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## 6 AQUATIC MICROBIAL BIOCHEMISTRY

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### 6.1. AQUATIC BIOCHEMICAL PROCESSES

Microorganisms—**bacteria**, **fungi**, **protozoa**, and **algae**—are living catalysts that enable a vast number of chemical processes to occur in water and soil. A majority of the important chemical reactions that take place in water, particularly those involving organic matter and oxidation-reduction processes, occur through bacterial intermediaries. Algae are the primary producers of biological organic matter (biomass) in water. Microorganisms are responsible for the formation of many sediment and mineral deposits; they also play the dominant role in secondary waste treatment. Some of the effects of microorganisms on the chemistry of water in nature are illustrated in [Figure 6.1](#).

Pathogenic microorganisms must be eliminated from water purified for domestic use. In the past, major epidemics of typhoid, cholera, and other water-borne diseases resulted from pathogenic microorganisms in water supplies. Even today, constant vigilance is required to ensure that water for domestic use is free of pathogens.

Although they are not involved in aquatic chemical transformations, which constitute most of this chapter, special mention should be made of viruses in water. Viruses cannot grow by themselves, but reproduce in the cells of host organisms. They are only about 1/30-1/20 the size of bacterial cells, and they cause a number of diseases, such as polio, viral hepatitis, and perhaps cancer. It is thought that many of these diseases are waterborne.

Because of their small size (0.025-0.100  $\mu\text{m}$ ) and biological characteristics, viruses are difficult to isolate and culture. They often survive municipal water treatment, including chlorination. Thus, although viruses have no effect upon the overall environmental chemistry of water, they are an important consideration in the treatment and use of water.

Microorganisms are divided into the two broad categories of **prokaryotes** and **eukaryotes**; the latter have well-defined cell nuclei enclosed by a nuclear membrane, whereas the former lack a nuclear membrane and the nuclear genetic material

is more diffuse in the cell. Other differences between these two classes of organisms include location of cell respiration, means of photosynthesis, means of motility, and reproductive processes. All classes of microorganisms produce **spores**, metabolically inactive bodies that form and survive under adverse conditions in a “resting” state until conditions favorable for growth occur.

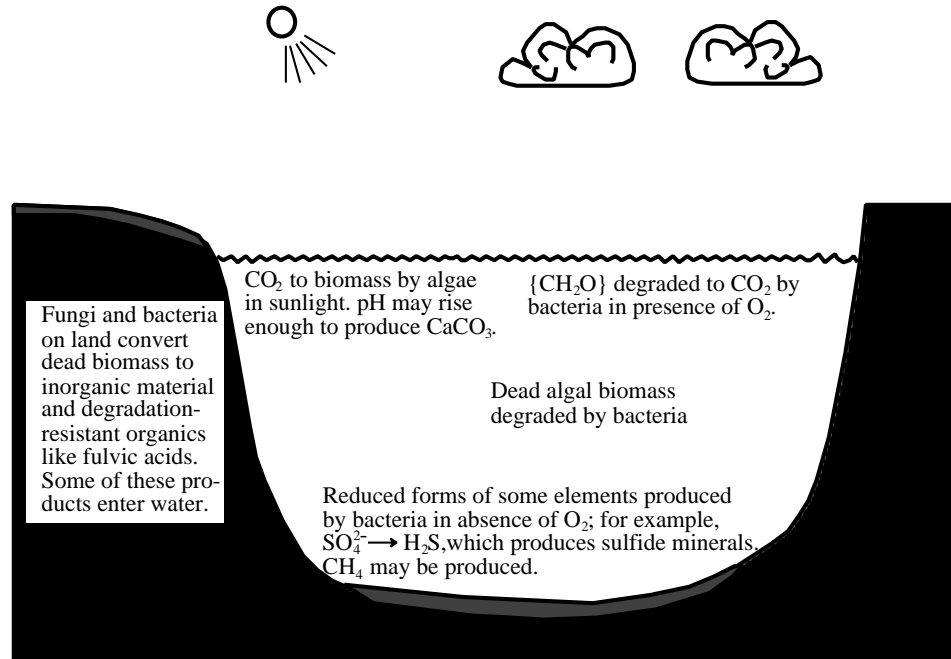


Figure 6.1. Effects of microorganisms on the chemistry of water in nature.

Fungi, protozoa, and bacteria (with the exception of photosynthetic bacteria and protozoa) are classified as **reducers**, which break down chemical compounds to more simple species and thereby extract the energy needed for their growth and metabolism. Algae are classified as **producers** because they utilize light energy and store it as chemical energy. In the absence of sunlight, however, algae utilize chemical energy for their metabolic needs. In a sense, therefore, bacteria, protozoa, and fungi may be looked upon as environmental catalysts, whereas algae function as aquatic solar fuel cells.

All microorganisms can be put into one of the four following classifications based on the sources of energy and carbon that they utilize: chemoheterotrophs, chemoautotrophs, photoheterotrophs, and photoautotrophs. These classifications are based upon (1) the energy source and (2) the carbon source utilized by the organism. **Chemotrophs** use chemical energy derived from oxidation-reduction reactions of simple inorganic chemical species for their energy needs. **Phototrophs** utilize light energy from photosynthesis. **Heterotrophs** obtain their carbon from other organisms; **autotrophs** use carbon dioxide and ionic carbonates for the C that they require. Figure 6.2 summarizes the classifications into which microorganisms may be placed with these definitions.

<b>Energy source</b> → <b>Carbon sources</b> ↓	<b>Chemical</b>	<b>Photochemical (light)</b>
<b>Organic matter</b>	<b>Chemoheterotrophs</b> All fungi and protozoans, most bacteria. Chemoheterotrophs use organic sources for both energy and carbon.	<b>Photoheterotrophs</b> A few specialized bacteria that use photoenergy, but are dependent on organic matter for a carbon source
<b>Inorganic carbon (CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>)</b>	<b>Chemoautotrophs</b> Use CO <sub>2</sub> for biomass and oxidize substances such as H <sub>2</sub> ( <i>Pseudomonas</i> ), NH <sub>4</sub> <sup>+</sup> ( <i>Nitrosomonas</i> ), S ( <i>Thiobacillus</i> ) for energy	<b>Photoautotrophs</b> Algae, cyanobacteria (“blue-green algae”), photosynthetic bacteria that use light energy to convert CO <sub>2</sub> (HCO <sub>3</sub> <sup>-</sup> ) to biomass by photosynthesis

Figure 6.2. Classification of microorganisms among chemoheterotrophs, chemoautotrophs, photoheterotrophs, and photoautotrophs.

## Microorganisms at Interfaces

Aquatic microorganisms tend to grow at interfaces. Many such microorganisms grow on solids that are suspended in water or are present in sediments. Large populations of aquatic bacteria typically reside on the surface of water at the air-water interface.<sup>1</sup> In addition to being in contact with air that aerobic microorganisms need for their metabolic processes, this interface also accumulates food in the form of lipids (oils, fats), polysaccharides, and proteins. Bacteria at this interface are generally different from those in the body of water and may have a hydrophobic cell character. When surface bubbles burst, bacteria at the air-water interface can be incorporated into aerosol water droplets and carried by wind. This is a matter of some concern with respect to sewage treatment plants as a possible vector for spreading disease-causing microorganisms.

### 6.2. ALGAE

For the purposes of discussion here, **algae** may be considered as generally microscopic organisms that subsist on inorganic nutrients and produce organic matter from carbon dioxide by photosynthesis.<sup>2</sup> In addition to single cells, algae grow as filaments, sheets, and colonies. Some algae, particularly the marine kelps, are huge multicellular organisms. The study of algae is called **phycology**.

The four main classes of unicellular algae of importance in environmental chemistry are the following:

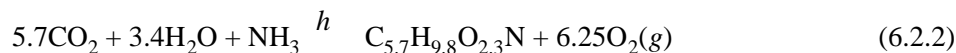
- **Chrysophyta**, which contain pigments that give these organisms a yellow-green or golden-brown color. Chrysophyta are found in both freshwater and marine systems. They store food as carbohydrate or oil. The most well-known of these algae are **diatoms**, characterized by silica-containing cell walls.
- **Chlorophyta**, commonly known as green algae, are responsible for most of the primary productivity in fresh waters.
- **Pyrrophyta**, commonly known as dinoflagellates, are motile with structures that enable them to move about in water. (In some cases the distinction between algae and single-celled “animal” protozoa is blurred; see the example of *Pfiesteria* discussed in Section 6.4.) Pyrrophyta occur in both marine and freshwater environments. “Blooms” of *Gymnodinium* and *Gonyaulax* species release toxins that cause harmful “red tides.”
- **Euglenophyta** likewise exhibit characteristics of both plants and animals. Though capable of photosynthesis, these algae are not exclusively photoautotrophic (see Figure 6.2), and they utilize biomass from other sources for at least part of their carbon needs

The general nutrient requirements of algae are carbon (obtained from CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup>), nitrogen (generally as NO<sub>3</sub><sup>-</sup>), phosphorus (as some form of orthophosphate), sulfur (as SO<sub>4</sub><sup>2-</sup>), and trace elements including sodium, potassium, calcium, magnesium, iron, cobalt, and molybdenum.

In a highly simplified form, the production of organic matter by algal photosynthesis is described by the reaction



where {CH<sub>2</sub>O} represents a unit of carbohydrate and *h* stands for the energy of a quantum of light. Fogg<sup>3</sup> has represented the overall formula of the algae *Chlorella* as C<sub>5.7</sub>H<sub>9.8</sub>O<sub>2.3</sub>NP<sub>0.06</sub>. Using Fogg’s formula for algal biomass exclusive of the phosphorus, the overall reaction for photosynthesis is:



In the absence of light, algae metabolize organic matter in the same manner as do nonphotosynthetic organisms. Thus, algae may satisfy their metabolic demands by utilizing chemical energy from the degradation of stored starches or oils, or from the consumption of algal protoplasm itself. In the absence of photosynthesis, the metabolic process consumes oxygen, so during the hours of darkness an aquatic system with a heavy growth of algae may become depleted in oxygen.

Symbiotic relationships of algae with other organisms are common. There are even reports of unicellular green algae growing inside hairs on polar bears, which are hollow for purposes of insulation; the sight of a green polar bear is alleged to have driven more than one arctic explorer to the brink of madness. The most common

symbiotic relationship involving algae is that of **lichen** in which algae coexist with fungi; both kinds of organisms are woven into the same thallus (tubular vegetative unit). The fungus provides moisture and nutrients required by the algae, which generates food photosynthetically. Lichen are involved in weathering processes of rocks.

The main role of algae in aquatic systems is the production of biomass. This occurs through photosynthesis, which fixes carbon dioxide and inorganic carbon from dissolved carbonate species as organic matter, thus providing the basis of the food chain for the other organisms in the system. Unless it occurs to an excessive extent, leading to accumulation of biomass that exhausts dissolved oxygen when it decays (eutrophication), the production of biomass is beneficial to the other organisms in the aquatic system. Under some conditions, the growth of algae can produce metabolites that are responsible for odor and even toxicity in water.<sup>4</sup>

### 6.3. FUNGI

Fungi are nonphotosynthetic, often filamentous, organisms exhibiting a wide range of morphology (structure).<sup>5</sup> Some fungi are as simple as the microscopic unicellular yeasts, whereas other fungi form large, intricate toadstools. The microscopic filamentous structures of fungi generally are much larger than bacteria, and usually are 5-10  $\mu\text{m}$  in width. Fungi are aerobic (oxygen-requiring) organisms and generally can thrive in more acidic media than can bacteria. They are also more tolerant of higher concentrations of heavy metal ions than are bacteria.

Perhaps the most important function of fungi in the environment is the breakdown of cellulose in wood and other plant materials. To accomplish this, fungal cells secrete an extracellular enzyme (exoenzyme), *cellulase*, that hydrolyzes insoluble cellulose to soluble carbohydrates that can be absorbed by the fungal cell.

Fungi do not grow well in water. However, they play an important role in determining the composition of natural waters and wastewaters because of the large amount of their decomposition products that enter water. An example of such a product is humic material, which interacts with hydrogen ions and metals (see Section 3.17).

### 6.4. PROTOZOA

**Protozoa** are microscopic animals consisting of single eukaryotic cells. The numerous kinds of protozoa are classified on the bases of morphology (physical structure), means of locomotion (flagella, cilia, pseudopodia), presence or absence of chloroplasts, presence or absence of shells, ability to form cysts (consisting of a reduced-size cell encapsulated in a relatively thick skin that can be carried in the air or by animals in the absence of water), and ability to form spores. Protozoa occur in a wide variety of shapes and their movement in the field of a microscope is especially fascinating to watch. Some protozoa contain chloroplasts and are photosynthetic.

Protozoa play a relatively small role in environmental biochemical processes, but are nevertheless significant in the aquatic and soil environment for the following reasons:

- Several devastating human diseases, including malaria, sleeping sickness, and some kinds of dysentery, are caused by protozoa that are parasitic to the human body.
- Parasitic protozoa can cause debilitating, even fatal, diseases in livestock and wildlife.
- Vast limestone ( $\text{CaCO}_3$ ) deposits have been formed by the deposition of shells from the *foramifera* group of protozoa.
- Protozoa are active in the oxidation of degradable biomass, particularly in sewage treatment.
- Protozoa may affect bacteria active in degrading biodegradable substances by “grazing” on bacterial cells.

Though they are single-celled, protozoa have a fascinating variety of structures that enable them to function. The protozoal cell membrane is protected and supported by a relatively thick pellicle, or by a mineral shell that may act as an exoskeleton. Food is ingested through a structure called a cytosome from which it is concentrated in a cytopharynx or oral groove, then digested by enzymatic action in a food vacuole. Residue from food digestion is expelled through a cytopyege and soluble metabolic products, such as urea or ammonia, are eliminated by a contractile vacuole, which also expels water from the cell interior.

One of the most troublesome aquatic protozoans in recent times is *Pfiesteria piscicida*, a single-celled organism that is reputed to have more than 20 life stages, including flagellated, amoeboid, and encysted forms, some of which are capable of photosynthesis and some of which are capable of living as parasites on fish.<sup>6</sup> In certain amoeboid or dinoflagellate stages, which are induced to form by substances in fish excreta, these organisms secrete a neurotoxin that incapacitates fish, enabling the *Pfiesteria* to attach to the fish and cause often fatal lesions. Large outbreaks of *Pfiesteria* occurred in North Carolina, and in the Pocomoke River of Maryland and the Rappahannock River of Virginia in the mid-late 1990s. In addition to killing fish, these microorganisms have caused symptoms in exposed humans, particularly a condition manifested by short-term memory loss. These “blooms” of *Pfiesteria* have been attributed to excessive enrichment of water with nitrogen and phosphorus, particularly from sewage and from runoff of swine-raising operations, leading to excessive algal growth and eutrophication.

## 6.5. BACTERIA

**Bacteria** are single-celled prokaryotic microorganisms that may be shaped as rods (**bacillus**), spheres (**coccus**), or spirals (**vibrios**, **spirilla**, **spirochetes**). Bacteria cells may occur individually or grow as groups ranging from two to millions of individual cells. Most bacteria fall into the size range of 0.5-3.0 micrometers. However, considering all species, a size range of 0.3-50  $\mu\text{m}$  is observed. Characteristics of most bacteria include a semirigid cell wall, motility with flagella for those capable of movement, unicellular nature (although clusters of cloned bacterial cells are common), and multiplication by binary fission in which each of two daughter cells is

genetically identical to the parent cell. Like other microorganisms, bacteria produce spores.

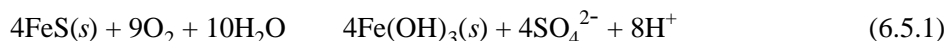
The metabolic activity of bacteria is greatly influenced by their small size. Their surface-to-volume ratio is extremely large, so that the inside of a bacterial cell is highly accessible to a chemical substance in the surrounding medium. Thus, for the same reason that a finely divided catalyst is more efficient than a more coarsely divided one, bacteria may bring about very rapid chemical reactions compared to those mediated by larger organisms. Bacteria excrete exoenzymes that break down solid food material to soluble components which can penetrate bacterial cell walls, where the digestion process is completed.

Although individual bacteria cells cannot be seen by the naked eye, bacterial colonies arising from individual cells are readily visible. A common method of counting individual bacterial cells in water consists of spreading a measured volume of an appropriately diluted water sample on a plate of agar gel containing bacterial nutrients. Wherever a viable bacterial cell adheres to the plate, a bacterial colony consisting of many cells will grow. These visible colonies are counted and related to the number of cells present initially. Because bacteria cells may already be present in groups, and because individual cells may not live to form colonies or even have the ability to form colonies on a plate, plate counts tend to grossly underestimate the number of viable bacteria.

## Autotrophic and Heterotrophic Bacteria

Bacteria may be divided into two main categories, autotrophic and heterotrophic. **Autotrophic bacteria** are not dependent upon organic matter for growth and thrive in a completely inorganic medium; they use carbon dioxide or other carbonate species as a carbon source. A number of sources of energy may be used, depending upon the species of bacteria; however, a biologically mediated chemical reaction always supplies the energy.

An example of autotrophic bacteria is *Gallionella*. In the presence of oxygen, these bacteria are grown in a medium consisting of  $\text{NH}_4\text{Cl}$ , phosphates, mineral salts,  $\text{CO}_2$  (as a carbon source), and solid  $\text{FeS}$  (as an energy source). It is believed that the following is the energy-yielding reaction for this species:



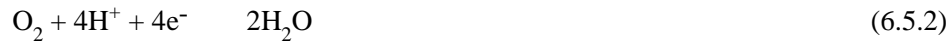
Starting with the simplest inorganic materials, autotrophic bacteria must synthesize all of the complicated proteins, enzymes, and other materials needed for their life processes. It follows, therefore, that the biochemistry of autotrophic bacteria is quite complicated. Because of their consumption and production of a wide range of minerals, autotrophic bacteria are involved in many geochemical transformations.

**Heterotrophic bacteria** depend upon organic compounds, both for their energy and for the carbon required to build their biomass. They are much more common in occurrence than autotrophic bacteria. Heterotrophic bacteria are the microorganisms primarily responsible for the breakdown of pollutant organic matter in water, and of organic wastes in biological waste-treatment processes.



## Aerobic and Anaerobic Bacteria

Another classification system for bacteria depends upon their requirement for molecular oxygen. **Aerobic bacteria** require oxygen as an electron receptor:



**Anaerobic bacteria** function only in the complete absence of molecular oxygen. Frequently, molecular oxygen is quite toxic to anaerobic bacteria.

A third class of bacteria, **facultative bacteria**, utilize free oxygen when it is available and use other substances as electron receptors (oxidants) when molecular oxygen is not available. Common oxygen substitutes in water are nitrate ion (see Section 6.11) and sulfate ion (see Section 6.12).

## 6.6. THE PROKARYOTIC BACTERIAL CELL

Figure 6.3 illustrates a generic prokaryotic bacterial cell. Bacterial cells are enclosed in a **cell wall**, which holds the contents of the bacterial cell and determines the shape of the cell. The cell wall in many bacteria is frequently surrounded by a **slime layer** (capsule). This layer protects the bacteria and helps the bacterial cells to adhere to surfaces.

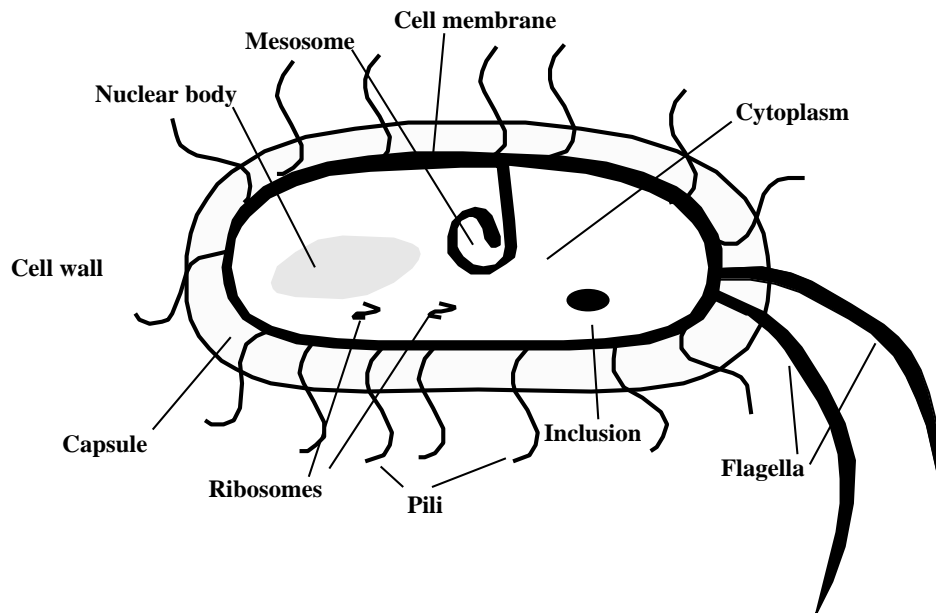


Figure 6.3. Generic prokaryotic bacterial cell illustrating major cell features.

The **cell membrane** or **cytoplasmic membrane** composed of protein and phospholipid occurs as a thin layer only about 7 nanometers in thickness on the inner surface of the cell wall enclosing the cellular cytoplasm. The cytoplasmic membrane

is of crucial importance to cell function in that it controls the nature and quantity of materials transported into and out of the cell. It is also very susceptible to damage from some toxic substances.

Folds in the cytoplasmic membrane called **mesosomes** serve several functions. One of these is to increase the surface area of the membrane to enhance transport of materials through it. Another function is to act as a site for division of the cell during reproduction. Bacterial DNA is separated at the mesosome during cell division.

Hairlike **pili** on the surface of a bacterial cell enable the cell to stick to surfaces. Specialized **sex pili** enable nucleic acid transfer between bacterial cells during an exchange of genetic material. Somewhat similar to pili — but larger, more complex, and fewer in number — are **flagella**, moveable appendages that cause bacterial cells to move by their whipping action. Bacteria with flagella are termed **motile**.

Bacterial cells are filled with an aqueous solution and suspension containing proteins, lipids, carbohydrates, nucleic acids, ions, and other materials. Collectively, these materials are referred to as **cytoplasm**, the medium in which the cell's metabolic processes are carried out. The major constituents of cytoplasm are the following:

- **Nuclear body** consisting of a single DNA macromolecule that controls metabolic processes and reproduction.
- **Inclusions** of reserve food material consisting of fats, carbohydrates, and even elemental sulfur.
- **Ribosomes**, which are sites of protein synthesis and which contain protein and RNA.

## 6.7. KINETICS OF BACTERIAL GROWTH

The population size of bacteria and unicellular algae as a function of time in a growth culture is illustrated by [Figure 6.4](#), which shows a **population curve** for a bacterial culture. Such a culture is started by inoculating a rich nutrient medium with a small number of bacterial cells. The population curve consists of four regions. The first region is characterized by little bacterial reproduction and is called the **lag phase**. The lag phase occurs because the bacteria must become acclimated to the new medium. Following the lag phase comes a period of very rapid bacterial growth. This is the **log phase**, or exponential phase, during which the population doubles over a regular time interval called the **generation time**. This behavior can be described by a mathematical model in which growth rate is proportional to the number of individuals present and there are no limiting factors such as death or lack of food:

$$\frac{dN}{dt} = kN \quad (6.7.1)$$

This equation can be integrated to give

$$\ln \frac{N}{N_0} = kt \text{ or } N = N_0 e^{kt} \quad (6.7.2)$$

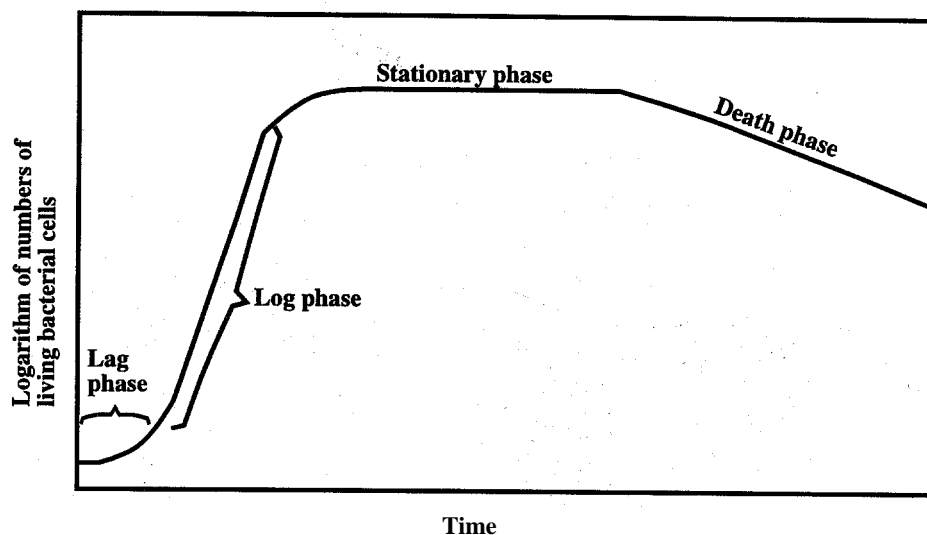


Figure 6.4. Population curve for a bacterial culture.

where  $N$  is the population at time  $t$  and  $N_0$  is the population at time  $t = 0$ . Thus, another way of describing population growth during the log phase is to say that the logarithm of bacterial population increases linearly with time. The generation time, or doubling time, is  $(\ln 2)/k$ , analogous to the half-life of radioactive decay. Fast growth during the log phase can cause very rapid microbial transformations of chemical species in water.

The log phase terminates and the **stationary phase** begins when a limiting factor is encountered. Typical factors limiting growth are depletion of an essential nutrient, build-up of toxic material, and exhaustion of oxygen. During the stationary phase, the number of viable cells remains virtually constant. After the stationary phase, the bacteria begin to die faster than they reproduce, and the population enters the **death phase**.

## 6.8. BACTERIAL METABOLISM

Bacteria obtain the energy and raw materials needed for their metabolic processes and reproduction by mediating chemical reactions. Nature provides a large number of such reactions, and bacterial species have evolved that utilize many of these. As a consequence of their participation in such reactions, bacteria are involved in many biogeochemical processes in water and soil. Bacteria are essential participants in many important elemental cycles in nature, including those of nitrogen, carbon, and sulfur. They are responsible for the formation of many mineral deposits, including some of iron and manganese. On a smaller scale, some of these deposits form through bacterial action in natural water systems and even in pipes used to transport water.

**Bacterial metabolism** addresses the biochemical processes by which chemical species are modified in bacterial cells. It is basically a means of deriving energy and

cellular material from nutrient substances. [Figure 6.5](#) summarizes the essential features of bacterial metabolism. The two major divisions of bacterial metabolism are **catabolism**, energy-yielding degradative metabolism which breaks macromolecules down to their small monomeric constituents, and **anabolism**, synthetic metabolism in which small molecules are assembled into large ones.

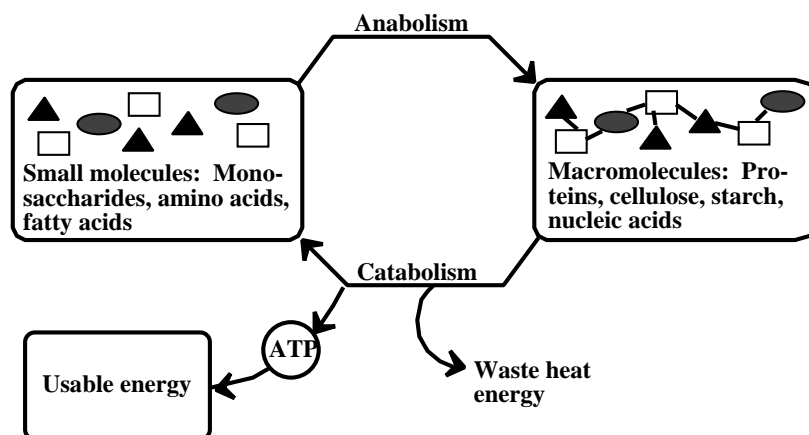


Figure 6.5. Bacterial metabolism and energy production.

A key distinction among bacteria has to do with the terminal electron acceptor in the electron transport chain involved in the process by which bacteria gain energy by oxidizing food materials. If the terminal electron acceptor is molecular  $O_2$ , the process is **aerobic respiration**. If it is another reducible species, commonly including  $SO_4^{2-}$ ,  $NO_3^-$ ,  $HCO_3^-$ , or iron(III), the process is called **anaerobic respiration**. As examples, *Desulfovibrio* bacteria convert  $SO_4^{2-}$  to  $H_2S$ , *Methanobacterium* reduce  $HCO_3^-$  to  $CH_4$ , and assorted bacteria reduce  $NO_3^-$  to  $NO_2^-$ ,  $N_2O$ ,  $N_2$ , or  $NH_4^+$ .

### Factors Affecting Bacterial Metabolism

Bacterial metabolic reactions are mediated by enzymes, biochemical catalysts endogenous to living organisms that are discussed in detail in Chapter 21. Enzymatic processes in bacteria are essentially the same as those in other organisms. At this point, however, it is useful to review several factors that influence bacterial enzyme activity and, therefore, bacterial growth.

[Figure 6.6](#) illustrates the effect of **substrate concentration** on enzyme activity, where a substrate is a substance upon which an enzyme acts. It is seen that enzyme activity increases in a linear fashion up to a value that represents saturation of the enzyme activity. Beyond this concentration, increasing substrate levels do not result in increased enzyme activity. This kind of behavior is reflected in bacterial activity which increases with available nutrients up to a saturation value. Superimposed on this plot in a bacterial system is increased bacterial population which, in effect, increases the amount of available enzyme.

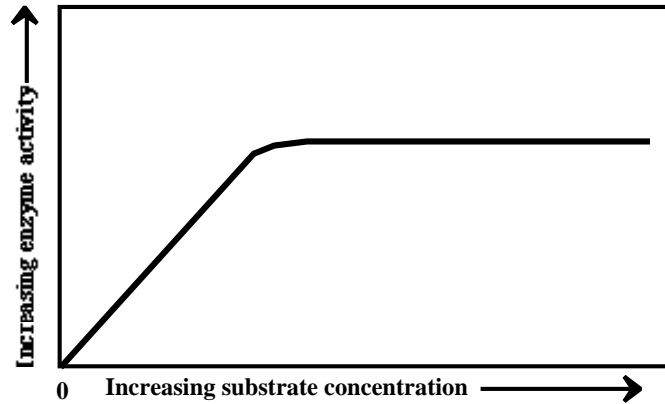


Figure 6.6. Effect of increasing substrate concentration on enzyme activity. Bacterial metabolism parallels such a plot.

Figure 6.7 shows the effect of **temperature** on enzyme activity and on bacterial growth and metabolism. It is seen that over a relatively short range of temperature, a plot of enzyme activity as a function of the reciprocal of the absolute temperature,  $1/T$ , is linear (an Arrhenius plot). The curve shows a maximum growth rate with an optimum temperature that is skewed toward the high temperature end of the curve, and exhibits an abrupt dropoff beyond the temperature maximum. This occurs because enzymes are destroyed by being denatured at temperatures not far above the optimum. Bacteria show different temperature optima. **Psychrophilic bacteria** are bacteria having temperature optima below approximately  $20^{\circ}\text{C}$ . The temperature optima of **mesophilic bacteria** lie between  $20^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ . Bacteria having temperature optima above  $45^{\circ}\text{C}$  are called **thermophilic bacteria**. The temperature range for optimum growth of bacteria is remarkably wide, with some bacteria being able to grow at  $0^{\circ}\text{C}$ , and some thermophilic bacteria existing in boiling hot water.

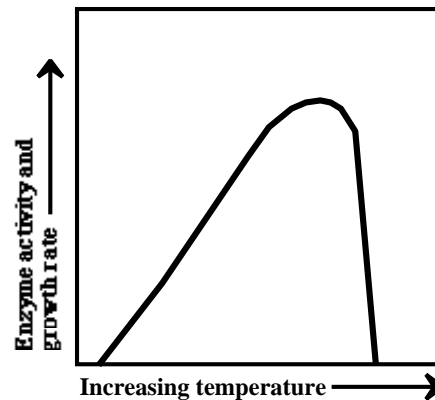


Figure 6.7. Enzyme activity as a function of temperature. A plot of bacterial growth vs. temperature has the same shape.

Figure 6.8 is a plot of pH vs. bacterial enzyme activity. Although the optimum pH will vary somewhat, enzymes typically have a pH optimum around neutrality.

Enzymes tend to become denatured at pH extremes. For some bacteria, such as those that generate sulfuric acid by the oxidation of sulfide or that produce organic acids by fermentation of organic matter, the pH optimum may be quite acidic, illustrating the ability of bacteria to adapt to very extreme environments.

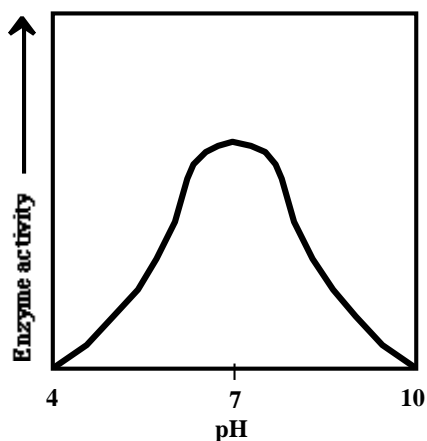


Figure 6.8. Enzyme activity and bacterial growth rate as a function of pH.

## Microbial Oxidation and Reduction

The metabolic processes by which bacteria obtain their energy involve mediation of oxidation-reduction reactions. The most environmentally important oxidation-reduction reactions occurring in water and soil through the action of bacteria are summarized in [Table 6.1](#). Much of the remainder of this chapter is devoted to a discussion of important redox reactions mediated by bacteria, particularly those summarized in [Table 6.1](#).

### 6.9. MICROBIAL TRANSFORMATIONS OF CARBON

Carbon is an essential life element and composes a high percentage of the dry weight of microorganisms. For most microorganisms, the bulk of net energy-yielding or energy-consuming metabolic processes involve changes in the oxidation state of carbon. These chemical transformations of carbon have important environmental implications. For example, when algae and other plants fix  $\text{CO}_2$  as carbohydrate, represented as  $\{\text{CH}_2\text{O}\}$ ,



carbon changes from the +4 to the 0 oxidation state. Energy from sunlight is stored as chemical energy in organic compounds. However, when the algae die, bacterial decomposition occurs through aerobic respiration in the reverse of the biochemical process represented by the above reaction for photosynthesis, energy is released, and oxygen is consumed.

**Table 6.1. Principal Microbially Mediated Oxidation and Reduction Reactions**

Oxidation	$pE^0(w)^1$
(1) $1/4\{CH_2O\} + 1/4H_2O \rightarrow 1/4CO_2 + H^+(w) + e^-$	-8.20
(1a) $1/2HCOO^- \rightarrow 1/2CO(g) + 1/2H^+(w) + e^-$	-8.73
(1b) $1/2\{CH_2O\} + 1/2H_2O \rightarrow 1/2HCOO^- + 3/2H^+(w) + e^-$	-7.68
(1c) $1/2CH_3OH \rightarrow 1/2\{CH_2O\} + H^+(w) + e^-$	-3.01
(1d) $1/2CH_4(g) + 1/2H_2O \rightarrow 1/2CH_3OH + H^+(w) + e^-$	-2.88
(2) $1/8HS^- + 1/2H_2O \rightarrow 1/8SO_4^{2-} + 9/8H^+(w) + e^-$	-3.75
(3) $1/8NH_4^+ + 3/8H_2O \rightarrow 1/8NO_3^- + 5/4H^+(w) + e^-$	+6.16
(4) <sup>1</sup> $FeCO_3(s) + 2H_2O \rightarrow FeOOH(s) + HCO_3^-(10^{-3}) + 2H^+(w) + e^-$	-1.67
(5) <sup>1</sup> $1/2MnCO_3(s) + H_2O \rightarrow 1/2MnO_2 + 1/2HCO_3^-(10^{-3}) + 3/2H^+(w) + e^-$	-8.5
<b>Reduction</b>	
(A) $1/4O_2(g) + H^+(w) + e^- \rightarrow 1/2H_2O$	+13.75
(B) $1/5NO_3^- + 6/5H^+(w) + e^- \rightarrow 1/10N_2 + 3/5H_2O$	+12.65
(C) $1/8NO_3^- + 5/4H^+(w) + e^- \rightarrow 1/8NH_4^+ + 3/8H_2O$	+6.15
(D) $1/2\{CH_2O\} + H^+(w) + e^- \rightarrow 1/2CH_3OH$	-3.01
(E) $1/8SO_4^{2-} + 9/8H^+(w) + e^- \rightarrow 1/8HS^- + 1/2H_2O$	-3.75
(F) $1/8CO_2(g) + H^+(w) + e^- \rightarrow 1/8CH_4(g) + 1/4H_2O$	-4.13
(G) $1/6N_2 + 4/3H^+(w) + e^- \rightarrow 1/3NH_4^+$	-4.68

*Sequence of Microbial Mediation*

*Model 1:* Excess of organic material (water initially contains  $O_2$ ,  $NO_3^-$ ,  $SO_4^{2-}$ , and  $HCO_3^-$ ). Examples: Hypolimnion of a eutrophic lake, sediments, sewage treatment plant digester.

	Combination	$pE^0(w)^2$	$G^0(w)$ , kcal
Aerobic respiration	(1) + (A)	21.95	-29.9
Denitrification	(1) + (B)	20.85	-28.4
Nitrate reduction	(1) + (C)	14.36	-19.6
Fermentation <sup>3</sup>	(1b) + (D)	4.67	-6.4
Sulfate reduction	(1) + (E)	4.45	-5.9
Methane fermentataion	(1) + (F)	4.07	-5.6
N-fixation	(1) + (G)	3.52	-4.8

*Model 2:* Excess  $O_2$  (water initially contains organic matter  $SH^-$ ,  $NH_4^+$ , and possibly Fe(II) and Mn(II)). Examples: aerobic waste treatment, self-purification in streams, epilimnion of lake.

**Table 6.1 (cont.)**

	Combination	$pE^0(w)^2$	$G^0(w)$ , kcal
Aerobic respiration	(A) + (1)	21.95	-29.9
Sulfide oxidation	(A) + (2)	17.50	-23.8
Nitrification	(A) + (3)	7.59	-10.3
Iron(II) oxidation <sup>4</sup>	(A) + (4)	15.42	21.0
Manganese(II) oxidation <sup>4</sup>	(A) + (5)	5.75	-7.2

<sup>1</sup> These  $pE^0$  values are at  $H^+$  ion activity of  $1.00 \times 10^{-7}$ ;  $H^+(w)$  designates water in which  $[H^+] = 1.00 \times 10^{-7}$ .  $pE^0$  values for half-reactions (1)-(5) are given for reduction, although the reaction is written as an oxidation.

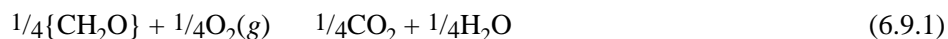
<sup>2</sup>  $pE^0$  values =  $\log K(w)$  for a reaction written for a one-electron transfer. The term  $K(w)$  is the equilibrium constant for the reaction in which the activity of the hydrogen ion has been set at  $1.00 \times 10^{-7}$  and incorporated into the equilibrium constant.

<sup>3</sup> Fermentation is interpreted as an organic redox reaction where one organic substance is reduced by oxidizing another organic substance (for example, alcoholic fermentation; the products are metastable thermodynamically with respect to  $CO_2$  and  $CH_4$ ).

<sup>4</sup> The data for  $pE^0(w)$  or  $G^0(w)$  of these reactions correspond to an activity of  $HCO_3^-$  ion of  $1.00 \times 10^{-3}$  rather than unity.

Source: Stumm, Werner, and James J. Morgan, *Aquatic Chemistry*, Wiley-Interscience, New York, 1970, pp. 336-337. Reproduced with permission of John Wiley & Sons, Inc.

In the presence of oxygen, the principal energy-yielding reaction of bacteria is the oxidation of organic matter. Since it is generally more meaningful to compare reactions on the basis of the reaction of one electron-mole, the aerobic degradation of organic matter is conveniently written as



for which the free-energy change is -29.9 kcal (see aerobic respiration, Table 6.1). From this general type of reaction, bacteria and other microorganisms extract the energy needed to carry out their metabolic processes, to synthesize new cell material, for reproduction, and for locomotion.

Partial microbial decomposition of organic matter is a major step in the production of peat, lignite, coal, oil shale, and petroleum. Under reducing conditions, particularly below water, the oxygen content of the original plant material (approximate empirical formula,  $\{CH_2O\}$ ) is lowered, leaving materials with relatively higher carbon contents.

### Methane-Forming Bacteria

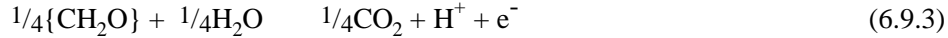
The production of methane in anoxic (oxygenless) sediments is favored by high organic levels and low nitrate and sulfate levels. Methane production plays a key role in local and global carbon cycles as the final step in the anaerobic decomposition of organic matter. This process is the source of about 80% of the methane entering the atmosphere.



The carbon from microbially produced methane can come from either the reduction of CO<sub>2</sub> or the fermentation of organic matter, particularly acetate. The anoxic production of methane can be represented in the following simplified manner. When carbon dioxide acts as an electron receptor in the absence of oxygen, methane gas is produced:



This reaction is mediated by methane-forming bacteria. When organic matter is degraded microbially, the half-reaction for one electron-mole of {CH<sub>2</sub>O} is



Adding half-reactions 6.9.2 and 6.9.3 yields the overall reaction for the anaerobic degradation of organic matter by methane-forming bacteria, which involves a free-energy change of -5.55 kcal per electron-mole:



This reaction, in reality a series of complicated processes, is a **fermentation reaction**, defined as a redox process in which both the oxidizing agent and reducing agent are organic substances. It may be seen that only about one-fifth as much free energy is obtained from one electron-mole of methane formation as from a one electron-mole reaction involving complete oxidation of one electron-mole of the organic matter, Reaction 6.9.1.

There are four main categories of methane-producing bacteria. These bacteria, differentiated largely by morphology, are *Methanobacterium*, *Methanobacillus*, *Methanococcus*, and *Methanosarcina*. The methane-forming bacteria are *obligately anaerobic*; that is, they cannot tolerate the presence of molecular oxygen. The necessity of avoiding any exposure to oxygen makes the laboratory culture of these bacteria very difficult.

Methane formation is a valuable process responsible for the degradation of large quantities of organic wastes, both in biological waste-treatment processes (see Chapter 8) and in nature. Methane production is used in biological waste treatment plants to further degrade excess sludge from the activated sludge process. In the bottom regions of natural waters, methane-forming bacteria degrade organic matter in the absence of oxygen. This eliminates organic matter which would otherwise require oxygen for its biodegradation. If this organic matter were transported to aerobic water containing dissolved O<sub>2</sub>, it would exert a biological oxygen demand (BOD). Methane production is a very efficient means for the removal of BOD. The reaction,



shows that 1 mole of methane requires 2 moles of oxygen for its oxidation to CO<sub>2</sub>. Therefore, the production of 1 mole of methane and its subsequent evolution from water are equivalent to the removal of 2 moles of oxygen demand. In a sense,

therefore, the removal of 16 grams (1 mole) of methane is equivalent to the addition of 64 grams (2 moles) of available oxygen to the water.

In favorable cases, methane fuel can be produced cost-effectively as a renewable resource from anaerobic digestion of organic wastes. Some installations use cattle feedlot wastes. Methane is routinely generated by the action of anaerobic bacteria and is used for heat and engine fuel at sewage treatment plants (see Chapter 8). Methane produced underground in municipal landfills is being tapped by some municipalities; however, methane seeping into basements of buildings constructed on landfill containing garbage has caused serious explosions and fires.

### **Bacterial Utilization of Hydrocarbons**

Methane is oxidized under aerobic conditions by a number of strains of bacteria. One of these, *Methanomonas*, is a highly specialized organism that cannot use any material other than methane as an energy source. Methanol, formaldehyde, and formic acid are intermediates in the microbial oxidation of methane to carbon dioxide. As discussed in Section 6.10, several types of bacteria can degrade higher hydrocarbons and use them as energy and carbon sources.

### **Microbial Utilization of Carbon Monoxide**

Carbon monoxide is removed from the atmosphere by contact with soil. It has been found that carbon monoxide is removed rapidly from air in contact with soil. Since neither sterilized soil nor green plants grown under sterile conditions show any capacity to remove carbon monoxide from air, this ability must be due to microorganisms in the soil. Fungi capable of CO metabolism include some commonly-occurring strains of the ubiquitous *Penicillium* and *Aspergillus*. It is also possible that some bacteria are involved in CO removal. Whereas some microorganisms metabolize CO, other aquatic and terrestrial organisms produce this gas.

## **6.10. BIODEGRADATION OF ORGANIC MATTER**

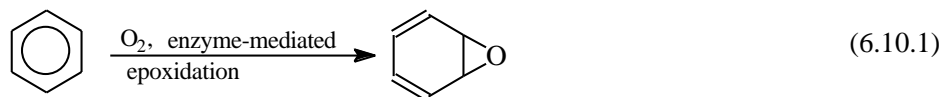
The biodegradation of organic matter in the aquatic and terrestrial environments is a crucial environmental process. Some organic pollutants are biocidal; for example, effective fungicides must be antimicrobial in action. Therefore, in addition to killing harmful fungi, fungicides frequently harm beneficial saprophytic fungi (fungi that decompose dead organic matter) and bacteria. Herbicides, which are designed for plant control, and insecticides, which are used to control insects, generally do not have any detrimental effect upon microorganisms.

The biodegradation of organic matter by microorganisms occurs by way of a number of stepwise, microbially catalyzed reactions. These reactions will be discussed individually with examples.

### **Oxidation**

**Oxidation** occurs by the action of oxygenase enzymes (see Chapter 21 for a discussion of biochemical terms). The microbially catalyzed conversion of aldrin to

dieldrin is an example of epoxide formation, a major step in many oxidation mechanisms. **Epoxidation** consists of adding an oxygen atom between two C atoms in an unsaturated system as shown below:



a particularly important means of metabolic attack upon aromatic rings that abound in many xenobiotic compounds.

### Microbial Oxidation of Hydrocarbons

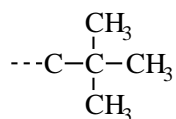
The degradation of hydrocarbons by microbial oxidation is an important environmental process because it is the primary means by which petroleum wastes are eliminated from water and soil. Bacteria capable of degrading hydrocarbons include *Micrococcus*, *Pseudomonas*, *Mycobacterium*, and *Nocardia*.

The most common initial step in the microbial oxidation of alkanes involves conversion of a terminal  $-\text{CH}_3$  group to a  $-\text{CO}_2$  group. More rarely, the initial enzymatic attack involves the addition of an oxygen atom to a nonterminal carbon, forming a ketone. After formation of a carboxylic acid from the alkane, further oxidation normally occurs by a process illustrated by the following reaction, a  $-\text{oxidation}$ :

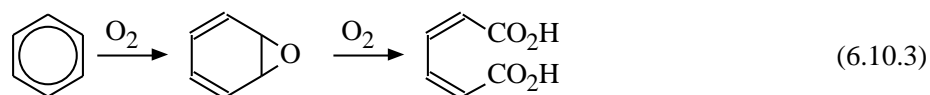


Since 1904, it has been known that the oxidation of fatty acids involves oxidation of the  $-\text{carbon}$  atom, followed by removal of two-carbon fragments. A complicated cycle with a number of steps is involved. The residue at the end of each cycle is an organic acid with two fewer carbon atoms than its precursor at the beginning of the cycle.

Hydrocarbons vary significantly in their biodegradability, and microorganisms show a strong preference for straight-chain hydrocarbons. A major reason for this preference is that branching inhibits  $-\text{oxidation}$  at the site of the branch. The presence of a quaternary carbon (below) particularly inhibits alkane degradation.

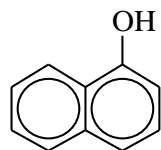


Despite their chemical stability, aromatic (aryl) rings are susceptible to microbial oxidation. The overall process leading to ring cleavage is

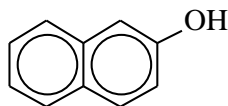


in which cleavage is preceded by addition of  $-\text{OH}$  to adjacent carbon atoms. Among

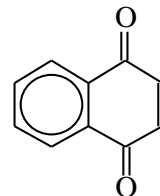
the microorganisms that attack aromatic rings is the fungus *Cunninghamella elegans*.<sup>7</sup> It metabolizes a wide range of hydrocarbons including: C<sub>3</sub>-C<sub>32</sub> alkanes; alkenes; and aryls, including toluene, naphthalene, anthracene, biphenyl, and phenanthrene. A study of the metabolism of naphthalene by this organism led to the isolation of the following metabolites (the percentage yields are given in parentheses):



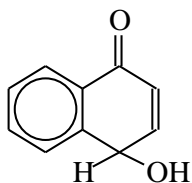
1-Naphthol (67.9%)



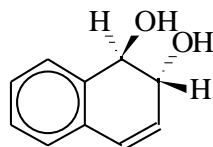
2-Naphthol (6.3%)



1,4-Naphthoquinone (2.8%)

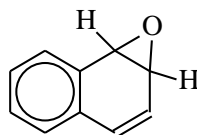


4-Hydroxy-1-tetralone (16.7%)



*Trans* -1,2-dihydroxy-  
1,2-dihydronaphthalene (5.3%)

The initial attack of oxygen on naphthalene produces 1,2-naphthalene oxide (below), which reacts to form the other products shown above.



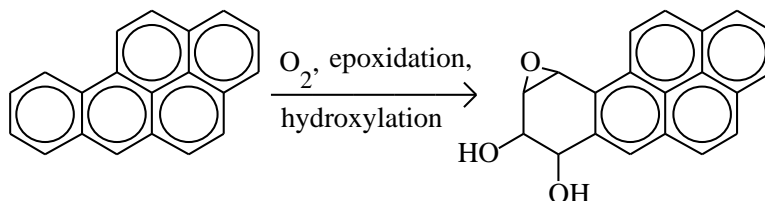
The biodegradation of petroleum is essential to the elimination of oil spills (of the order of a million metric tons per year). This oil is degraded by both marine bacteria and filamentous fungi. In some cases, the rate of degradation is limited by available nitrate and phosphate.

The physical form of crude oil makes a large difference in its degradability. Degradation in water occurs at the water-oil interface. Therefore, thick layers of crude oil prevent contact with bacterial enzymes and O<sub>2</sub>. Apparently, bacteria synthesize an emulsifier that keeps the oil dispersed in the water as a fine colloid and therefore accessible to the bacterial cells.

**Hydroxylation** often accompanies microbial oxidation. It is the attachment of -OH groups to hydrocarbon chains or rings. In the biodegradation of foreign compounds, hydroxylation often follows epoxidation as shown by the following rearrangement reaction for benzene epoxide:



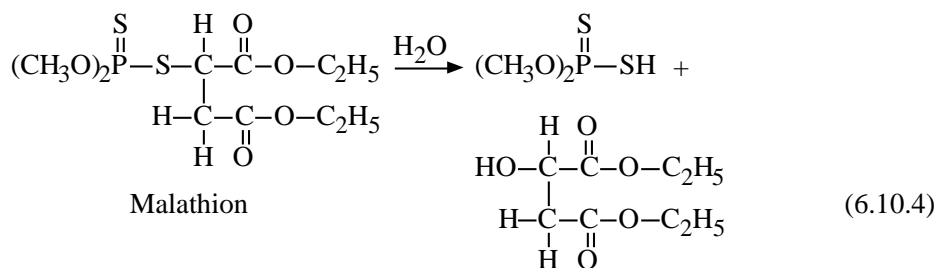
Hydroxylation can consist of the addition of more than one hydroxide group. An example of epoxidation and hydroxylation is the metabolic production of the 7,8-diol-9,10-epoxide of benzo(a)pyrene as illustrated below:



The metabolism of benzo(a)pyrene according to the above reaction is particularly important from the environmental and toxicological viewpoints because one of the stereoisomers of the 7,8-diol-9,10-epoxide binds strongly to cellular DNA, which can cause cancer in an organism.

## Other Biochemical Processes

**Hydrolysis**, which involves the addition of  $H_2O$  to a molecule accompanied by cleavage of the molecule into two products, is a major step in microbial degradation of many pollutant compounds, especially pesticidal esters, amides, and organophosphate esters. The types of enzymes that bring about hydrolysis are **hydrolase enzymes**; those that enable the hydrolysis of esters are called **esterases**, whereas those that hydrolyze amides are **amidases**. At least one species of *Pseudomonas* hydrolyzes malathion in a type of hydrolysis reaction typical of those by which pesticides are degraded:



**Reductions** are carried out by **reductase enzymes**; for example, nitroreductase enzyme catalyzes the reduction of the nitro group. Table 6.2 gives the major kinds of functional groups reduced by microorganisms.

**Dehalogenation** reactions of organohalide compounds involve the bacterially-mediated replacement of a covalently-bound halogen atom (F, Cl, Br, I) with  $-OH$ , and are discussed in more detail in Section 6.13.

**Table 6.2. Functional Groups that Undergo Microbial Reduction**

Reactant	Process	Product
$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{H} \end{array}$	Aldehyde reduction	$\begin{array}{c} \text{H} \\   \\ \text{R}-\text{C}-\text{OH} \\   \\ \text{H} \end{array}$
$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{R}' \end{array}$	Ketone reduction	$\begin{array}{c} \text{OH} \\   \\ \text{R}-\text{C}-\text{R}' \\   \\ \text{H} \end{array}$
$\begin{array}{c} \text{O} \\    \\ \text{R}-\text{S}-\text{R}' \end{array}$	Sulfoxide reduction	$\text{R}-\text{S}-\text{R}'$
$\text{R}-\text{SS}-\text{R}'$	Disulfide reduction	$\text{R}-\text{SH}, \text{R}'-\text{SH}$
$\begin{array}{c} \text{R} \quad \text{H} \\ \diagdown \quad / \\ \text{C}=\text{C} \\ / \quad \diagdown \\ \text{H} \quad \text{R}' \end{array}$	Alkene reduction	$\begin{array}{c} \text{H} \quad \text{H} \\   \quad   \\ \text{R}-\text{C}-\text{C}-\text{R}' \\   \quad   \\ \text{H} \quad \text{H} \end{array}$
$\text{R}-\text{NO}_2$	Nitro group reduction	$\text{R}-\text{NO}, \text{R}-\text{NH}_2,$ $\begin{array}{c} \text{H} \\   \\ \text{R}-\text{N} \\   \\ \text{OH} \end{array}$

**Ring cleavage** is a crucial step in the ultimate degradation of organic compounds having aryl rings. Normally, ring cleavage follows the addition of  $-\text{OH}$  groups (hydroxylation).

Many environmentally significant organic compounds contain alkyl groups, such as the methyl ( $-\text{CH}_3$ ) group, attached to atoms of O, N, and S. An important step in the microbial metabolism of many of these compounds is **dealkylation**, replacement of alkyl groups by H as shown in Figure 6.9. Examples of these kinds of reactions include O-dealkylation of methoxychlor insecticides, N-dealkylation of carbaryl insecticide, and S-dealkylation of dimethyl sulfide. Alkyl groups removed by dealkylation usually are attached to oxygen, sulfur, or nitrogen atoms; those attached to carbon are normally not removed directly by microbial processes.

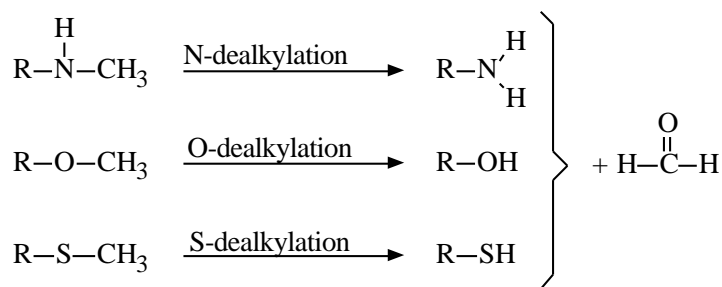


Figure 6.9. Metabolic dealkylation reactions shown for the removal of  $\text{CH}_3$  from N, O, and S atoms in organic compounds.

## 6.11. MICROBIAL TRANSFORMATIONS OF NITROGEN

Some of the most important microorganism-mediated chemical reactions in aquatic and soil environments are those involving nitrogen compounds. They are summarized in the **nitrogen cycle** shown in Figure 6.10. This cycle describes the dynamic processes through which nitrogen is interchanged among the atmosphere, organic matter, and inorganic compounds. It is one of nature's most vital dynamic processes.

Among the biochemical transformations in the nitrogen cycle are nitrogen fixation, whereby molecular nitrogen is fixed as organic nitrogen; nitrification, the process of oxidizing ammonia to nitrate; nitrate reduction, the process by which nitrogen in nitrate ion is reduced to form compounds having nitrogen in a lower oxidation state; and denitrification, the reduction of nitrate and nitrite to  $N_2$ , with a resultant net loss of nitrogen gas to the atmosphere. Each of these important chemical processes will be discussed separately.

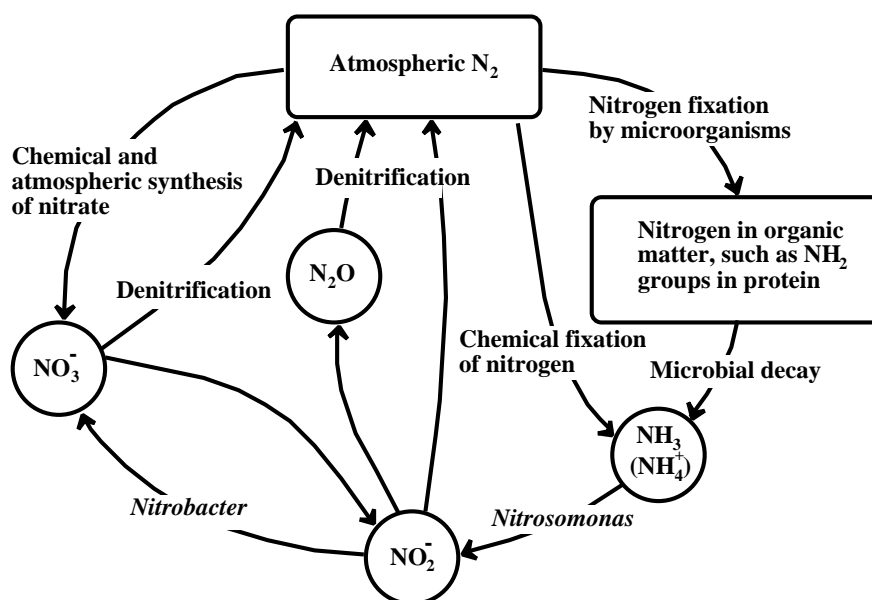


Figure 6.10. The nitrogen cycle.

### Nitrogen Fixation

The overall microbial process for **nitrogen fixation**, the binding of atmospheric nitrogen in a chemically combined form,



is actually quite complicated and not completely understood. Biological nitrogen fixation is a key biochemical process in the environment and is essential for plant growth in the absence of synthetic fertilizers.

Only a few species of aquatic microorganisms have the ability to fix atmospheric nitrogen. Among the aquatic bacteria having this capability are photosynthetic bacteria, *Azotobacter*, several species of *Clostridium*, and cyanobacteria, formerly called blue-green algae. In most natural fresh water systems, however, the fraction of nitrogen fixed by organisms in the water relative to that originating from the decay of organic material, fertilizer runoff and other external sources, is quite low.

The best-known and most important form of nitrogen-fixing bacteria is *Rhizobium*, which enjoys a symbiotic (mutually advantageous) relationship with leguminous plants such as clover or alfalfa. The *Rhizobium* bacteria are found in root nodules, special structures attached to the roots of legumes (see [Figure 16.2](#)). The nodules develop as a result of the bacteria “irritating” the root hairs of the developing legume plants. The nodules are connected directly to the vascular (circulatory) system of the plant, enabling the bacteria to derive photosynthetically-produced energy directly from the plant. Thus, the plant provides the energy required to break the strong triple bonds in the dinitrogen molecule, converting the nitrogen to a reduced form which is directly assimilated by the plant. When the legumes die and decay,  $\text{NH}_4^+$  ion is released and is converted by microorganisms to nitrate ion which is assimilable by other plants. Some of the ammonium ion and nitrate released may be carried into natural water systems.

Some nonlegume angiosperms fix nitrogen through the action of actinomycetes bacteria contained in root nodules. Shrubs and trees in the nitrogen-fixing category are abundant in fields, forests, and wetlands throughout the world. Their rate of nitrogen fixation is comparable to that of legumes.

Free-living bacteria associated with some grasses are stimulated by the grasses to fix nitrogen. One such bacterium is *Spirillum lipoferum*. In tropical surroundings, the amount of reduced nitrogen fixed by such bacteria can amount to the order of 100 kg per hectare per year.

Because of the cost of energy required to fix nitrogen synthetically, efforts are underway to increase the efficiency of natural means of nitrogen fixation. One approach uses recombinant DNA methodologies in attempts to transfer the nitrogen-fixing capabilities of nitrogen-fixing bacteria directly to plant cells. Though a fascinating possibility, this transfer has not yet been achieved on a practical basis. The other approach uses more conventional plant breeding and biological techniques in attempts to increase the range and effectiveness of the symbiotic relationship existing between some plants and nitrogen-fixing bacteria.

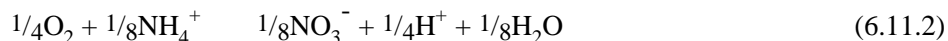
One matter of concern is that successful efforts to increase nitrogen fixation may upset the global nitrogen balance. Total annual global fixation of nitrogen is now more than 50% higher than the pre-industrial level of 150 million metric tons estimated for 1850. Potential accumulation of excess fixed nitrogen is the subject of some concern because of aquatic nitrate pollution and microbial production of  $\text{N}_2\text{O}$  gas. Some atmospheric scientists fear that excess  $\text{N}_2\text{O}$  gas may be involved in depletion of the protective atmospheric ozone layer (see Chapter 14).

## Nitrification

**Nitrification**, the conversion of N(-III) to N(V), is a very common and extremely important process in water and in soil. Aquatic nitrogen in thermodynamic



equilibrium with air is in the +5 oxidation state as  $\text{NO}_3^-$ , whereas in most biological compounds, nitrogen is present as N(-III), such as  $-\text{NH}_2$  in amino acids. The equilibrium constant of the overall nitrification reaction, written for one electron-mole,



is  $10^{7.59}$  (Table 6.1), showing that the reaction is highly favored from a thermodynamic viewpoint.

Nitrification is especially important in nature because nitrogen is absorbed by plants primarily as nitrate. When fertilizers are applied in the form of ammonium salts or anhydrous ammonia, a microbial transformation to nitrate enables maximum assimilation of nitrogen by the plants.

The nitrification conversion of ammoniacal nitrogen to nitrate ion takes place if extensive aeration is allowed to occur in the activated sludge sewage-treatment process (see Chapter 8). As the sewage sludge settles out in the settler, the bacteria in the sludge carry out denitrification while using this nitrate as an oxygen source (Reaction 6.11.8), producing  $\text{N}_2$ . The bubbles of nitrogen gas cause the sludge to rise, so that it does not settle properly. This can hinder the proper treatment of sewage through carryover of sludge into effluent water.

In nature, nitrification is catalyzed by two groups of bacteria, *Nitrosomonas* and *Nitrobacter*. *Nitrosomonas* bacteria bring about the transition of ammonia to nitrite,

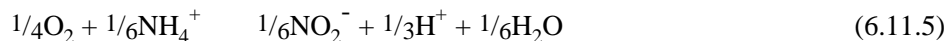


whereas *Nitrobacter* mediates the oxidation of nitrite to nitrate:



Both of these highly specialized types of bacteria are *obligate aerobes*; that is, they function only in the presence of molecular  $\text{O}_2$ . These bacteria are also *chemolithotrophic*, meaning that they can utilize oxidizable inorganic materials as electron donors in oxidation reactions to yield needed energy for metabolic processes.

For the aerobic conversion of one electron-mole of ammoniacal nitrogen to nitrite ion at pH 7.00,



the free-energy change is -10.8 kcal. The free-energy change for the aerobic oxidation of one electron-mole of nitrite ion to nitrate ion,

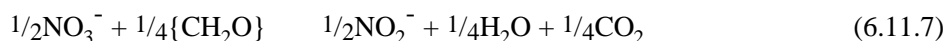


is -9.0 kcal. Both steps of the nitrification process involve an appreciable yield of free energy. It is interesting to note that the free-energy yield per electron-mole is approximately the same for the conversion of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  as it is for the conversion of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ , about 10 kcal/electron-mole.

## Nitrate Reduction

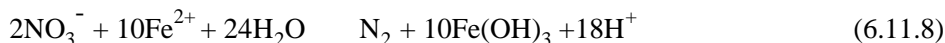
As a general term, **nitrate reduction** refers to microbial processes by which nitrogen in chemical compounds is reduced to lower oxidation states. In the absence of free oxygen, nitrate may be used by some bacteria as an alternate electron receptor. The most complete possible reduction of nitrogen in nitrate ion involves the acceptance of 8 electrons by the nitrogen atom, with the consequent conversion of nitrate to ammonia (+V to -III oxidation state). Nitrogen is an essential component of protein, and any organism that utilizes nitrogen from nitrate for the synthesis of protein must first reduce the nitrogen to the -III oxidation state (ammoniacal form). However, incorporation of nitrogen into protein generally is a relatively minor use of the nitrate undergoing microbially mediated reactions and is more properly termed nitrate assimilation.

Nitrate ion functioning as an electron receptor usually produces  $\text{NO}_2^-$ :



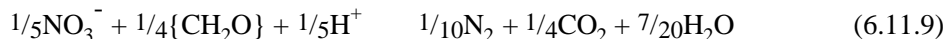
The free-energy yield per electron-mole is only about 2/3 of the yield when oxygen is the oxidant; however, nitrate ion is a good electron receptor in the absence of  $\text{O}_2$ . One of the factors limiting the use of nitrate ion in this function is its relatively low concentration in most waters. Furthermore, nitrite,  $\text{NO}_2^-$ , is relatively toxic and tends to inhibit the growth of many bacteria after building up to a certain level. Sodium nitrate has been used as a “first-aid” treatment in sewage lagoons that have become oxygen-deficient. It provides an emergency source of oxygen to reestablish normal bacterial growth.

Nitrate ion can be an effective oxidizing agent for a number of species in water that are oxidized by the action of microorganisms. One place in which this process is of interest is in biological sewage treatment. Nitrate has been shown to act as a microbial oxidizing agent for the conversion of iron(II) to iron(III) under conditions corresponding to the biological treatment of sewage.<sup>8</sup>



## Denitrification

An important special case of nitrate reduction is **denitrification**, in which the reduced nitrogen product is a nitrogen-containing gas, usually  $\text{N}_2$ . At pH 7.00, the free-energy change per electron-mole of reaction,



is -2.84 kcal. The free-energy yield per mole of nitrate reduced to  $\text{N}_2$  (5 electron-moles) is lower than that for the reduction of the same quantity of nitrate to nitrite. More important, however, the reduction of a nitrate ion to  $\text{N}_2$  gas consumes 5 electrons, compared to only 2 electrons for the reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ .

Denitrification is an important process in nature. It is the mechanism by which fixed nitrogen is returned to the atmosphere. Denitrification is also used in advanced

water treatment for the removal of nutrient nitrogen (see Chapter 8). Because nitrogen gas is a nontoxic volatile substance that does not inhibit microbial growth, and since nitrate ion is a very efficient electron acceptor, denitrification allows the extensive growth of bacteria under anaerobic conditions.

Loss of nitrogen to the atmosphere may also occur through the formation of  $N_2O$  and  $NO$  by bacterial action on nitrate and nitrite catalyzed by the action of several types of bacteria. Production of  $N_2O$  relative to  $N_2$  is enhanced during denitrification in soils by increased concentrations of  $NO_3^-$ ,  $NO_2^-$ , and  $O_2$ .

### Competitive Oxidation of Organic Matter by Nitrate Ion and Other Oxidizing Agents

The successive oxidation of organic matter by dissolved  $O_2$ ,  $NO_3^-$ , and  $SO_4^{2-}$  brings about an interesting sequence of nitrate-ion levels in sediments and hypolimnion waters initially containing  $O_2$  but lacking a mechanism for reaeration.<sup>9</sup> This is shown in Figure 6.11, where concentrations of dissolved  $O_2$ ,  $NO_3^-$ , and  $SO_4^{2-}$  are

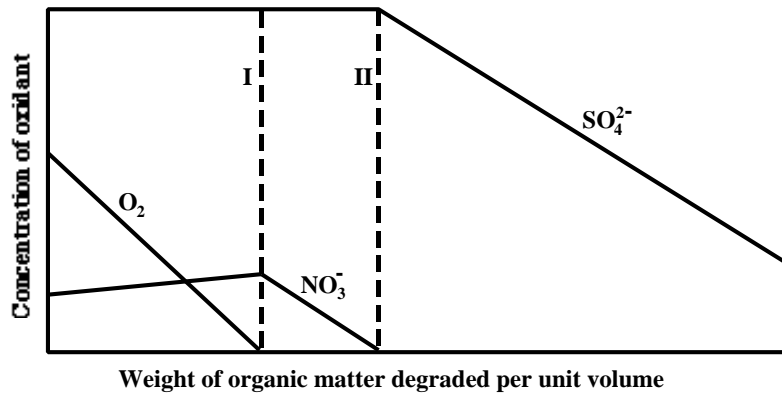


Figure 6.11. Oxidation of organic matter by  $O_2$ ,  $NO_3^-$ , and  $SO_4^{2-}$ .

plotted as a function of total organic matter metabolized. This behavior can be explained by the following sequence of biochemical processes:



So long as some  $O_2$  is present, some nitrate may be produced from organic matter. After exhaustion of molecular oxygen, nitrate is the favored oxidizing agent, and its concentration falls from a maximum value (I) to zero (II). Sulfate, which is usually present in a large excess over the other two oxidants, then becomes the favored electron receptor, enabling biodegradation of organic matter to continue.

## 6.12. MICROBIAL TRANSFORMATIONS OF PHOSPHORUS AND SULFUR

### Phosphorus Compounds

Biodegradation of phosphorus compounds is important in the environment for two reasons. The first of these is that it provides a source of algal nutrient orthophosphate from the hydrolysis of polyphosphates (see Section 3.16). Secondly, biodegradation deactivates highly toxic organophosphate compounds, such as the organophosphate insecticides.

The organophosphorus compounds of greatest environmental concern tend to be sulfur-containing **phosphorothionate** and **phosphorodithioate** ester insecticides with the general formulas illustrated in Figure 6.12, where R and R' represents a hydro-carbon substituted hydrocarbon moieties. These are used because they exhibit higher ratios of insect:mammal toxicity than do their nonsulfur analogs. The metabolic conversion of P=S to P=O (oxidative desulfuration, such as in the conversion of parathion to paraoxon) in organisms is responsible for the insecticidal activity and mammalian toxicity of phosphorothionate and phosphorodithioate insecticides. The biodegradation of these compounds is an important environmental chemical process. Fortunately, unlike the organohalide insecticides that they largely displaced, the organophosphates readily undergo biodegradation and do not bioaccumulate.

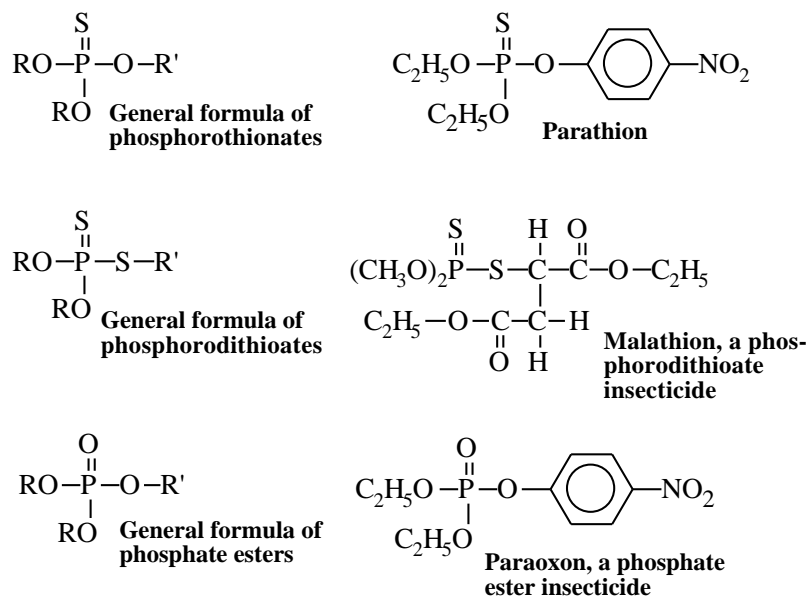
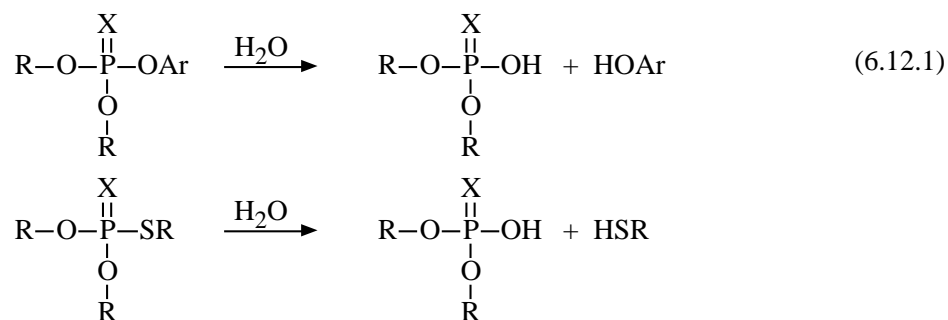


Figure 6.12. Phosphorothionate, phosphorodithioate, and phosphate ester insecticides.

Hydrolysis is an important step in the biodegradation of phosphorothionate, phosphorodithioate, and phosphate ester insecticides as shown by the following general reactions where R is an alkyl group, Ar is a substituent group that is frequently aromatic, and X is either S or O:



## Sulfur Compounds

Sulfur compounds are very common in water. Sulfate ion,  $\text{SO}_4^{2-}$ , is found in varying concentrations in practically all natural waters. Organic sulfur compounds, both those of natural origin and pollutant species, are very common in natural aquatic systems, and the degradation of these compounds is an important microbial process. Sometimes the degradation products, such as odiferous and toxic  $\text{H}_2\text{S}$ , cause serious problems with water quality.

There is a strong analogy between sulfur in the environment and nitrogen in the environment. Sulfur in living material is present primarily in its most reduced state, for example, as the hydrosulfide group,  $-\text{SH}$ . Nitrogen in living material is present in the (-III) oxidation state, for example, as  $-\text{NH}_2$ . When organic sulfur compounds are decomposed by bacteria, the initial sulfur product is generally the reduced form,  $\text{H}_2\text{S}$ . When organic nitrogen compounds are decomposed by microorganisms, the reduced form of nitrogen,  $\text{NH}_3$  or  $\text{NH}_4^+$ , is produced. Just as some microorganisms can produce elemental nitrogen from nitrogen compounds, some bacteria produce and store elemental sulfur from sulfur compounds. In the presence of oxygen, some bacteria convert reduced forms of sulfur to the oxidized form in  $\text{SO}_4^{2-}$  ion, whereas other bacteria catalyze the oxidation of reduced nitrogen compounds to nitrate ion.

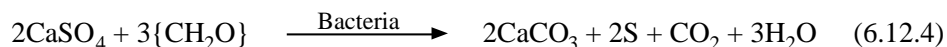
### *Oxidation of $\text{H}_2\text{S}$ and Reduction of Sulfate by Bacteria*

Although organic sulfur compounds often are the source of  $\text{H}_2\text{S}$  in water, they are not required as the sulfur source for  $\text{H}_2\text{S}$  formation. The bacteria *Desulfovibrio* can reduce sulfate ion to  $\text{H}_2\text{S}$ . In so doing, they utilize sulfate as an electron acceptor in the oxidation of organic matter. The overall reaction for the microbially-mediated oxidation of biomass with sulfate is,



and it requires other bacteria besides *Desulfovibrio* to oxidize organic matter completely to  $\text{CO}_2$ . The oxidation of organic matter by *Desulfovibrio* generally terminates with acetic acid, and accumulation of acetic acid is evident in bottom waters. Because of the high concentration of sulfate ion in seawater, bacterially-mediated formation of  $\text{H}_2\text{S}$  causes pollution problems in some coastal areas and is a major source of atmospheric sulfur. In waters where sulfide formation occurs, the sediment is often black in color due to the formation of  $\text{FeS}$ .

Bacterially-mediated reduction of sulfur in calcium sulfate deposits produces elemental sulfur interspersed in the pores of the limestone product. The highly generalized chemical reaction for this process is



although the stoichiometric amount of free sulfur is never found in these deposits due to the formation of volatile  $\text{H}_2\text{S}$ , which escapes.

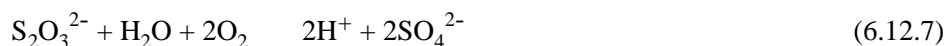
Whereas some bacteria can reduce sulfate ion to  $\text{H}_2\text{S}$ , others can oxidize hydrogen sulfide to higher oxidation states. The purple sulfur bacteria and green sulfur bacteria derive energy for their metabolic processes through the oxidation of  $\text{H}_2\text{S}$ . These bacteria utilize  $\text{CO}_2$  as a carbon source and are strictly anaerobic. The aerobic colorless sulfur bacteria may use molecular oxygen to oxidize  $\text{H}_2\text{S}$ ,



elemental sulfur,



or thiosulfate ion:

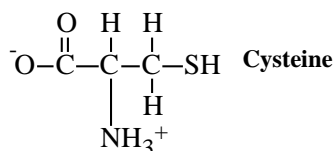


Oxidation of sulfur in a low oxidation state to sulfate ion produces sulfuric acid, a strong acid. One of the colorless sulfur bacteria, *Thiobacillus thiooxidans* is tolerant of 1 normal acid solutions, a remarkable acid tolerance. When elemental sulfur is added to excessively alkaline soils, the acidity is increased because of a microorganism-mediated reaction (6.12.6), which produces sulfuric acid. Elemental sulfur may be deposited as granules in the cells of purple sulfur bacteria and colorless sulfur bacteria. Such processes are important sources of elemental sulfur deposits.

### Microorganism-Mediated Degradation of Organic Sulfur Compounds

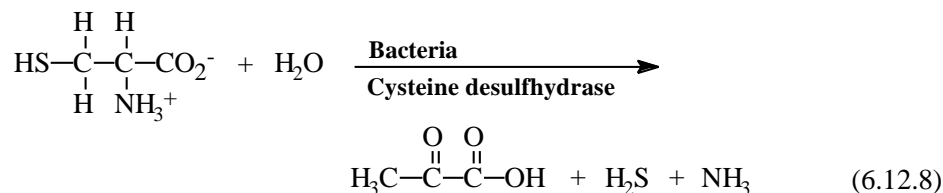
Sulfur occurs in many types of biological compounds. As a consequence, organic sulfur compounds of natural and pollutant origin are very common in water. The degradation of these compounds is an important microbial process having a strong effect upon water quality.

Among some of the common sulfur-containing functional groups found in aquatic organic compounds are hydrosulfide ( $-\text{SH}$ ), disulfide ( $-\text{SS}-$ ), sulfide ( $-\text{S}-$ ), sulfoxide ( $-\overset{\text{O}}{\underset{||}{\text{S}}}-$ ), sulfonic acid ( $-\text{SO}_2\text{OH}$ ), thioketone ( $-\overset{\text{S}}{\underset{||}{\text{C}}}-$ ), and thiazole (a heterocyclic sulfur group). Protein contains some amino acids with sulfur functional groups—cysteine, cystine, and methionine—whose breakdown is important in natural waters. The amino acids are readily degraded by bacteria and fungi.



The biodegradation of sulfur-containing amino acids can result in production of volatile organic sulfur compounds such as methane thiol,  $\text{CH}_3\text{SH}$ , and dimethyl disulfide,  $\text{CH}_3\text{SSCH}_3$ . These compounds have strong, unpleasant odors. Their formation, in addition to that of  $\text{H}_2\text{S}$ , accounts for much of the odor associated with the biodegradation of sulfur-containing organic compounds.

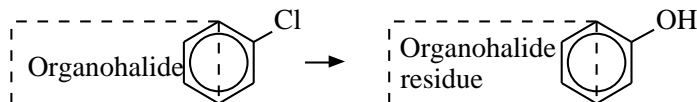
Hydrogen sulfide is formed from a large variety of organic compounds through the action of a number of different kinds of microorganisms. A typical sulfur-cleavage reaction producing  $\text{H}_2\text{S}$  is the conversion of cysteine to pyruvic acid through the action of cysteine desulfhydrase enzyme in bacteria:



Because of the numerous forms in which organic sulfur may exist, a variety of sulfur products and biochemical reaction paths must be associated with the biodegradation of organic sulfur compounds.

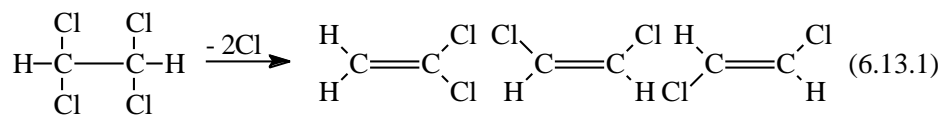
### 6.13. MICROBIAL TRANSFORMATIONS OF HALOGENS AND ORGANOHALIDES

**Dehalogenation** reactions involving the replacement of a halogen atom, for example,

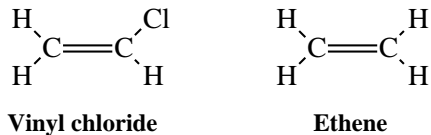


represent a major pathway for the biodegradation of organohalide hydrocarbons. In some cases, organohalide compounds serve as sole carbon sources, sole energy sources, or electron acceptors for anaerobic bacteria.<sup>10</sup> Microorganisms need not utilize a particular organohalide compound as a sole carbon source in order to cause its degradation. This is due to the phenomenon of **cometabolism**, which results from a lack of specificity in the microbial degradation processes. Thus, bacterial degradation of small amounts of an organohalide compound may occur while the microorganism involved is metabolizing much larger quantities of another substance.

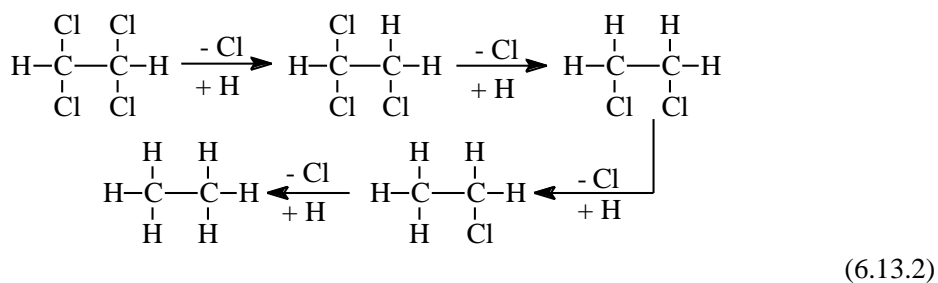
Organohalide compounds can undergo biodegradation anaerobically as shown by the example of 1,1,2,2-tetrachloroethane.<sup>11</sup> Microbially mediated dichloroelimination from this compound can produce one of three possible isomers of dichloroethylene.



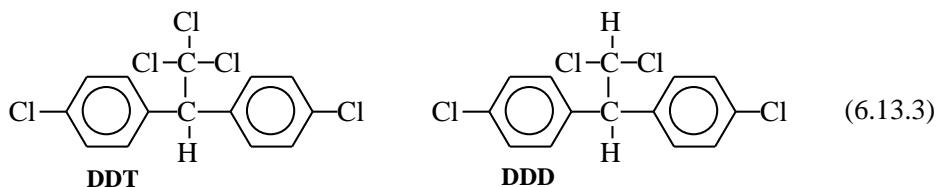
Successive hydrogenolysis reactions can produce vinyl chloride and ethene (ethylene).



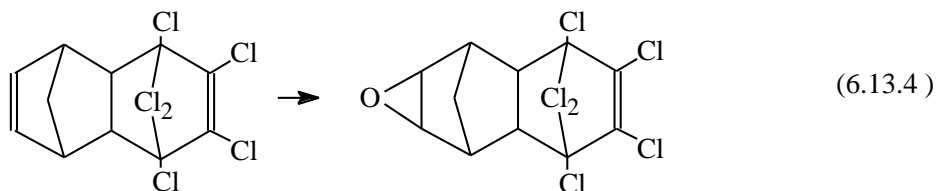
Successive hydrogenolysis reactions of 1,1,2,2-tetrachloroethane can produce ethane derivatives with 3, 2, 1, and 0 chlorine atoms.



Bioconversion of DDT to replace Cl with H yields DDD:



The latter compound is more toxic to some insects than DDT and has even been manufactured as a pesticide. The same situation applies to microbially mediated conversion of aldrin to dieldrin:



## 6.14. MICROBIAL TRANSFORMATIONS OF METALS AND METALLOIDS

Some bacteria, including *Ferrobacillus*, *Gallionella*, and some forms of *Sphaerotilus*, utilize iron compounds in obtaining energy for their metabolic needs.

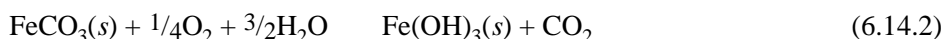


These bacteria catalyze the oxidation of iron(II) to iron(III) by molecular oxygen:



The carbon source for some of these bacteria is  $\text{CO}_2$ . Since they do not require organic matter for carbon, and because they derive energy from the oxidation of inorganic matter, these bacteria may thrive in environments where organic matter is absent.

The microorganism-mediated oxidation of iron(II) is not a particularly efficient means of obtaining energy for metabolic processes. For the reaction



the change in free energy is approximately 10 kcal/electron-mole. Approximately 220 g of iron(II) must be oxidized to produce 1.0 g of cell carbon. The calculation assumes  $\text{CO}_2$  as a carbon source and a biological efficiency of 5%. The production of only 1.0 g of cell carbon would produce approximately 430 g of solid  $\text{Fe(OH)}_3$ . It follows that large deposits of hydrated iron(III) oxide form in areas where iron-oxidizing bacteria thrive.

Some of the iron bacteria, notably *Gallionella*, secrete large quantities of hydrated iron(III) oxide in the form of intricately branched structures. The bacterial cell grows at the end of a twisted stalk of the iron oxide. Individual cells of *Gallionella*, photographed through an electron microscope, have shown that the stalks consist of a number of strands of iron oxide secreted from one side of the cell (Figure 6.13).

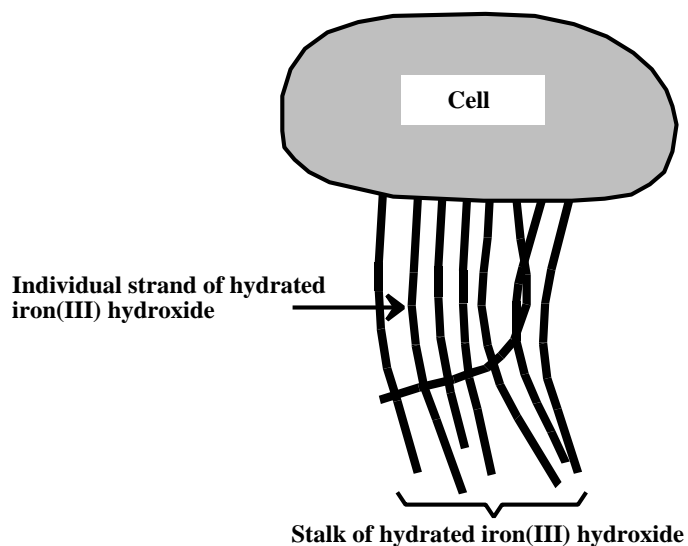


Figure 6.13. Sketch of a cell of *Gallionella* showing iron(III) oxide secretion.

At nearly neutral pH values, bacteria deriving energy by mediating the air oxidation of iron(II) must compete with direct chemical oxidation of iron(II) by  $\text{O}_2$ . The latter process is relatively rapid at pH 7. As a consequence, these bacteria tend

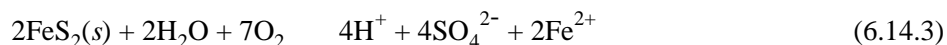
to grow in a narrow layer in the region between the oxygen source and the source of iron(II). Therefore, iron bacteria are sometimes called *gradient organisms*, and they grow at intermediate pE values.

Bacteria are strongly involved in the oceanic manganese cycle. Manganese nodules, a potentially important source of manganese, copper, nickel, and cobalt that occur on ocean floors, yield different species of bacteria which enzymatically mediate both the oxidation and reduction of manganese.

## Acid Mine Waters

One consequence of bacterial action on metal compounds is acid mine drainage, one of the most common and damaging problems in the aquatic environment. Many waters flowing from coal mines and draining from the “gob piles” left over from coal processing and washing are practically sterile due to high acidity.

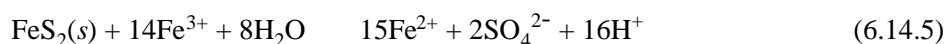
Acid mine water results from the presence of sulfuric acid produced by the oxidation of pyrite, FeS<sub>2</sub>. Microorganisms are closely involved in the overall process, which consists of several reactions. The first of these reactions is the oxidation of pyrite:



The next step is the oxidation of iron(II) ion to iron(III) ion,



a process that occurs very slowly at the low pH values found in acid mine waters. Below pH 3.5, the iron oxidation is catalyzed by the iron bacterium *Thiobacillus ferrooxidans*, and in the pH range 3.5-4.5 it may be catalyzed by a variety of *Metallogenium*, a filamentous iron bacterium. Other bacteria that may be involved in acid mine water formation are *Thiobacillus thiooxidans* and *Ferrobacillus ferrooxidans*. The Fe<sup>3+</sup> ion further dissolves pyrite,



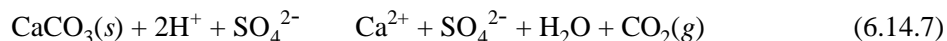
which in conjunction with Reaction 6.14.4 constitutes a cycle for the dissolution of pyrite. Fe(H<sub>2</sub>O)<sub>6</sub><sup>3+</sup> is an acidic ion and at pH values much above 3, the iron(III) precipitates as the hydrated iron(III) oxide:



The beds of streams afflicted with acid mine drainage often are covered with “yellowboy,” an unsightly deposit of amorphous, semigelatinous Fe(OH)<sub>3</sub>. The most damaging component of acid mine water, however, is sulfuric acid. It is directly toxic and has other undesirable effects.

In past years, the prevention and cure of acid mine water has been one of the major challenges facing the environmental chemist. One approach to eliminating excess acidity involves the use of carbonate rocks. When acid mine water is treated

with limestone, the following reaction occurs:



Unfortunately, because iron(III) is generally present,  $\text{Fe}(\text{OH})_3$  precipitates as the pH is raised (Reaction 6.11.6). The hydrated iron(III) oxide product covers the particles of carbonate rock with a relatively impermeable layer. This armoring effect prevents further neutralization of the acid.

## Microbial Transitions of Selenium

Directly below sulfur in the periodic table, selenium is subject to bacterial oxidation and reduction. These transitions are important because selenium is a crucial element in nutrition, particularly of livestock. Diseases related to either selenium excesses or deficiency have been reported in at least half of the states of the U.S. and in 20 other countries, including the major livestock-producing countries. Livestock in New Zealand, in particular, suffer from selenium deficiency.

Microorganisms are closely involved with the selenium cycle, and microbial reduction of oxidized forms of selenium has been known for some time. Reductive processes under anaerobic conditions can reduce both  $\text{SeO}_3^{2-}$  and  $\text{SeO}_4^{2-}$  ions to elemental selenium, which can accumulate as a sink for selenium in anoxic sediments. Some bacteria such as selected strains of *Thiobacillus* and *Leptothrix* can oxidize elemental selenium to selenite,  $\text{SeO}_3^{2-}$ , thus remobilizing this element from deposits of  $\text{Se}(0)$ .<sup>12</sup>

## 6.15. MICROBIAL CORROSION

Corrosion is a redox phenomenon and was discussed in Section 4.12. Much corrosion is bacterial in nature.<sup>13,14</sup> Bacteria involved with corrosion set up their own electrochemical cells in which a portion of the surface of the metal being corroded forms the anode of the cell and is oxidized. Structures called tubercles form in which bacteria pit and corrode metals as shown in [Figure 6.14](#).

It is beyond the scope of this book to discuss corrosion in detail. However, its significance and effects should be kept in mind by the environmental chemist.

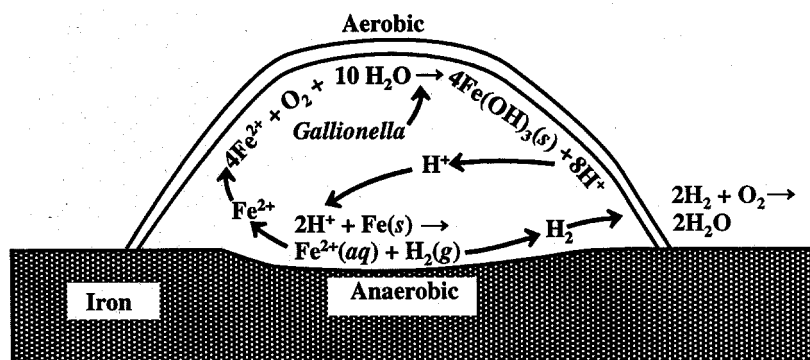


Figure 6.14. Tubercle in which the bacterially-mediated corrosion of iron occurs through the action of *Gallionella*.

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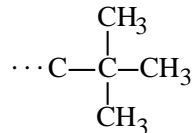
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## QUESTIONS AND PROBLEMS

1. As  $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$  biodegrades in several steps to carbon dioxide and water, various chemical species are observed. What stable chemical species would be observed as a result of the first step of this degradation process?
2. Which of the following statements is true regarding the production of methane in water: (a) it occurs in the presence of oxygen, (b) it consumes oxygen, (c) it removes biological oxygen demand from the water, (d) it is accomplished by aerobic bacteria, (e) it produces more energy per electron-mole than does aerobic respiration.
3. At the time zero, the cell count of a bacterial species mediating aerobic respiration of wastes was  $1 \times 10^6$  cells per liter. At 30 minutes it was  $2 \times 10^6$ ; at 60 minutes it was  $4 \times 10^6$ ; at 90 minutes,  $7 \times 10^6$ ; at 120 minutes,  $10 \times 10^6$ ; and at 150 minutes,  $13 \times 10^6$ . From these data, which of the following logical conclusions would you draw? (a) The culture was entering the log phase at the end of the 150-minute period, (b) the culture was in the log phase throughout the 150-minute period, (c) the culture was leaving the log phase at the end of the 150-minute period, (d) the culture was in the lag phase throughout the 150-minute period, (e) the culture was in the death phase throughout the 150-minute period.
4. What may be said about the biodegradability of a hydrocarbon containing the following structure?



5. Suppose that the anaerobic fermentation of organic matter,  $\{\text{CH}_2\text{O}\}$ , in water yields 15.0 L of  $\text{CH}_4$  (at standard temperature and pressure). How many grams of oxygen would be consumed by the aerobic respiration of the same quantity of  $\{\text{CH}_2\text{O}\}$ ? (Recall the significance of 22.4 L in chemical reaction of gases.)

6. What weight of  $\text{FeCO}_3(\text{s})$ , using Reaction (A) + (4) in Table 6.1, gives the same free energy yield as 1.00 g of organic matter, using Reaction (A) + (1), when oxidized by oxygen at pH 7.00?
7. How many bacteria would be produced after 10 hours by one bacterial cell, assuming exponential growth with a generation time of 20 minutes?
8. Referring to Reaction 6.11.2, calculate the concentration of ammonium ion in equilibrium with oxygen in the atmosphere and  $1.00 \times 10^{-5} \text{ M NO}_3^-$  at pH 7.00.
9. When a bacterial nutrient medium is inoculated with bacteria grown in a markedly different medium, the lag phase (Fig. 6.4) often is quite long, even if the bacteria eventually grow well in the new medium. Can you explain this behavior?
10. Most plants assimilate nitrogen as nitrate ion. However, ammonia ( $\text{NH}_3$ ) is a popular and economical fertilizer. What essential role do bacteria play when ammonia is used as a fertilizer? Do you think any problems might occur when using ammonia in a waterlogged soil lacking oxygen?
11. Why is the growth rate of bacteria as a function of temperature (Fig. 6.7) not a symmetrical curve?
12. Discuss the analogies between bacteria and a finely divided chemical catalyst.
13. Would you expect autotrophic bacteria to be more complex physiologically and biochemically than heterotrophic bacteria? Why?
14. Wastewater containing 8 mg/L  $\text{O}_2$  (atomic weight O = 16),  $1.00 \times 10^{-3} \text{ M NO}_3^-$ , and  $1.00 \times 10^{-2} \text{ M}$  soluble organic matter,  $\{\text{CH}_2\text{O}\}$ , is stored isolated from the atmosphere in a container richly seeded with a variety of bacteria. Assume that denitrification is one of the processes which will occur during storage. After the bacteria have had a chance to do their work, which of the following statements will be true? (a) No  $\{\text{CH}_2\text{O}\}$  will remain, (b) some  $\text{O}_2$  will remain, (c) some  $\text{NO}_3^-$  will remain, (d) denitrification will have consumed more of the organic matter than aerobic respiration, (e) the composition of the water will remain unchanged.
15. Of the four classes of microorganisms—algae, fungi, bacteria, and virus—which has the least influence on water chemistry?
16. Figure 6.3 shows the main structural features of a bacterial cell. Which of these do you think might cause the most trouble in water-treatment processes such as filtration or ion exchange, where the maintenance of a clean, unfouled surface is critical? Explain.
17. A bacterium capable of degrading 2,4-D herbicide was found to have its maximum growth rate at  $32^\circ\text{C}$ . Its growth rate at  $12^\circ\text{C}$  was only 10% of the maximum. Do you think there is another temperature at which the growth rate would also be 10% of the maximum? If you believe this to be the case, of the following temperatures, choose the one at which it is most plausible for the bacterium to also have a growth rate of 10% of the maximum:  $52^\circ\text{C}$ ,  $37^\circ\text{C}$ ,  $8^\circ\text{C}$ ,  $20^\circ\text{C}$ .

18. The day after a heavy rain washed a great deal of cattle feedlot waste into a farm pond, the following counts of bacteria were obtained:

<u>Time</u>	<u>Thousands of viable cells per mL</u>
6:00 a.m.	0.10
7:00 a.m.	0.11
8:00 a.m.	0.13
9:00 a.m.	0.16
10:00 a.m.	0.20
11:00 a.m.	0.40
12:00 Noon	0.80
1:00 p.m.	1.60
2:00 p.m.	3.20

To which portion of the bacterial growth curve, [Figure 6.3](#), does this time span correspond?

19. Addition of which two half-reactions in [Table 6.1](#) is responsible for: (a) elimination of an algal nutrient in secondary sewage effluent using methanol as a carbon source, (b) a process responsible for a bad-smelling pollutant when bacteria grow in the absence of oxygen, (c) A process that converts a common form of commercial fertilizer to a form that most crop plants can absorb, (d) a process responsible for the elimination of organic matter from wastewater in the aeration tank of an activated sludge sewage-treatment plant, (e) a characteristic process that occurs in the anaerobic digester of a sewage treatment plant.
20. What is the surface area in square meters of 1.00 gram of spherical bacterial cells, 1.00  $\mu\text{m}$  in diameter, having density of 1.00  $\text{g}/\text{cm}^3$ ?
21. What is the purpose of exoenzymes in bacteria?
22. Match each species of bacteria listed in the left column with its function on the right.
- |                                      |   |
|--------------------------------------|---|
| (a) <i>Spirillum lipoferum</i>       | (1) Reduces sulfate to $\text{H}_2\text{S}$                     |
| (b) <i>Rhizobium</i>                 | (2) Catalyzes oxidation of $\text{Fe}^{2+}$ to $\text{Fe}^{3+}$ |
| (c) <i>Thiobacillus ferrooxidans</i> | (3) Fixes nitrogen in grasses                                   |
| (d) <i>Desulfovibrio</i>             | (4) On legume roots   |
23. What factors favor the production of methane in anoxic surroundings?