as host to sulphur-oxidising bacteria. Energy and reducing power derived from sulphide oxidation are used to fix CO_2 via the Calvin cycle and provide the worm with organic carbon. Reproduced from Prescott, LM, et al. (2002) Microbiology, 5th edn, with permission from McGraw-Hill.

A number of bacteria, viruses, fungi, protozoans and even algae act as pathogens in animals, and cause millions of human deaths every year. Examples of diseases caused by each group are discussed in Chapter 15.

13.2 Microbial associations with plants

The roots of almost all plants form mutualistic associations with fungi, known as *mycorrhizae*, which serve to enhance the uptake of water and mineral

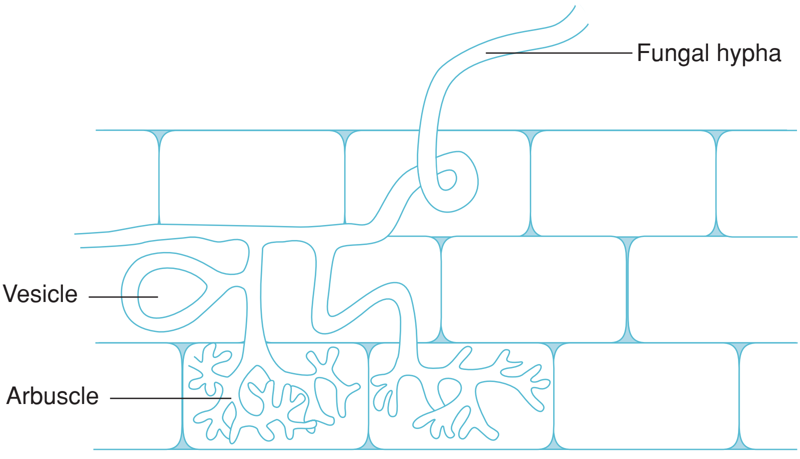

Figure 13.2 Endomycorrhizae. Section through a plant root colonised by an endomycorrhizal fungus. Note the spreading 'treelike' arbuscles.

nutrients, especially phosphate, by the plants. The beneficial effect of a mycorrhizal association is particularly noticeable in soils with a low phosphorus content. In return, the plant supplies reduced carbon in the form of carbohydrates to the fungi. Unlike other plant–microorganism interactions that occur in the rhizosphere, mycorrhizal associations involve the formation of a distinct, integrated structure comprising root cells and fungal hyphae. In *ectomycorrhizae* the plant partner is always a tree; the fungus surrounds the root tip, and hyphae spread between (but do not enter) root cells. In the case of the more common *endomycorrizae*, the fungal hyphae actually penetrate the cells by releasing cellulolytic enzymes. Arbuscular mycorrhizal fungi (AMF) belong to the phylum Glomeromycota, and are found in most plant types, including 'lower' plants (mosses, ferns). They form highly branched *arbuscules* within the root cells that gradually lyse, releasing nutrients into the plant cells (Figure 13.2). The plants benefit particularly from the increased uptake of phosphorus that results from the association, whereas the AMF receives a supply of hexose sugars. In contrast to pathogenic fungi, mycorrhizal fungi are often rather non-specific in their choice of 'partner' plant.

> A *mycorrhiza* is a mutually beneficial association between plant roots and a species of fungus.

> The **rhizosphere** is the region around the surface of a plant's root system.

The ability of crop plants to thrive is frequently limited by the supply of available nitrogen; although there is a lot of it in the atmosphere, plants are unable to utilise it, and instead must rely on an inorganic supply (both naturally occurring and in the form of fertilisers). As we saw in Chapter 7,

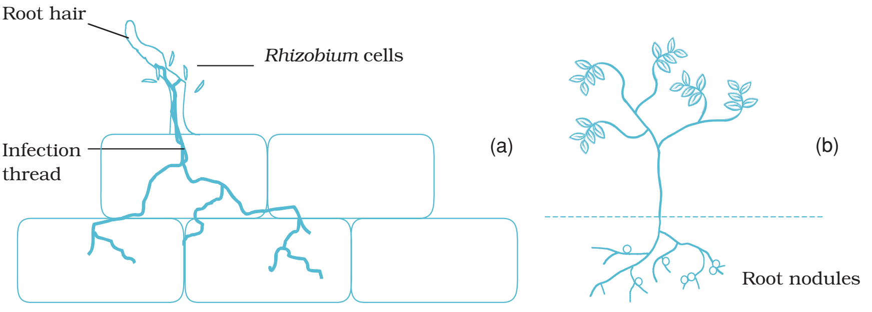

Figure 13.3 Nitrogen-fixing bacteria form root nodules in legumes. (a) Nitrogen-fixing *Rhizobium* proliferate inside root cells of leguminous plants as bacteroids. (b) Root nodule formation.

however, certain bacterial species are able to 'fix' atmospheric nitrogen into a usable form. Some of these, notably *Rhizobium* spp., form a mutualistic relationship with leguminous plants such as peas, beans and clover, converting nitrogen to ammonia, which the legume can incorporate into amino acids. In return, the bacteria receive a supply of organic carbon, which they can use as an energy source for the fixation of nitrogen.

> *Root nodules* are tumour-like growths on the roots of legumes, where nitrogen fixation takes place.

The free-living *Rhizobium* enters the plant via its root hairs, forming an infection thread and infecting more and more cells (Figure 13.3). Normally rod-shaped, they proliferate as irregularly shaped *bacteroids*, densely packing the cells and causing them to swell, forming *root nodules*. *Rhizobium* requires oxygen as a terminal electron acceptor in oxidative phosphorylation, but as you may recall from Chapter 7, the nitrogenase enzyme that fixes the nitrogen is sensitive to oxygen. The correct microaerophilic conditions are maintained by means of a unique oxygen-binding pigment, *leghaemoglobin*. This is only synthesised by means of a collaboration between both partners. Nitrogen fixation requires a considerable input of energy in the form of ATP (16 molecules for every molecule of nitrogen), so when ammonia is in plentiful supply the synthesis of the nitrogenase enzyme is repressed.

Farmers have long recognised the value of incorporating a legume into a crop rotation system; the nodules left behind in the soil after harvesting the crop appreciably enhance the nitrogen content of the soil.

> Unlike higher plants, ferns do not possess true roots, stems and leaves. The structure equivalent to a leaf is called a *frond*.

Legumes are not the only plants able to benefit from the nitrogen-fixing capabilities of bacteria. The water fern *Azolla,* which grows prolifically in the paddy fields of southeast Asia, has its nitrogen supplied by the blue-green bacterium *Anabaena.* When the fern dies, it acts as a natural fertiliser for the rice crop. *Anabaena* does not form root nodules, but takes up residence in small pores in the *Azolla* fronds. Nitrogen fixation takes place in heterocysts, specialised cells whose thick walls slow down the rate at which oxygen can diffuse into the cell, providing appropriate conditions for the oxygen-sensitive nitrogenase.

The alder tree (*Alnus* spp.) is able to grow in soils with a poor nitrogen content due to its association in root nodules with the nitrogen-fixing actinomycete *Frankia*. The filamentous *Frankia* solves the problem of nitrogenase's sensitivity to oxygen by compartmentalising it in thick-walled vesicles at the tips of its hyphae, which serve the same function as the heterocysts of *Anabaena*.

Several genera of bacteria live inside the cells of insects and other arthropods. Perhaps the best known of these are members of the genus *Wolbachia,* whose relationship with their host ranges from parasitism to obligate mutualism. *Wolbachia* bacteria have the ability to alter the reproductive capabilities of some hosts by specifically infecting reproductive cells. The result can be males being killed off, males turned into females, or parthenogenesis (asexual reproduction of female with no male involvement). *Wolbachia* also infect species of nematode worms, including some serious parasites of humans. Since in some cases the nematode is completely dependent on *Wolbachia* for survival, some disease-control strategies target the bacterium with antibiotics, rather than trying to kill off the worms directly. *Carsonella ruddii* is an endosymbiont of psyllids, which are sap-sucking insects. It provides its host with amino acids that are missing from the psyllid's sugar-rich but protein-poor diet. *C. ruddii* has the smallest genome known so far, just 159 662 base pairs, encoding a mere 182 proteins. It lacks many essential genes such as those responsible for DNA replication and key metabolic pathways, and is thus entirely dependent on its host for survival. A significant proportion of its small genome is devoted to amino acid synthesis. Since it lacks so many of the genes necessary for independent existence, some argue that *C. ruddi* has a status closer to that of an organelle rather than that of an organism in its own right.

Many microorganisms, particularly bacteria and yeasts, are to be found living as harmless commensals on the surface structures of plants such as leaves, stems and fruits.

Organisms that grow on the surface of a plant are called *epiphytes*. They frequently live as commensals.

13.2.1 Plant diseases

Plant disease may be caused by viruses, bacteria, fungi or protozoans. These frequently have an impact on humans, especially if the plant affected is a commercially important crop. Occasionally the effect on a human population can be catastrophic, as with the Irish famine of the 1840s brought about by potato blight. A number of microbial diseases of plants are listed in Table 13.5.

Table 13.5 Some microbial diseases of plants

Causative agent	Type of microorganism	Host	Disease
Heterobasidion	Fungus	Pine trees	Heart rot
Ceratocystis	Fungus	Elm trees	Dutch elm disease
Puccinia graminis	Fungus	Wheat	Wheat rust
Phytophthora infestans	Water mould	Potato	Potato blight
Erwinia amylovora	Bacterium	Apple, pear tree	Fire blight
Pseudomonas syringae	Bacterium	Various	Chlorosis
Agrobacterium	Bacterium	Various	Crown gall disease
Tobacco mosaic virus	Virus	Tobacco	Tobacco mosaic disease

We have already encountered the soil bacterium *Agrobacterium tumefaciens* in Chapter 12, where we saw how it has been exploited as a means of genetically modifying plants. *A. tumefaciens* is useful for introducing foreign DNA because it is a natural pathogen of plants, entering wounds and causing *crown gall disease*, a condition characterised by areas of uncontrolled growth, analogous to tumour formation in animals. This proliferation is caused by the expression within the plant cell of genes that encode the sequence for enzymes involved in the synthesis of certain plant hormones. The genes are carried on the T-DNA, part of an *A. tumefaciens* plasmid, which integrates into a host chromosome. Also on the T-DNA are genes that code for amino acids called opines. These are of no value to the plant, but are utilised by the *A. tumefaciens* as a food source.

13.3 Microbial associations with other microorganisms

A *lichen* is a mutually beneficial association between a fungus and an alga or cyanobacterium (blue green).

The most familiar example of mutualism between microorganisms is that of *lichens*, which comprise a close association between the cells of a fungus (usually belonging to the Ascomycota) and a photosynthetic alga or cyanobacterium. Although many different fungal species may take part in lichens, only a limited number of algae or cyanobacteria do so. Lichens are typically found on exposed hard surfaces such as rocks, tree bark and the roofs of buildings, and grow very slowly at a rate of a millimetre or two per year. They often occupy particularly harsh environments, from the polar regions to the hottest deserts. The photosynthetic partner usually exists as a layer of cells scattered among fungal hyphae (Figure 13.4). Often unicellular, it fixes carbon dioxide as organic matter, which the heterotrophic fungus absorbs and utilises.

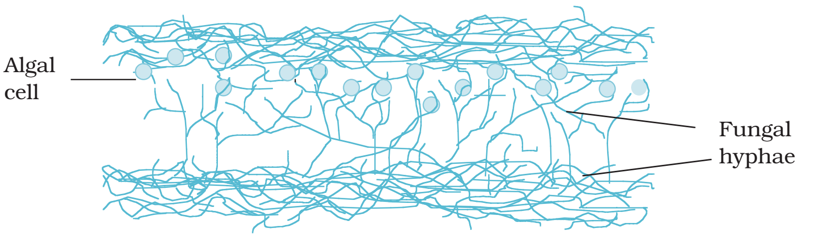

Figure 13.4 Algae and fungi combine to form lichens. Algal cells are embedded among the fungal hyphae just below the surface, where light is able to penetrate. Organic carbon and oxygen produced by photosynthesis are used by the fungus, while it provides water, minerals and shelter for its algal partner.

The fungal member provides anchorage and supplies inorganic nutrients and water, as well as protecting the alga from excessive exposure to sunlight.

Although lichens are tolerant of extremes of temperature and water loss, they have a well-known sensitivity to atmospheric pollutants such as the oxides of nitrogen and sulphur. Their presence in an urban setting is therefore a useful indicator of air quality. Lichens were used for many years as a source of brightly coloured dyes for the textile industry and also used in the perfume industry. The dye used in litmus paper is derived from a lichen belonging to the genus *Roccella*.

It should be stressed that lichens are not just a mixture of fungal and algal cells. They are distinctive structures with properties not possessed by either of their component species. Indeed, the relationship between the two partners of a lichen is so intimate that the composite organisms are given taxonomic status, and many thousands of species of lichen have been identified. Their classification is based on their fungal component.

As noted in the definitions at the beginning of this chapter, a mutualistic relationship need not always be as intimate and essential to both partners as it is in a lichen. The sulphate-reducing bacterium *Desulfovibrio*, for example, can obtain the sulphate and organic substrates it needs from the photosynthetic purple sulphur bacterium *Chromatium*, which in turn receives the carbon dioxide and hydrogen sulphide it requires (Figure 13.5). This protocooperation is not essential to either bacterium, however, as each is able to satisfy its requirements by alternative means. In another bacterial relationship, the gut bacteria *E. coli* and *Enterococcus faecalis* join forces to utilise arginine. As shown in Figure 13.6, neither can usefully metabolise this amino acid on its own, but neither is dependent on the cooperation.

Commensal relationships, in which one partner benefits and neither suffer, are common among microorganisms; such associations are rarely obligatory. A common basis for microbial commensalism is for one partner to benefit as a coincidental consequence of the normal metabolic activities of the other.

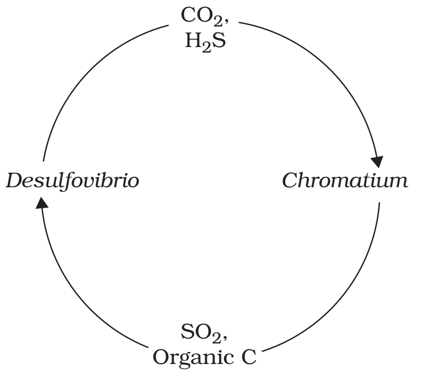

Figure 13.5 Protocooperation in bacteria. The sulphate-reducing *Desulfovibrio* and the sulphur-oxidising *Chromatium* can supply each other with the raw materials required for energy production. Neither is completely dependent on the association.

Thus one may excrete vitamins or amino acids that can be utilised by the other, or a facultative anaerobe may assist its obligate anaerobe neighbour by removing oxygen from the atmosphere, thus providing the conditions for the latter to grow.

The nature of a role played by an organism in a symbiotic relationship may alter according to the prevailing conditions. In a soil already rich in nitrogen, for example, the legume derives no benefit from the *Rhizobium*, which is then more accurately classed as a parasite, as it continues to utilise organic carbon produced by the plant. In humans, harmless gut symbionts such as *E. coli* can become *opportunistic pathogens,* and cause infections if introduced to an inappropriate site such as a wound or the urinary tract.

Box 13.1 Once bitten, twice shy

An interesting example of mutualistic association concerns the endophytic (= 'inside plant') fungus *Acremonium.* It derives reduced carbon compounds and shelter by living within the tissues of the grass, *Stipa robusta*, and in return deters animals from grazing on it. It does this by producing various alkaloids that, if ingested in sufficient amounts, are powerful enough to send a horse to sleep for several days! The horse clearly does not relish the experience, as it avoids the grass thereafter. The *Acremonium* passes to future generations through the seeds, so the relationship between plant and fungus is perpetuated. The nickname of 'sleepy grass' is self-explanatory!

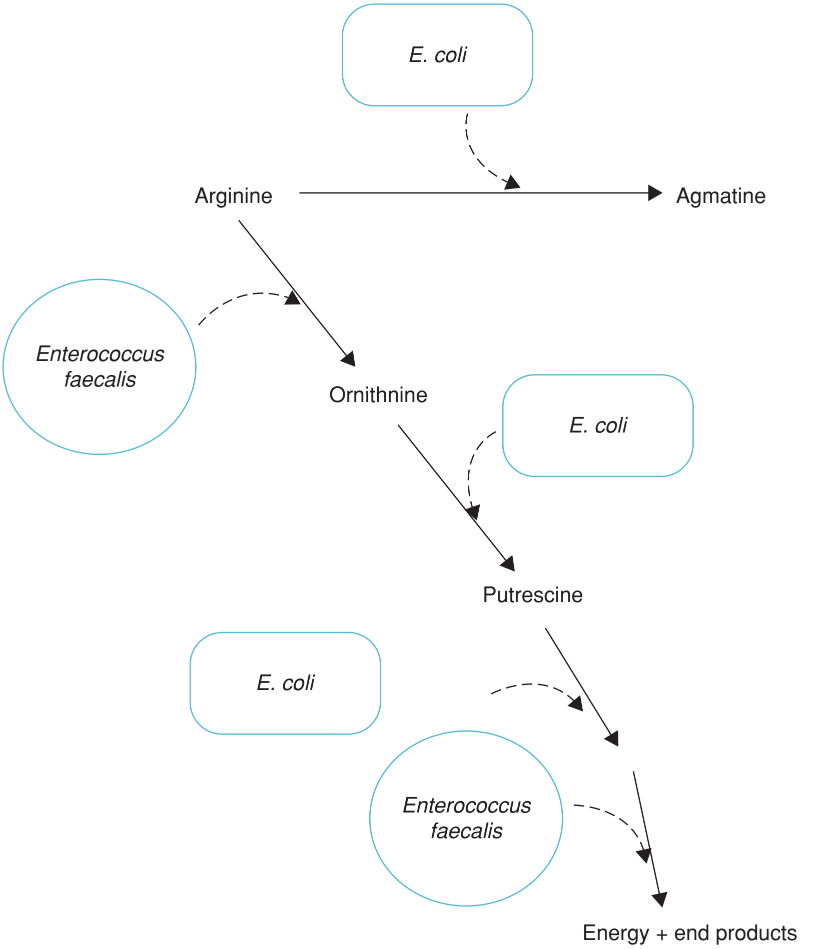

Figure 13.6 Protocooperation can make available an otherwise unutilisable substrate. Individually, neither *Enterococcus faecalis* nor *E. coli* is able to utilise arginine; however, working together they can convert it into putrescine, which can then be metabolised further by either organism to produce energy.

> An *endoparasite* fully enters its host and lives inside it. An *ectoparasite* attaches to the outside.

A limited number of microorganisms exist by living *parasitically* inside another. Viruses (see Chapter 10) are all obligate endoparasites that form an association with a specific host. This host may be microbial: bacteria, fungi, protozoans and algae all act as hosts to their own viruses. Viruses of bacteria are termed *bacteriophages* and are only able to replicate themselves inside an actively metabolising bacterial cell (see Chapter 10 for bacteriophage replication cycles). An unusual bacterium belonging to the genus *Bdellovibrio* also parasitises bacteria, but as it does not properly enter the cell, it is more properly thought of as an ectoparasite or even a predator (see Chapter 7). Other nonviral parasitism involves bacteria or fungi on protozoans, and fungi on algae and on other fungi.

Bdellovibrio itself may be parasitised by bacteriophages; this is known as *hyperparasitism*!

14 Microorganisms in the Environment

At various points in this book we have referred to the different environments in which particular microorganisms are to be found. Like other living organisms, they live as components of *ecosystems*, and we shall consider the three main types of ecosystem – terrestrial, freshwater and marine – later in this chapter.

Living organisms, together with their physical surroundings, make up an *ecosystem*.

Firstly, however, we must turn our attention again to the subject of energy relations in living things. In Chapter 4, we looked at the different ways in which microorganisms can derive and utilise energy from various sources. We now need to put these processes into a global perspective. All organisms may be placed into one of three categories with respect to their part in the global flow of energy:

- *(Primary) Producers*: autotrophs that obtain energy from the Sun or chemical sources (e.g. green plants, photosynthetic bacteria, chemolithotrophic bacteria). They use the energy to synthesise organic material from carbon dioxide and water.
- *Consumers*: heterotrophs that derive energy through the consumption of other organisms (producers or other consumers). They may serve as a link between the primary producers and the decomposers.
- *Decomposers*: organisms that break down the remains and waste products of producers and consumers, obtaining energy and releasing nutrients, including CO_2, that can be reused by the producers.

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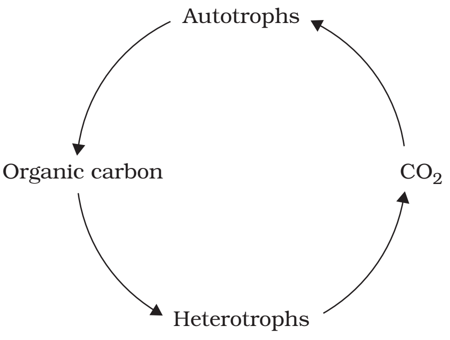


Figure 14.1 The carbon cycle. Autotrophs fix CO_2 as an organic compound, which heterotrophs convert back to CO_2. The recycling of carbon satisfies the requirements of both nutritional types.

Natural systems exist in a balance. Carbon and all the other elements that make up living things are subject to repeated *recycling*, so that they are available to different organisms in different forms. Think back to Chapter 6, in which we discussed how algae, green plants and certain bacteria capture light energy, then use it to synthesise organic carbon compounds from carbon dioxide and water. What happens to all this organic carbon? It doesn't just accumulate, but is recycled by other living things, which convert it back to carbon dioxide by respiration. This can be seen in its simplest form in Figure 14.1. Many other elements such as sulphur, nitrogen and iron are similarly transformed from one form to another in this way, by a cyclic series of reactions. Microorganisms are responsible for most of these reactions, oxidising and reducing the elements according to their metabolic needs. The continuation of life on Earth is dependent on the cycling of finite resources in this way.

14.1 The carbon cycle

A more detailed scheme of the carbon cycle is shown in Figure 14.2. Both aerobic and anaerobic reactions contribute to the cycle. The numbers in parentheses in the following description refer to the pathways in Figure 14.2.

> The *carbon cycle* is the series of processes by which carbon from the environment is incorporated into living organisms and returned to the atmosphere as carbon dioxide.

Atmospheric CO_2 is fixed into organic compounds by plants, together with phototrophic and chemoautotrophic microorganisms (1). These compounds undergo cellular respiration, and CO_2 is returned to the atmosphere (2). The carbon may have been passed along a food chain to consumers before this

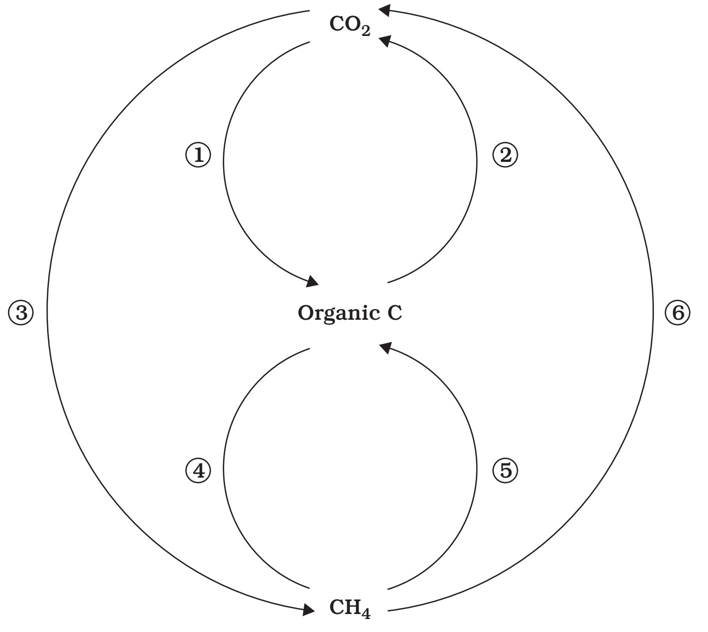


Figure 14.2 The carbon cycle: a closer look. Carbon is circulated as one of three forms, carbon dioxide, methane and organic compounds. Different organisms are able to utilise each form for their own metabolic requirements, converting it in the process to one of the others. Numbered arrows refer to reactions described in the text.

occurs. Carbon dioxide is also produced by the decomposition of dead plant, animal and microbial material by heterotrophic bacteria and fungi.

Methanogenic bacteria produce methane from organic carbon or CO_2 (3, 4). This in turn is oxidised by methanotrophic bacteria; carbon may be incorporated into organic material or lost as CO_2 (5, 6).

14.2 The nitrogen cycle

Nitrogen is essential to all living things as a component of proteins and nucleic acids. Although elemental nitrogen makes up three-quarters of the Earth's atmosphere, only a handful of life forms are able to utilise it for metabolic purposes. These are termed nitrogen-fixing bacteria, and incorporate the nitrogen into ammonia (Figure 14.3, reaction 1):

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_4^+ + 16ADP + 16Pi$$

The nitrogenase enzyme complex responsible for the reaction is very sensitive to oxygen, and is thought to have evolved early in the Earth's history,

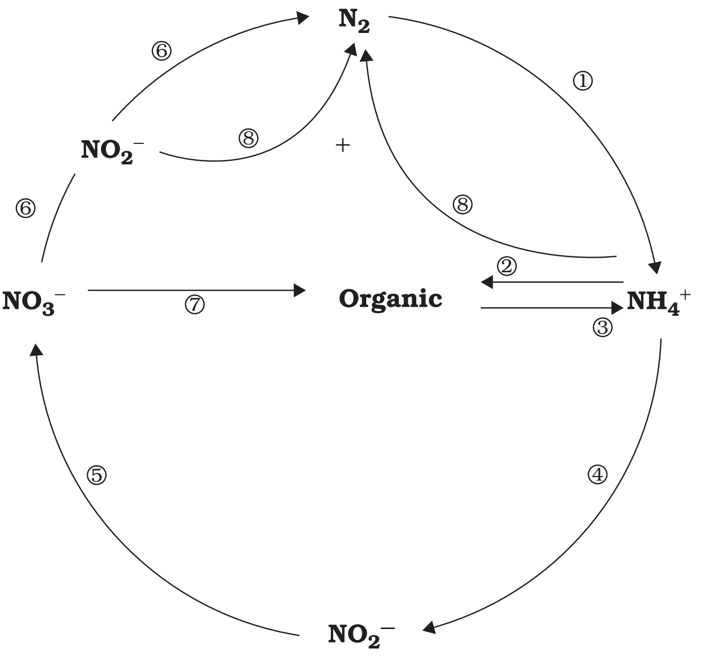


Figure 14.3 The nitrogen cycle. See the text for further details of reactions. Numbered arrows refer to reactions described in the text.

when the atmosphere was still largely oxygen-free. Many nitrogen-fixing bacteria are anaerobes; those that are not have devised ways of keeping the cell interior anoxic. *Azotobacter* spp., for example, utilise oxygen at a high rate, so that it never accumulates in the cell, and so does not inactivate the nitrogenase. Many cyanophytes (blue-greens) carry out nitrogen fixation in thick-walled heterocysts, which help maintain anoxic conditions.

Some nitrogen-fixing bacteria such as *Rhizobium* infect the roots of leguminous plants such as peas, beans and clover, where they produce nodules and form a mutually beneficial association.

Ammonia produced by nitrogen fixation is assimilated as amino acids, which can then form proteins and feed into pathways of nucleotide synthesis (2). Organic nitrogen in the form of dead plant and animal material plus excrement re-enters the environment, where it undergoes *mineralisation* (3) to an inorganic form at the hands of a range of microorganisms. This involves the deamination of amino acids to their corresponding organic acid. This process of mineralisation may occur aerobically or anaerobically, in a wide range of microorganisms:

The process by which microorganisms convert organic matter to an inorganic form such as CO_2, CH_4, NH_4^+ is termed *mineralisation*.

Alanine Pyruvate

The process of *nitrification*, by which ammonia is oxidised stepwise firstly to nitrite and then to nitrate, involves two different groups of bacteria (4, 5:

$$NH_4^+ \rightarrow NO_2^-$$
$$NO_2^- \rightarrow NO_3^-$$

The nitrate thus formed may suffer a number of fates. It may act as an electron acceptor in anaerobic respiration, becoming reduced to nitrogen via a series of intermediates including nitrite (6). This process of *denitrification* occurs in anaerobic conditions such as waterlogged soils. Alternatively, it can be reduced once again to ammonia and be converted to organic nitrogen (7).

> *Denitrification* is the reduction, under anaerobic conditions, of nitrite and nitrate to nitrogen gas.

A final pathway of nitrogen cycling has only been discovered relatively recently. It is known as *anammox* (anaerobic ammonia oxidation), and is carried out by members of a group of Gram-negative bacteria called the Planctomycetes (see Chapter 7). The reaction, which can be represented thus:

> *Anammox* is the formation of nitrogen gas by the anaerobic oxidation of ammonia and nitrite.

$$H_4^+ + NO_2^- \rightarrow N_2 + 2H_2O(8)$$

has considerable potential in the removal of nitrogen from wastewater.

14.3 The sulphur cycle

Sulphur is found in living organisms in the form of compounds such as amino acids, coenzymes and vitamins. It can be utilised by different types of organisms in several forms; Figure 14.4 shows the principal components of the sulphur cycle.

In its elemental form, sulphur is unavailable to most organisms; however, certain bacteria such as *Acidithiobacillus* are able to oxidise it to sulphate (1), a form that can be utilised by a much broader range of organisms (see Chapter 7):

$$2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$$

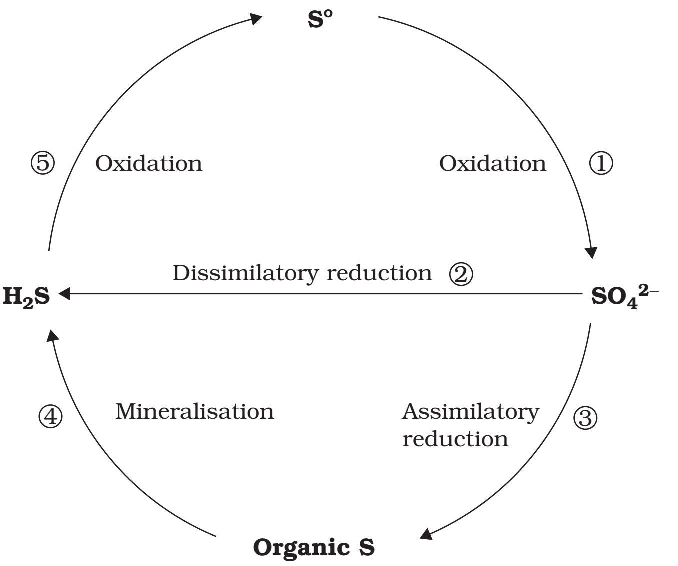


Figure 14.4 The sulphur cycle. See the text for further details of reactions. Numbered arrows refer to reactions described in the text.

Powdered sulphur is often added to alkaline soils in order to encourage this reaction and thereby reduce the pH.

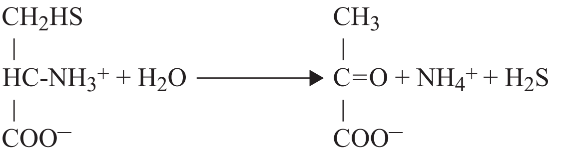
Sulphate-reducing bacteria convert the sulphate to hydrogen sulphide gas (2) using either an organic compound or hydrogen gas as electron donor:

$$8H^+ + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$$

These bacteria are obligate anaerobes, and the process is termed *dissimilatory* sulphate reduction.

Plants are also able to utilise sulphate, incorporating it into cellular constituents such as the amino acids methionine and cysteine (3) (*assimilatory* sulphate reduction).

When the plants die, these are broken down, again with the release of hydrogen sulphide (4) (see mineralisation, earlier).



Cysteine Pyruvate

Green and purple photosynthetic bacteria and some chemoautotrophs use hydrogen sulphide as an electron donor in the reduction of carbon dioxide, producing elemental sulphur and thus completing the cycle (5):

$$H_2S + CO_2 \rightarrow (CH_2O)_n + S^0$$

Phosphorus exists almost exclusively as phosphate; however, this is cycled between soluble and insoluble forms. This conversion is pH-dependent, and if phosphate is only present in an insoluble form, it will act as a limiting nutrient. This explains the sudden surge in the growth of plants, algae and cyanobacteria in water bodies when a source of soluble phosphate (typically fertiliser or detergent) enters a watercourse.

14.4 The microbiology of soil

In the following section it will be necessary to generalise, and treat soil as a homogeneous medium. In fact, it is no such thing; its precise make-up is dependent upon the underlying geology, and the climatic conditions both past and present. In addition, the microbial population of a soil will vary according to the amount of available water and organic matter, and different organisms colonise different strata in the soil.

> The *topsoil* is the top few centimetres of a soil, characterised by its high content of organic material in various stages of decomposition. It is distinguished from the succeeding layers underneath it, termed the subsoil, parent layer and bedrock.

The organic content of a soil derives from the remains of dead plants and animals. These are broken down in the soil by a combination of invertebrates and microorganisms (mainly bacteria and fungi) known as the *decomposers*. Their action results in the release of substances that can be used by plants and by other microorganisms. Much organic material is easily degraded, while the more resistant fraction is referred to as *humus*, and comprises lignin together with various other macromolecules. The humus content of a soil, then, is a reflection of how favourable (or otherwise) conditions are for its decomposition; the value usually lies between 2 and 10% by weight. The inorganic fraction of a soil derives from the weathering of minerals. Microorganisms may be present in soils in huge numbers, mostly attached to soil particles. Their numbers vary according to the availability of suitable nutrients. Bacteria (notably actinomycetes) form the largest fraction of the microbial population, together with much smaller numbers of fungi, algae and protozoans. Published values of bacterial numbers range from overestimates (those that do not distinguish between living and dead

> *Humus* is the complex organic content of a soil, comprising complex materials that remain after microbial degradation.

cells) and underestimates (those that depend on colony counts and therefore exclude those organisms we are not yet able to grow in the laboratory – 99% of species according to some experts!). Suffice to say that many millions (possibly billions) of bacteria may be present in a single gram of topsoil. In spite of being present in such enormous numbers, bacteria only represent a minute percentage of the volume of most soils. Fungi, although present in much smaller numbers than bacteria, form a higher proportion of the soil biomass, due to their greater size. The majority of soil microorganisms are aerobic heterotrophs, involved in the decomposition of organic substrates, thus microbial numbers diminish greatly the further down into the soil we go, away from organic matter and oxygen. The proportion of anaerobes increases with depth, but unless the soil is waterlogged, they are unlikely to predominate.

Other factors affecting microbial distribution include pH, temperature and moisture. Broadly speaking, neutral conditions favour bacteria, while fungi flourish in mildly acidic conditions (down to about pH 4), although extremophiles survive well outside these limits. Actinomycetes favour slightly alkaline conditions. Bacterial forms occurring commonly in soils include *Pseudomonas*, *Bacillus*, *Clostridium*, *Nitrobacter* and the nitrogen-fixing *Rhizobium* and *Azotobacter*, as well as cyanobacteria such as *Nostoc* and *Anabaena*. Commonly found actinomycetes include *Streptomyces* and *Nocardia*. As we shall see in Chapter 17, actinomycetes are notable for their secretion of antimicrobial compounds into their surroundings. This provides an example of how the presence of one type of microorganism in a soil population can influence the growth of others, forming a dynamic, interactive ecosystem. In addition, bacteria may serve as prey for predatory protozoans, and secondary colonisers may depend on a supply of nutrients from, for example, cellulose degraders. Important fungal genera common in soil include the familiar *Penicillium* and *Aspergillus*; these not only recycle nutrients by breaking down organic material, but also contribute to the fabric of the soil, by binding together microscopic soil particles. Soil protozoans are mostly predators, which ingest bacteria or protists such as yeasts or unicellular algae. All the major forms of protozoans may be present (flagellates, ciliates and amoebas), moving around the water-lined spaces between soil particles. Algae are of course phototrophic, and are therefore to be found mostly near the soil surface, although it will be recalled from Chapter 9 that some forms are capable of heterotrophic growth, and may thus survive further down.

The surface of soil particles is a good natural habitat for the development of *biofilms*, complex microenvironments comprising microbial cells held together in a polysaccharide matrix. The microorganisms themselves produce the polysaccharide, which also allows the passage of nutrients from the environment. Biofilms can form on almost any surface, and are often to be found in rapidly flowing waters. Microorganisms with different metabolic properties may occupy different positions in a biofilm, with, for example, phototrophic species near to the surface.

Although we have emphasised the importance of organic matter in soil ecosystems, microorganisms may also be found growing on or even within rocks. The growth of such organisms, together with the action of wind and rainfall, contribute to the weathering of rocks.

14.5 The microbiology of freshwater

The microbial population of freshwater is strongly influenced by the presence or absence of oxygen and light. A body of water such as a pond or lake is stratified into zones (Figure 14.5), each having its own characteristic microflora, determined by the availability of these factors. The *littoral* zone is the region situated close to land where the water is sufficiently shallow for sunlight to penetrate to the bottom. The *limnetic* zone occupies the same depth, but is in

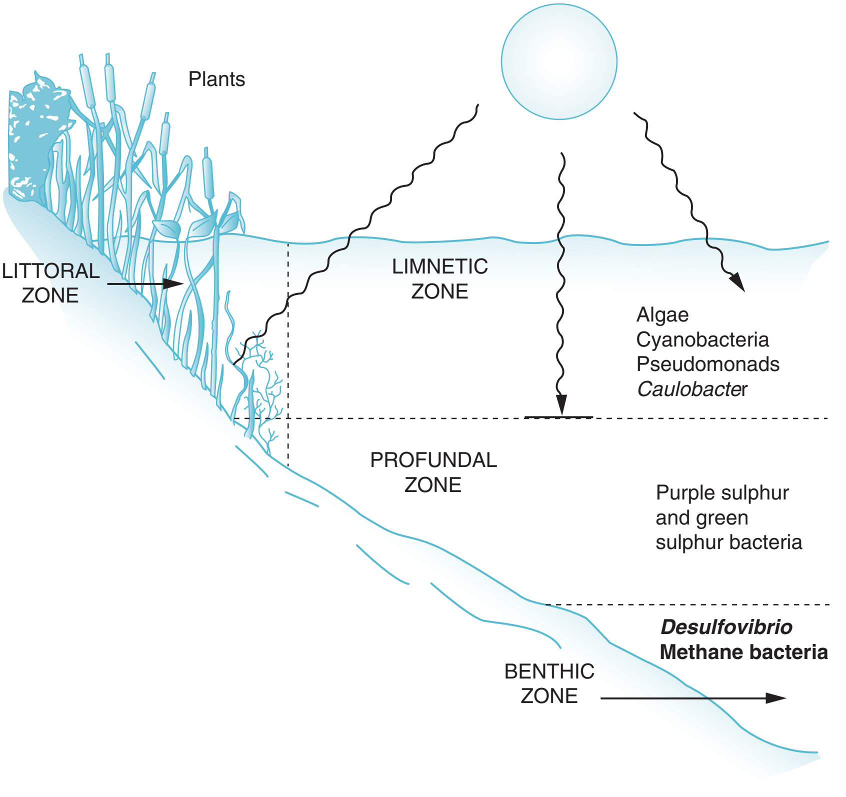


Figure 14.5 Vertical zonation in a lake or pond. Representative organisms are indicated for each zone. Reproduced from Black, JG (1999) Microbiology: Principles and Explorations, 4th edn, with permission from John Wiley & Sons.

open water, away from the shore. The *profundal* zone occupies deeper water, where sunlight is unable to penetrate, and finally the *benthic* zone comprises the sediment of mud and organic matter at the bottom of the pond or lake.

Oxygen is poorly soluble in water (9 mg/litre at 20°C), so its availability is often a limiting factor in determining the microbial population of a body of water. Oxygen availability in lakes and ponds is closely linked to oxygenic photosynthesis and therefore, indirectly, to the penetrability of light. Phototrophs such as algae and blue-greens are limited to those regions where light is able to penetrate. Oxygen is absent or very limited in the benthic zone, where anaerobic forms such as the methanogenic bacteria are to be found. Another factor influencing microbial populations is the organic content of the water; if this is high, the growth of decomposers will be encouraged, which will in turn deplete the oxygen. This is much less of an issue in rivers and streams, where physical agitation of the water generally ensures its continued oxygenation.

The temperature of freshwater ecosystems ranges between extremes (<0 to 90°C), and microorganisms may be found throughout this range.

Microorganisms play a central role in the purification of wastewaters, a topic we shall examine in more detail later in this chapter.

14.6 The microbiology of seawater

The world's oceans cover some 70% of the Earth's surface and have a fairly constant salt content of 3.5% (w/v). The depth to which light can penetrate varies, but is limited to the first 100 metres or so. A world of permanent darkness exists at greater depths; however, in spite of the absence of photosynthesis, oxygen is often still present. This is because the generally low levels of mineral nutrients in seawater limit the amount of primary production, and therefore heterotrophic activity. At extreme depths, however, anoxic conditions prevail.

Compared to freshwater habitats, marine ecosystems show much less variability in both temperature and pH, although there are exceptions to this general rule. A more pertinent issue in marine environments is that of *pressure*; this increases progressively in deeper waters, and at 1000 metres reaches around 100 times normal atmospheric pressure. Concomitant with this increase in pressure is a decrease in temperature and nutrients. Surprisingly, however, certain members of the Archaea have been isolated even from these extreme conditions.

> *Phytoplankton* is a collective term used to describe the unicellular photosynthesisers, which include cyanobacteria, dinoflagellates, diatoms and single-celled algae.

In contrast to terrestrial ecosystems, where plants are responsible for most of the energy fixation via photosynthesis, marine primary production is largely microbial, in the shape of members of the *phytoplankton*. As we have seen, such forms are restricted to those zones where light is able to penetrate. Also found here may be protozoans and fungi that feed on the phytoplankton. Because of the high salt concentration of seawater, the bacteria that are typically found in such environments differ from those in freshwater. In the last decade or so, the presence of *ultramicrobacteria* has been detected in marine ecosystems at relatively high densities; these are around one-tenth of the size of 'normal' bacteria. Marine bacteria are of necessity halophilic. Anaerobic decomposing bacteria inhabit the benthic zone, carrying out reactions similar to those that occur in freshwater sediments, whilst the profundal zone is largely free of microbial life.

Ultramicrobacteria are bacteria that are much smaller than normal forms, and some are able to pass through a 0.22 μm filter. They may represent a response to reduced nutrient conditions.

14.7 Detection and isolation of microorganisms in the environment

As we emphasised in the last chapter, microorganisms rarely, if ever, exist in nature as pure cultures but rather as members of mixed populations. Methods are required, therefore, for the detection and isolation of specific microbial types from such mixtures. The traditional method of isolation is the use of an enrichment culture, as described in Chapter 4. As examples, aerobic incubation with a supply of nitrite would assist in the isolation of nitrifying bacteria such as *Nitrobacter* from mud or sewage, whilst a minimal medium containing $FeSO_4$ at pH 2 would encourage the isolation of *Acidithiobacillus ferrooxidans* from a water sample.

We now know, however, that there are many types of microorganism in the environment that have so far resisted all attempts to culture them in the laboratory (often referred to as *viable but non-culturable*). Modern molecular techniques have helped us to identify the existence of a much broader range of bacteria and archaea than had previously been thought to exist. The extreme sensitivity of such methods means that we are able to demonstrate the presence of even a single individual of a particular bacterium in a mixed population. One such technique is called *fluorescence in situ hybridisation* or *FISH*. This uses an oligonucleotide probe comprising a short sequence of single-stranded DNA or RNA that is unique to a particular microorganism, attached to a fluorescent dye. The microorganisms are fixed to a glass slide and incubated with the probe. The rules of base pairing in nucleic acids mean that the probe will seek out its complementary sequence, and cells carrying this sequence can be visualised under a fluorescence microscope. The most commonly used 'target' is ribosomal RNA, since this shows sequence variation from one microbial type to another, and because there are multiple copies within each cell, providing a stronger response. The polymerase chain reaction (PCR, see Chapter 12) is another valuable tool in the identification of specific nucleic acid sequences. Other methods, not dependent on DNA, include the use of fluorescence-labelled antibodies raised against specific microorganisms.

Antibodies are proteins produced by the immune systems of higher animals in response to infection by a foreign organism; their main characteristic is their extreme specificity, thus they can be used to locate a specific protein.

14.8 Beneficial effects of microorganisms in the environment

The central role played by microorganisms in the recycling of essential elements on a global scale has already been stressed in this chapter. Many of their natural activities are exploited by humans for their own benefit. Some form the basis of industrial processes such as those used in the food and drink industries and are considered in Chapter 18, while the application of others is essentially environmental. Notable among these is the harnessing of natural processes of *biodegradation* to treat the colossal volumes of liquid and solid wastes generated by our society. These are reviewed briefly in the following section.

Biodegradation is the term used to describe the natural processes of breakdown of matter by microorganisms.

14.8.1 Solid waste treatment: composting and landfill

Those of us who live in the industrialised nations are often said to belong to a 'throwaway society'. On average, each of us generates around 2 tonnes of solid waste material per year, and all of this must be disposed of in some way! Most of it ends up in landfill sites – huge holes in the ground where refuse is deposited to prevent it being a hazard. The non-biodegradable components (metals, plastics, rubble, etc.) remain there more or less indefinitely; however, over a period of time biodegradable material (food waste, textiles, paper, etc.) undergoes a decomposition process. The rate at which this happens is dependent on the nature of the waste and the conditions of the landfill, but can take several decades. Aerobic processes give way to anaerobic ones and a significant result of the latter is the generation of methane. Modern landfill sites incorporate systems that remove this to prevent it being a fire/explosion hazard, and may put it to good use as a fuel source.

Many householders separate organic waste items such as vegetable peelings and grass cuttings and use them to make *compost*. This practice, apart from providing a useful gardening supplement, also substantially reduces the volume of material that has to be disposed of by other means (see earlier). We have already mentioned the role of microorganisms in the recycling of carbon in the biosphere; these same processes serve to degrade the organic waste, especially the cellulose, resulting in a considerable reduction of the bulk. Fungi and bacteria, particularly actinomycetes, break down the organic matter to produce CO_2, water and humus, a relatively stable organic end product. Compost is not really a fertiliser, since its nitrogen content is not high, but it nevertheless provides nutrients to a soil and generally helps to improve its condition. Composting is carried out on a large scale by local authorities using the waste generated in municipal parks and gardens.

14.8.2 Wastewater treatment

The aim of wastewater treatment is the removal of undesirable substances and hazardous microorganisms in order that the water may safely enter a watercourse such as a river or stream. Further purification procedures are required before it can be used as drinking water. Wastewater treatment is fundamental to any developed society, and greatly reduces the incidence of waterborne diseases such as cholera. Wastewater may come from domestic or commercial sources; highly toxic industrial effluents may require pretreatment before entering a water treatment system. *Sewage* is the term used to describe liquid wastes that contain faecal matter (human or animal).

The effectiveness of the treatment process is judged chiefly by the reduction of the wastewater's *biochemical oxygen demand* (BOD). This is a measure of the amount of oxygen needed by microorganisms to oxidise its organic content. A high BOD leads to the removal of oxygen from water, a certain indicator of pollution.

Wastewater treatment usually occurs in stages, the first of which (primary treatment) is purely physical, and involves the removal of floating objects followed by sedimentation, a process that removes up to one-third of the BOD value. Secondary treatment involves microbial oxidation, leading to a substantial further reduction in BOD. This may take one of two forms, both of which are aerobic, the traditional *trickling filter* and the more recent *activated sludge* process (Figure 14.6). In the former, the wastewater is passed slowly over beds of stones or pieces of moulded plastic. These develop a biofilm comprising bacteria, protozoans, fungi and algae, and the resulting treated water has its BOD reduced by some 80–85%. Activated sludge facilities achieve an even higher degree of BOD reduction. Here the wastewater is aerated in tanks that have been seeded with a mixed microbial sludge. The main component of this is the bacterium *Zoogloea,* which secretes slime, forming aggregates called *flocs*, around which other microorganisms such as protozoans attach. Some of the water's organic content is not immediately oxidised, but becomes incorporated into the flocs. After a few hours' residence

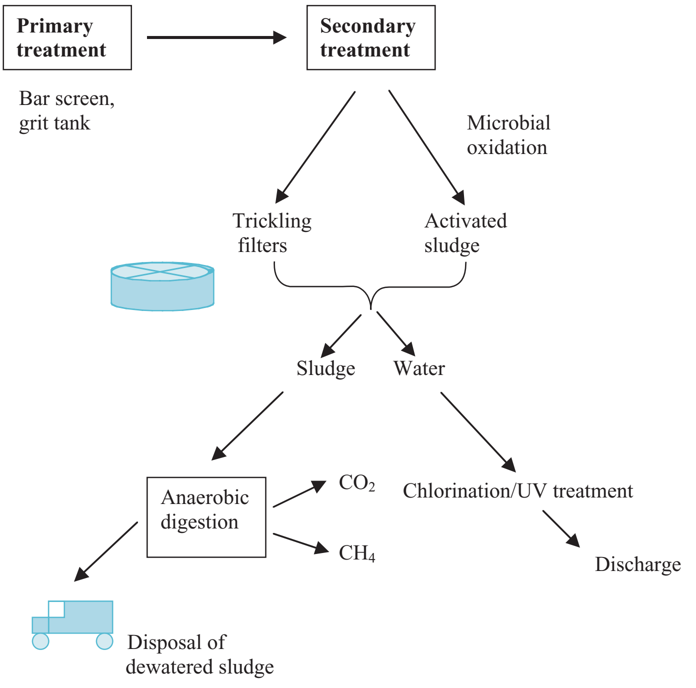


Figure 14.6 Wastewater treatment achieves a reduction of the biochemical oxygen demand of the water by primary (physical) and secondary (biological) treatment.

in the tank, the sludge is allowed to settle out, and the treated water passes out of the system. Before being discharged to a watercourse, it is treated with chlorine to remove any pathogenic microorganisms that may remain.

The principal operating problem encountered with activated sludge is that of *bulking*. This is caused by filamentous bacteria such as *Sphaerotilus natans*; it results in the sludge not settling properly and consequently passing out with the treated water.

Both secondary treatment processes result in some surplus sludge, which undergoes *anaerobic digestion*, resulting in the production of methane and CO_2. The methane can be used as a fuel to power the plant, and any remaining sludge is dewatered and used as a soil conditioner. Care must be taken in this context, however, that the sludge does not contain toxic heavy metals.

14.8.3 Bioremediation

One of the biggest problems facing the developed world in the early twenty-first century is that of pollution of the environment. Our dependence on the products of the chemical industries has resulted in the production of vast amounts of toxic waste material. One way of dealing with such (mostly organic) waste is to encourage the growth of bacteria and fungi that are able to oxidise the pollutants, a process known as *bioremediation*. Elsewhere in this book we have seen how microorganisms are able to utilise an enormous range of organic compounds as carbon sources. Many organisms can metabolise not only naturally occurring substances, but also synthetic ones, making them valuable allies in the process of bioremediation. Often the most effective microorganisms to use are those found living naturally at the contaminated site, since they have demonstrated a naturally developed ability to survive the toxic effects of the pollutant, although in other cases specially adapted or genetically modified bacteria may be introduced (*bioaugmentation*). Examples of the use of microorganisms include the treatment of toxic waste sites, chemical spills, pesticides in groundwater and oil spills. One of the first large-scale attempts at bioremediation came in the aftermath of the *Exxon Valdez* disaster in 1989, when thousands of tons of crude oil were released off the coast of Alaska. Depending on the circumstances, bioremediation procedures may occur *in situ*, or the contaminated soil or water may be removed to a specialist facility for treatment.

> *Bioremediation* is the use of biological processes to improve a specific environment, such as by the removal of a pollutant.

> *Bioaugmentation* is the deliberate introduction of specific microorganisms to an environment in order to assist in bioremediation.

14.9 Harmful effects of microorganisms in the environment

The natural processes of bioconversion that are so important in the global recycling of elements may have unwanted consequences for humans. One of these is *acid mine drainage*, a frequently encountered problem in mining regions. Bacterial oxidation of mineral sulphides, particularly the ubiquitous iron pyrite, leads to the release of a highly acidic leachate into streams and rivers. This also contains toxic dissolved metals such as copper and also ferric iron. When it mixes with stream water, the pH is raised sufficiently for the iron to precipitate as unsightly orange ferric hydroxides, blanketing the stream bed and wiping out plant and animal life. The main culprits in the formation of acid mine drainage are sulphur-oxidising bacteria, notably *Acidithiobacillus ferrooxidans;* as we shall see in Chapter 18, under controlled conditions this same organism can also provide economic benefits to the mining industry by extracting valuable metals from low-grade ores.

Biodeterioration is the damage caused to materials of economic importance due to biological (mainly microbial) processes.

Another area in which environmental microorganisms can have detrimental effects is that of *biodeterioration*, whereby economically important materials such as wood, paper, textiles, petroleum and even metals and concrete may be subject to damage by a range of microorganisms, mainly fungi and bacteria.

The most important microorganisms in the biodeterioration of wood are members of the Basidiomycota. Wood is only susceptible to fungal attack when its moisture level reaches around 30%. The major component of wood that is subject to microbial attack is *cellulose*, although some microorganisms can also degrade *lignin*. There are two main forms of rot: *white rot*, which involves the degradation of lignin as well as cellulose, and *brown rot*, in which the lignin is unaltered.

The dry rot fungus *Serpula lacrymans* produces thick strands of hyphae called *rhizomorphs*, which it uses to conduct water and nutrients from damper areas. These are very strong, and able to travel over brickwork and masonry barriers. *S. lacrymans* can generate water as a metabolic end-product and thus, once established, is able to grow even on dry wood. Dry rot flourishes in areas of static dampness such as badly ventilated, uninhabited properties.

Since cellulose is also an important component of paper and textiles, its breakdown is clearly of great economic importance. Degradation by fungi and, to a lesser extent, bacteria, results in a loss of strength of the material in question. The paper-making process provides warm, wet conditions rich in nutrients, ideal for microbial growth, which can clog up machinery and discolour the finished product. A variety of biocides are used in an effort to minimise microbial contamination.

The discoloration referred to above raises the point that biodeterioration of a material need not necessarily affect its physical or chemical make-up; aesthetic damage can lessen the economic value of a material by altering its appearance. The blackening of shower curtains by moulds growing on surface detritus, familiar to generations of students, is another example of this!