CHAPTER

14

Biogeochemical Cycling

RAINA M. MAIER

14.1 INTRODUCTION

14.1.1 Biogeochemical Cycles

What happens to the vast array of organic matter that is produced on the earth during photosynthetic processes? This material does not keep accumulating rather, it is consumed and degraded, and a delicate global balance of carbon is maintained; carbon dioxide is removed from the atmosphere during photosynthesis and released during respiration. This balance is a result of the biologically driven, characteristic cycling of carbon between biotic forms such as sugar or other cellular building blocks and abiotic forms such as carbon dioxide. Cycling between biotic and abiotic forms is not limited to carbon. All of the major elements found in biological organisms (see Table 14.1), as well as some of the minor and trace elements, are similarly cycled in predictable and definable ways.

Taken together, the various element cycles are called the **biogeochemical cycles.** Understanding these cycles allows scientists to understand and predict the development of microbial communities and activities in the environment. There are many activites that can be harnessed in a beneficial way, such as for remediation of organic and metal pollutants or for recovery of precious metals such as copper or uranium from low-grade ores. There are also detrimental aspects of the cycles that can cause global environmental problems such as the formation of acid rain and acid mine drainage, metal corrosion processes, and formation of nitrous oxide, which can deplete the earth's ozone layer (see Chapter 15). As these examples illustrate, the microbial activities that drive biogeochemical cycles are highly relevant to the field of environmental microbiology. Thus, the knowledge of these cycles is increasingly critical as the human population continues to grow and the impact of human activity on the earth's environment becomes more significant. In this chapter, the biogeochemical cycles pertaining to carbon, nitrogen, and sulfur are delineated while our discussion will be limited to these three cycles, it should be noted that there are a number of other cycles. These include the phosphorus cycle, the iron cycle, the calcium cycle and more (Dobrovolsky, 1994).

14.1.2 Gaia Hypothesis

In the early 1970s, James Lovelock theorized that Earth behaves like a superorganism, and this concept developed into what is now known as the **Gaia hypothesis.** To quote Lovelock (1995), "Living organisms and their material environment are tightly coupled. The coupled system is a superorganism, and as it evolves there emerges a new property, the ability to self-regulate climate and chemistry." The basic tenet of this hypothesis is that the earth's physicochemical properties are self-regulated so that they are maintained in a favorable range for life. As evidence for this, consider that the sun has heated up by 30% during the past 4–5 billion years. Given the earth's original carbon dioxide-rich atmosphere, the average surface temperature of a lifeless earth today would be approximately 290°C (Table 14.2). In fact, when one compares Earth's present-day atmosphere with the atmospheres found on our nearest neighbors Venus and Mars, one can see that something has drastically affected the development of Earth's atmosphere. According to the Gaia hypothesis, this is the development and continued presence of life. Micro-

TABLE 14.1 Chemical Composition of an *E. coli* Cell

Elemental breakdown	% dry mass of an *E. coli* cell
Major elements	
Carbon	50
Oxygen	20
Hydrogen	8
Nitrogen	14
Sulfur	1
Phosphorus	3
Minor elements	
Potassium	2
Calcium	0.05
Magnesium	0.05
Chlorine	0.05
Iron	0.2
Trace elements	
Manganese	all trace elements
Molybdenum	combined comprise 0.3%
Cobalt	of dry weight of cell
Copper	
Zinc	

Adapted from Neidhardt *et al.* (1990).

bial activity, and later the appearance of plants, have changed the original heat-trapping carbon dioxide–rich atmosphere to the present oxidizing, carbon dioxide-poor atmosphere. This has allowed Earth to maintain an average surface temperature of 13°C, which is favorable to the life that exists on Earth.

How do biogeochemical activities relate to the Gaia hypothesis? These biological activities have driven the response to the slow warming of the sun resulting in the major atmospheric changes that have occurred over the last 4–5 billion years. When Earth was formed 4–5 billion years ago, a reducing (anaerobic) atmosphere existed. The initial reactions that mediated the formation of organic carbon were abiotic, driven by large influxes of ultraviolet (UV) light. The resulting reservoir of organic matter was utilized by early anaerobic heterotrophic organisms. This was followed by the development of the ability of microbes to fix carbon dioxide photosynthetically. Evidence from **stromatolites** suggests that the ability to photosynthesize was developed at least 3.5 billion years ago. Stromatolites are fossilized laminated structures that have been found in Africa and Australia. These structures were formed primarily by cyanobacteria that grew in mats and entrapped or precipitated inorganic material as they grew. The evolution of photosynthetic organisms tapped into an unlimited source of energy, the sun, and provided a mechanism for carbon recycling, i.e., the first carbon cycle (Fig. 14.1). This first carbon cycle was maintained for approximately 1.5 billion years. Geologic evidence then suggests that approximately 2 billion years ago, photosynthetic microorganisms developed the ability to produce oxygen. This allowed oxygen to accumulate in the atmosphere, resulting, in time, in a change from reducing to oxidizing conditions. Further, oxygen accumulation in the atmosphere created an ozone layer, which reduced the influx of harmful UV radiation, allowing the development of higher forms of life to begin.

At the same time that the carbon cycle evolved, the nitrogen cycle emerged because nitrogen was a limiting element for microbial growth. Although molecular nitrogen was abundant in the atmosphere, microbial cells could not directly utilize nitrogen as N_2 gas. Cells required organic nitrogen compounds or reduced inorganic forms of nitrogen for growth. Therefore, under the reducing conditions found on early Earth, some organisms developed a mechanism for fixing nitrogen using the enzyme nitrogenase. Nitrogen fixation remains an important microbiological process, and to this day, the nitrogenase enzyme is totally inhibited in the presence of oxygen.

When considered over this geologic time scale of several billion years, it is apparent that biogeochemical activities have been unidirectional. This means that the predominant microbial activities on earth have evolved over this long period of time to produce changes and to respond to changes that have occurred in the atmosphere, i.e., the appearance of oxygen and

TABLE 14.2 Atmosphere and Temperatures Found on Venus, Mars, and Earth

	Planet			
Gas	Venus	Mars	Earth without life	Earth with life
Carbon dioxide	96.5%	95%	98%	0.03%
Nitrogen	3.5%	2.7%	1.9%	9%
Oxygen	trace	0.13%	0.0	21%
Argon	70 ppm	1.6%	0.1%	1%
Methane	0.0	0.0	0.0	1.7 ppm
Surface temperature (°C)	459	−53	290 ± 50	13

Adapted from Lovelock (1995).

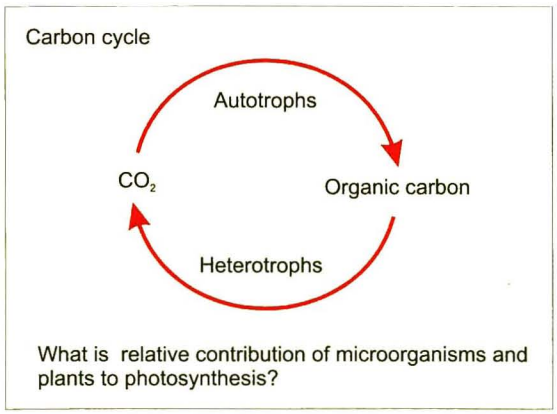


FIGURE 14.1 The carbon cycle is dependent on autotrophic organisms that fix carbon dioxide into organic carbon and heterotrophic organisms that respire organic carbon to carbon dioxide.

TABLE 14.3 Global Carbon Reservoirs

Carbon reservoir	Metric tons carbon	Actively cycled
Atmosphere		
CO_2	6.7×10^{11}	Yes
Ocean		
Biomass	4.0×10^{9}	No
Carbonates	3.8×10^{13}	No
Dissolved and particulate organics	2.1×10^{12}	Yes
Land		
Biota	5.0×10^{11}	Yes
Humus	1.2×10^{12}	Yes
Fossil fuel	1.0×10^{13}	Yes
Earth's crust[a]	1.2×10^{17}	No

[a] This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Data from Dobrovolsky, 1994.)

the decrease in carbon dioxide content. Presumably these changes will continue to occur, but they occur so slowly that we do not have the capacity to observe them. However, one can also consider biogeochemical activities on a more contemporary time scale, that of tens to hundreds of years. On this much shorter time scale, biogeochemical activities are regular and cyclic in nature, and it is these activities that are addressed in this chapter. On the one hand, the presumption that earth is a superorganism and can respond to drastic environmental changes is heartening when one considers that human activity is effecting unexpected changes in the atmosphere, such as ozone depletion and buildup of carbon dioxide. However, it is important to point out that the response of a superorganism is necessarily slow (thousands to millions of years), and as residents of earth we must be sure not to overtax Earth's ability to respond to change by artificially changing the environment in a much shorter time frame.

14.2 CARBON CYCLE

14.2.1 Carbon Reservoirs

A **reservoir** is a sink or source of an element such as carbon. There are various global reservoirs of carbon, some of which are immense in size and some of which are relatively small (Table 14.3). The largest carbon reservoir is carbonate rock found in the earth's sediments. This reservoir is four orders of magnitude larger than the carbonate reservoir found in the ocean and six orders of magnitude larger than the carbon reservoir found as carbon dioxide in the atmosphere. If one considers these three reservoirs, it is obvious that the carbon most available for photosynthesis is in the smallest of the reservoirs, the atmosphere. Therefore, it is the smallest reservoir that is most actively cycled. It is small, actively cycled reservoirs such as atmospheric carbon dioxide that are subject to perturbation from human activity. In fact, since global industrialization began in the late 1800s, humans have affected several of the smaller carbon reservoirs. Utilization of fossil fuels (an example of a small, inactive carbon reservoir) and deforestation (an example of a small, active carbon reservoir) are two activities that have reduced the amount of fixed organic carbon in these reservoirs and added to the atmospheric carbon dioxide reservoir (Table 14.4).

The increase in atmospheric carbon dioxide has not been as great as expected. This is because the reservoir of carbonate found in the ocean acts as a buffer between the atmospheric and sediment carbon reservoirs through the equilibrium equation shown below.

$$H_2CO_3 \rightleftarrows HCO_3^- \rightleftarrows CO_2$$

Thus, some of the excess carbon dioxide that has been released has been absorbed by the oceans. However, there has still been a net efflux of carbon dioxide into the atmosphere of approximately 7×10^9 metric tons/year. The problem with this imbalance is that be-

TABLE 14.4 Net Carbon Flux between Selected Carbon Reservoirs

Carbon source	Flux (metric tons carbon/year)
Release by fossil fuel combustion	7×10^9
Land clearing	3×10^9
Forest harvest and decay	6×10^9
Forest regrowth	-4×10^9
Net uptake by oceans (diffusion)	-3×10^9
Annual flux	9×10^9

cause atmospheric carbon dioxide is a small carbon reservoir, the result of a continued net efflux over the past 100 years or so has been a 28% increase in atmospheric carbon dioxide from 0.026% to 0.033%. A consequence of the increase in atmospheric carbon dioxide is that it may contribute to global warming through the **greenhouse effect.** The greenhouse effect is caused by gases in the atmosphere that trap heat from the sun and cause the earth to warm up. This effect is not solely due to carbon dioxide; other gases such as methane, chlorofluorocarbons (CFCs), and nitrous oxide add to the problem.

14.2.2 Carbon Fixation and Energy Flow

The ability to photosynthesize allows sunlight energy to be trapped and stored. In this process carbon dioxide is fixed into organic matter (Fig. 14.1). Photosynthetic organisms, also called **primary producers,** include plants and microorganisms such as algae, cyanobacteria, some bacteria, and some protozoa. As shown in Fig. 14.2, the efficiency of sunlight trapping is very low; less than 0.1% of the sunlight energy that hits the earth is actually utilized. As the fixed sunlight energy moves up each level of the food chain, up to 90% or more of the trapped energy is lost through respiration. Despite this seemingly inefficient trapping, photoautotrophic primary producers support most of the considerable ecosystems found on the earth. Productivity varies widely among different ecosystems depending on the climate, the type of primary producer, and whether the system is a managed one (Table 14.5). For example, one of the most productive natural areas is the coral reefs. Managed agricultural systems such as corn and sugarcane systems are also very productive, but it should be remembered that a significant amount of energy is put into these systems in terms of fertilizer addition and care. The open ocean has much lower productivity, but covers a majority of the earth's surface and so is a major contributor to primary production. In fact, aquatic and terrestrial environments contribute almost equally to global primary production. Plants predominate in terrestrial environments, but with the exception of immediate coastal zones, microorganisms are responsible for most primary production in aquatic environments. It follows that microorganisms are responsible for approximately one half of all primary production on the earth.

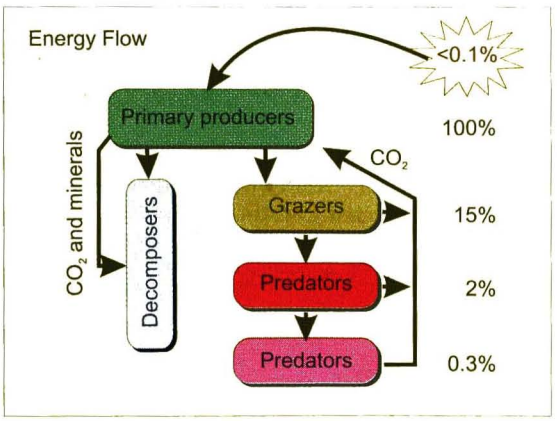


FIGURE 14.2 Diagram of the efficiency of sunlight energy flow from primary producers to consumers.

TABLE 14.5 Net Primary Productivity of Some Natural and Managed Ecosystems

Description of ecosystem	Net primary productivity (g dry organic matter/m^2/year)
Tundra	400
Desert	200
Temperate grassland	Up to 1500
Temperate or deciduous forest	1200–1600
Tropical rain forest	Up to 2800
Cattail swamp	2500
Freshwater pond	950–1500
Open ocean	100
Coastal seawater	200
Upwelling area	600
Coral reef	4900
Corn field	1000–6000
Rice paddy	340–1200
Sugarcane field	Up to 9400

Adapted from Atlas and Bartha (1993).

14.2.3 Carbon Respiration

Carbon dioxide that is fixed into organic compounds as a result of photoautotrophic activity is available for consumption or respiration by animals and heterotrophic microorganisms. This is the second half of the carbon cycle shown in Fig. 14.1. The end-products of respiration are carbon dioxide and new cell mass. An interesting question to consider is the following: if respiration were to stop, how long would it take for photosynthesis to use up all of the carbon dioxide reservoir in the atmosphere? Based on estimates of global photosynthesis, it has been estimated that it would take 30 to 300 years. This illustrates the importance of both legs of the carbon cycle in maintaining a carbon balance.

The following sections discuss the most common organic compounds found in the environment and the microbial catabolic activities that have evolved in response. These include organic polymers, humus, and

C1 compounds such as methane (CH_4). It is important to understand the fate of these naturally occurring organic compounds. This is because degradative activities that evolved for these compounds form the basis for degradation pathways that may be applicable to organic contaminants that are spilled in the environment (see Chapter 16). But before looking more closely at the individual carbon compounds, it should be pointed out that the carbon cycle is actually not quite as simple as depicted in Fig. 4.1. This simplified figure does not include anaerobic processes, which were predominant on the early earth and remain important in carbon cycling even today. A more complex carbon cycle containing anaerobic activity is shown in Fig. 14.3. Under anaerobic conditions, which predominated for the first several billion years on earth, some cellular components were less degradable than others (Fig. 14.4). This is especially true for highly reduced molecules such as cellular lipids. These components were therefore left over and buried with sediments over time and became the present-day fossil fuel reserves. Another carbon compound produced under anaerobic conditions is methane. Methane is produced in soils as an end product of anaerobic respiration (see Eq. 3.20, Chapter 3). Methane is also produced under the anaerobic conditions found in ruminants such as cows.

14.2.3.1 Organic Polymers

What are the predominant types of organic carbon found in the environment? They include plant polymers, polymers used to build fungal and bacterial cell walls, and arthropod exoskeletons (Fig. 14.5). Because these polymers constitute the majority of organic carbon, they are the basic food supply available to support heterotrophic activity. The three most common polymers are the plant polymers cellulose, hemicellulose, and lignin (Table 14.6) (Wagner and Wolf, 1998). There are also various other polymers including starch, chitin, and peptidoglycan. These various polymers can be divided into two groups on the basis of their structures: the carbohydrate-based polymers, which include the majority of the polymers found in the environment, and the phenylpropane-based polymer, lignin.

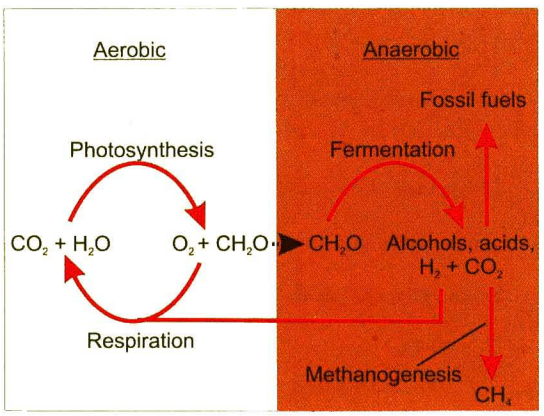


FIGURE 14.3 The carbon cycle, showing both aerobic and anaerobic contributions.

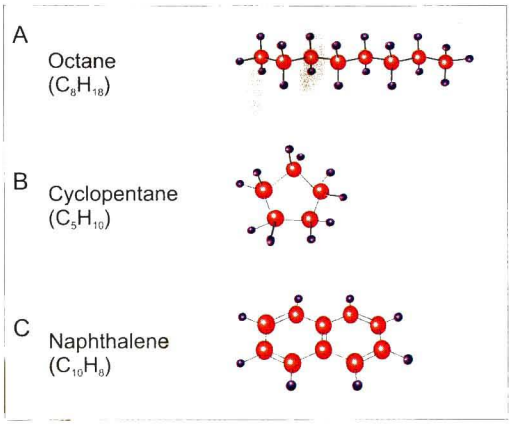
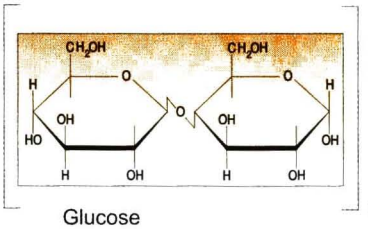


FIGURE 14.4 Examples of petroleum constituents: (A) an alkane, (B) an alicyclic, and (C) an aromatic compound. A crude oil contains some of each of these types of compounds but the types and amounts vary in different petroleum reservoirs.

Carbohydrate-based Polymers

Cellulose is not only the most abundant of the plant polymers, it is also the most abundant polymer found on the earth. It is a linear molecule containing β-1,4 linked glucose subunits (Fig. 14.5a). Each molecule contains 1000 to 10,000 subunits with a resulting molecular weight of up to 1.8×10^6. These linear molecules are arranged in microcrystalline fibers that help make up the woody structure of plants. Cellulose is not only a large molecule, it is also insoluble in water. How then do microbial cells get such a large, insoluble molecule across their walls and membranes? The answer is that they have developed an alternative strategy, which is to make and release enzymes, called **extracellular enzymes**, that can begin the polymer degradation process outside the cell (Deobald and Crawford, 1997). There are two extracellular enzymes that initiate cellulose degradation. These are β-1,4-endoglucanase and β-1,4-exoglucanase. The endoglucanase hydrolyzes cellulose molecules randomly within the polymer, producing smaller and smaller cellulose molecules (Fig. 14.6). The exoglucanase consecutively hydrolyzes two glucose subunits from the reducing end of the cellulose molecule, releasing the disaccharide cellobiose. A third enzyme, known as β-glucosidase or cellobiase, then hydrolyzes cellobiose to glucose. Cellobiase can be found as both an extracellular and an intracellular enzyme.

a

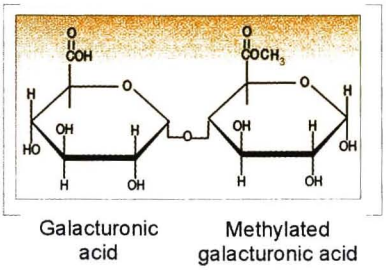


Cellulose

Molecular weight up to 1.8×10^6

Glucose subunits
β-1 - 4 linked

b

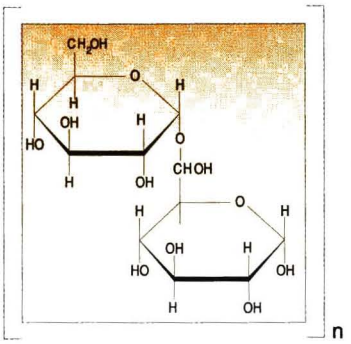


Hemicellulose
(e.g., pectin)

Molecular weight ~ 40,000

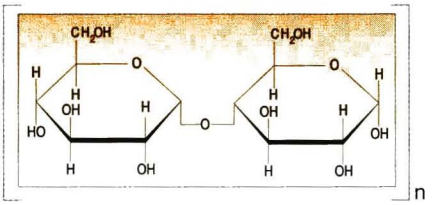
c

Starch



Amylopectin

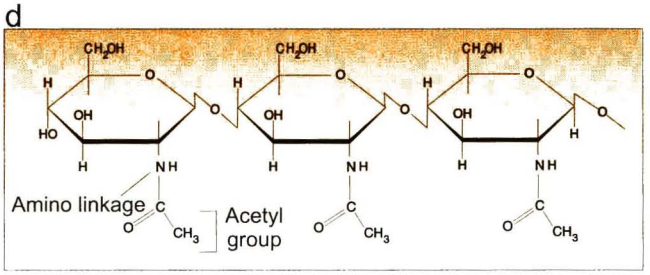
Glucose subunits
α-1-6 linked branch points



Amylose

glucose subunits
α-1-4 linked

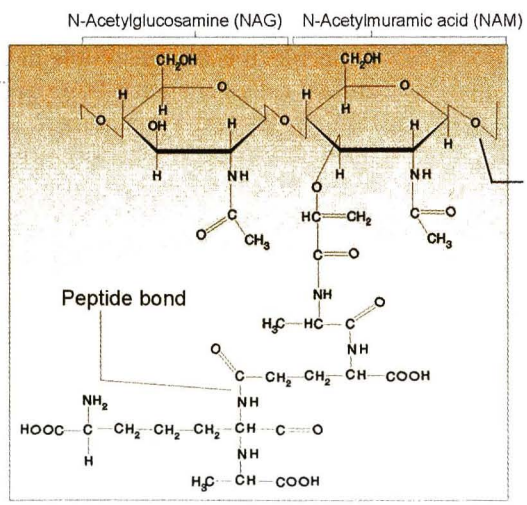
d



Chitin

N-acetylglucosamine subunits
β-1-4 linked

e



Peptidoglycan

NAG-NAM subunits
β-1 - 4 linked

Lysozyme - sensitive bond

L - Alanine

D - Glutamic acid

Meso - Diaminopimelic acid

D - Alanine

Table 14.6 Major Types of Organic Components of Plants

Plant component	% dry mass of plant
Cellulose	15–60
Hemicellulose	10–30
Lignin	5–30
Protein and nucleic acids	2–15

Both cellobiose and glucose can be taken up by many bacterial and fungal cells.

Hemicellulose is the second most common plant polymer. This molecule is more heterogeneous than cellulose, consisting of a mixture of several monosaccharides including various hexoses and pentoses as well as uronic acids. In addition, the polymer is branched instead of linear. An example of a hemicellulose polymer is the pectin molecule shown in Fig. 14.5b, which contains galacturonic acid and methylated galacturonic acid. Degradation of hemicellulose is similar to the process described for cellulose except that, because the molecule is more heterogeneous, many more extracellular enzymes are involved.

In addition to the two major plant polymers, several other important organic polymers are carbohydrate based. One of these is **starch,** a polysaccharide synthesized by plants to store energy (Fig. 14.5c). Starch is formed from glucose subunits and can be linear (α-1,4 linked), a structure known as amylose, or can be branched (α-1,4 and α-1,6 linked), a structure known as amylopectin. Amylases (α-1,4–linked exo- and endoglucanases) are extracellular enzymes produced by many bacteria and fungi. Amylases produce the disaccharide maltose, which can be taken up by cells and mineralized. Another common polymer is **chitin,** which is formed from β-1,4–linked subunits of *N*-acetylglucosamine (Fig. 14.5d). This linear, nitrogen-containing polymer is an important component of fungal cell walls and of the exoskeleton of arthropods. Finally, there is **peptidoglycan,** a polymer of *N*-acetylglucosamine and *N*-acetylmuramic acid, which is an important component of bacterial cell walls (Fig. 14.5e).

Lignin

Lignin is the third most common plant polymer and is strikingly different in structure from all of the carbohydrate-based polymers. The basic building blocks of lignin are the two aromatic amino acids tyrosine and phenylalanine. These are converted to phenylpropene subunits such as coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Then 500 to 600 phenylpropene subunits are randomly polymerized, resulting in the formation of the amorphous aromatic polymer known as lignin. In plants lignin surrounds cellulose microfibrils and strengthens the cell wall. Lignin also helps make plants more resistant to pathogens.

Biodegradation of lignin is slower and less complete than degradation of other organic polymers. This is shown experimentally in Figure 14.7a, and visually in Figure 14.7b. Lignin degrades slowly because it is constructed as a highly heterogeneous polymer and in addition contains aromatic residues rather than carbohydrate residues. The great heterogeneity of the molecule precludes the evolution of specific degradative enzymes comparable to cellulose. Instead, a nonspecific extracellular enzyme, H_2O_2-dependent lignin peroxidase, is used in conjunction with an extracellular oxidase enzyme that generates H_2O_2. The peroxidase enzyme and H_2O_2 system generate oxygen-based free radicals that react with the lignin polymer to release phenylpropene residues (Morgan *et al.*, 1993). These residues are taken up by microbial cells and degraded as shown in Fig. 14.8. Biodegradation of intact lignin polymers occurs only aerobically, which is not surprising because reactive oxygen is needed to release lignin residues. However, once residues are released, they can be degraded under anaerobic conditions.

Phenylpropene residues are aromatic in nature, similar in structure to several types of organic pollutant molecules such as the BTEX (benzene, toluene, ethylbenzene, xylene) and polyaromatic hydrocarbon compounds found in crude oil and gasoline and creosote compounds found in wood preservatives (see Fig. 16.13). These naturally occurring aromatic biodegrada-

FIGURE 14.5 Common organic polymers found in the environment. (A) Cellulose is the most common plant polymer. It is a linear polymer of β-1,4-linked glucose subunits. Each polymer contains 1000 to 10,000 subunits. (B) Hemicellulose is the second most common polymer. This molecule is more heterogeneous, consisting of hexoses, pentoses, and uronic acids. An example of a hemicellulose polymer is pectin. (C) Starch is a polysaccharide synthesized by plants to store energy. Starch is formed from glucose subunits and can be linear (α-1,4-linked), a structure known as amylose, or can be branched (α-1,4 and α-1,6 linked), known as amylopectin. (D) Chitin is formed from subunits of *N*-acetylglucosamine linked β-1,4. This polymer is found in fungal cell walls. (E) Bacterial cell walls are composed of polymers of *N*-acetylglucosamine and *N*-acetylmuramic acid connected by β-1,4 linkages.

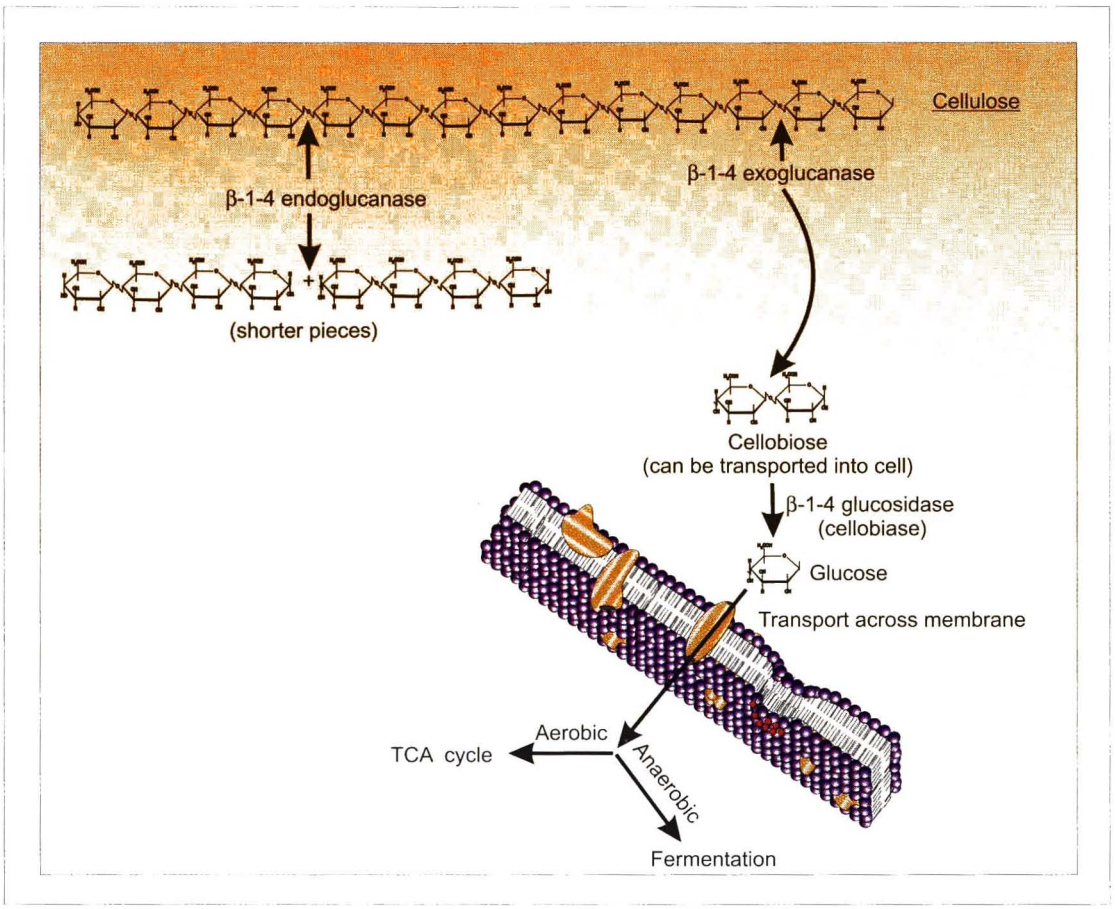


FIGURE 14.6 The degradation of cellulose begins outside the cell with a series of extracellular enzymes called cellulases. The resulting smaller glucose subunit structures can be taken up by the cell and metabolized.

tion pathways are of considerable importance in the field of bioremediation. In fact, a comparison of the pathway shown in Fig. 14.8 with the pathway for degradation of aromatics presented in Chapter 16 shows that they are very similar. Lignin is degraded by a variety of microbes including fungi, actinomycetes, and bacteria. The best studied organism with respect to lignin degradation is the white rot fungus *Phanerochaete chrysosporium*. This organism is also capable of degrading several pollutant molecules with structures similar to those of lignin residues (see Case Study Chapter 16).

14.2.3.2 Humus

Humus was introduced in Chapter 4.2.2 and its structure is shown in Fig. 4.8. How does humus form? It forms in a two-stage process that involves the formation of reactive monomers during the degradation of organic matter, followed by the spontaneous polymerization of some of these monomers into the humus molecule. Although the majority of organic matter that is released into the environment is respired to form new cell mass and carbon dioxide, a small amount of this carbon becomes available to form humus. To understand this spontaneous process, consider the degradation of the common organic polymers found in soil that were described in the preceding sections. Each of these polymers requires the production of extracellular enzymes that begin the polymer degradation process. In particular, for lignin these extracellular enzymes are nonspecific and produce hydrogen peroxide and oxygen radicals. It is not surprising, then, that some of the reactive residues released during polymer degradation might repolymerize and result in the production of humus. In addition, nucleic acid and protein residues that are released from dying and decaying cells contribute to the pool of molecules available for humus formation. This process is illustrated in Fig. 14.9. Considering the wide array of residues that

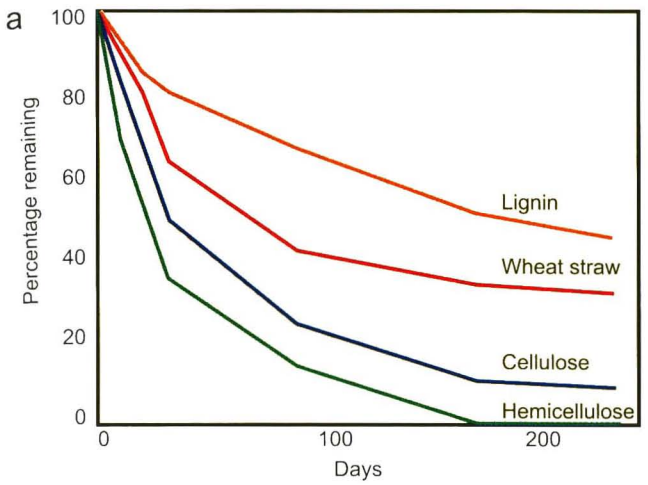


FIGURE 14.7 (a) Decomposition of wheat straw and its major constituents in a silt loam. The initial composition of the wheat straw was 50% cellulose, 25% hemicellulose, and 20% lignin. (Adapted from Wagner and Wolf, 1998.) (b) Leaves from a rain forest in different stages of decomposition. Cellulose and hemicellulose are degraded first, leaving the lignin skeleton. (Photo taken in Henry Pittier National Park, Venezuela, courtesy C. M. Miller.)

can contribute to humus formation, it is not surprising that humus is even more heterogeneous than lignin. Table 14.7 compares the different properties of these two complex molecules.

Humus is the most complex organic molecule found in soil, and as a result it is the most stable organic molecule. The turnover rate for humus ranges from 2 to 5% per year, depending on climatic conditions (Wagner and Wolf, 1998). This can be compared with the degradation of lignin shown in Fig. 14.7a, where approximately 50% of lignin added to a silt loam was degraded in 250 days. Thus, humus provides a slowly released source of carbon and energy for indigenous autochthonous microbial populations. The release of humic residues most likely occurs in a manner similar to release of lignin residues. Because the humus content of most soils does not change, the rate of formation of humus must be similar to the rate of turnover. Thus, humus can be thought of as a molecule that is in a state of dynamic equilibrium (Haider, 1992).

14.2.3.3 Methane

Methanogenesis

The formation of methane, **methanogenesis,** is predominantly a microbial process, although a small amount of methane is generated naturally through volcanic activity (Table 14.8) (Ehrlich, 1981). Methanogenesis is an anaerobic process and occurs extensively in specialized environments including water-saturated areas such as wetlands and paddy fields; anaerobic niches in the soil; landfills; the rumen; and termite guts. Methane is an end-product of anaerobic degradation (see Chapter 3.4) and as such is associated with petroleum, natural gas, and coal deposits.

At present a substantial amount of methane is released to the atmosphere as a result of energy harvesting and utilization. A second way in which methane is released is through landfill gas emissions. It is estimated that landfills in the United States alone generate 10×10^6 metric tons of methane per year. Although methane makes a relatively minor carbon contribution to the global carbon cycle (compare Table 14.8 with Table 14.3), methane emission is of concern from several environmental aspects. First, like carbon dioxide, meth-ane is a greenhouse gas and contributes to global warming. In fact, it is the second most common greenhouse gas emitted to the atmosphere. Further, it is 22 times more effective than carbon dioxide at trapping heat. Second, localized production of methane in landfills can create safety and health concerns. Methane is an explosive gas at concentrations as small as 5%. Thus, to avoid accidents, the methane generated in a landfill must be managed in some way. If methane is present in concentrations higher than 35%, it can be collected and used for energy. Alternatively, the methane can be burned off at concentrations of 15% percent or higher. However, most commonly, it is simply vented to the atmosphere to prevent it from building up in high enough concentrations to ignite. Although venting landfill gas to the atmosphere does help prevent explosions, it clearly adds to the global warming problem.

The organisms responsible for methanogenesis are a group of obligately anaerobic archaebacteria called the **methanogens.** The basic metabolic pathway used by the methanogens is:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^{\circ\prime} = -130.7 \text{ kJ} \qquad \text{(Eq. 14.1)}$$

This is an exothermic reaction where CO_2 acts as the TEA and H_2 acts as the electron donor providing en-

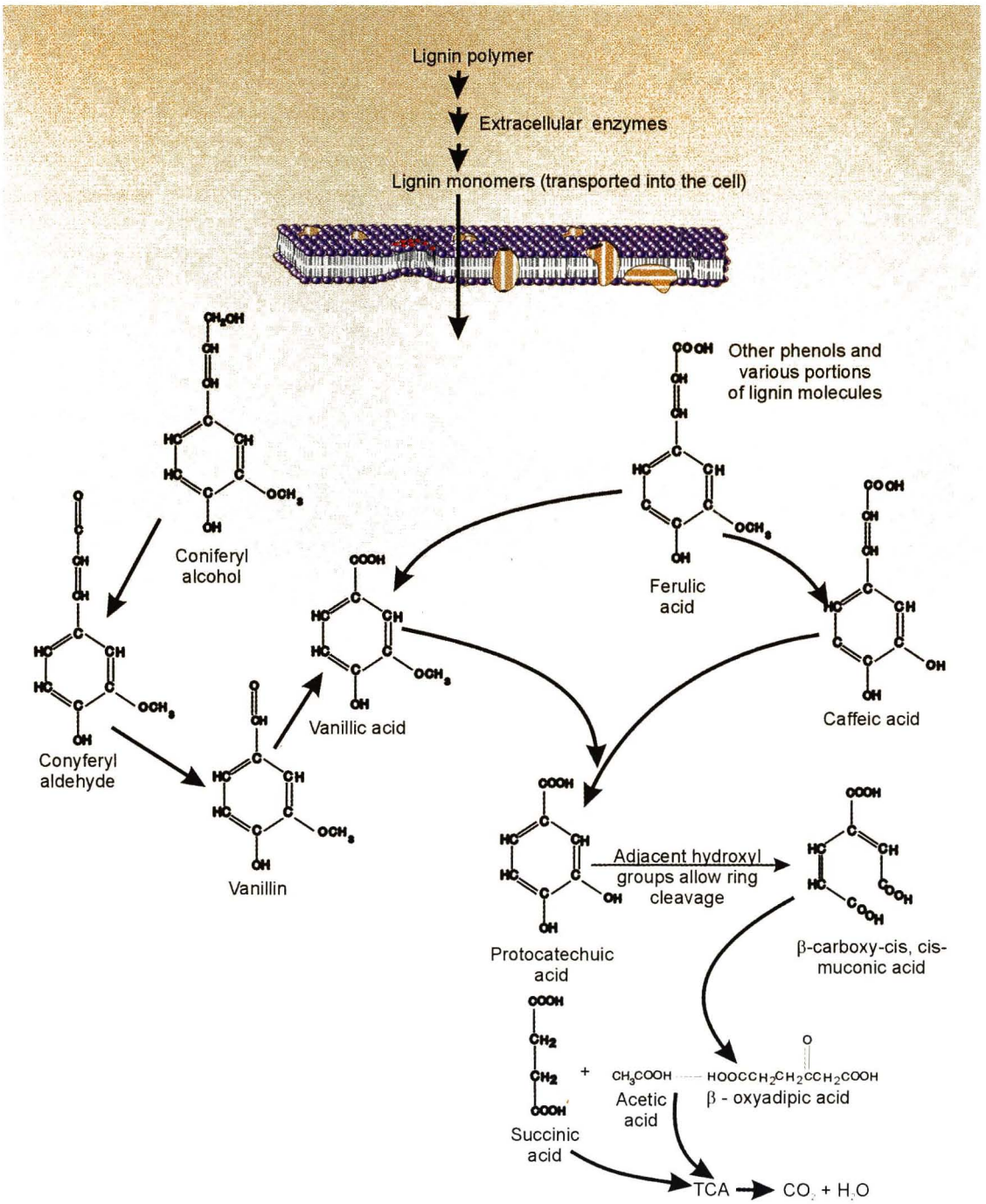


FIGURE 14.8 Lignin degradation. (Adapted from Wagner and Wolf, 1998.)

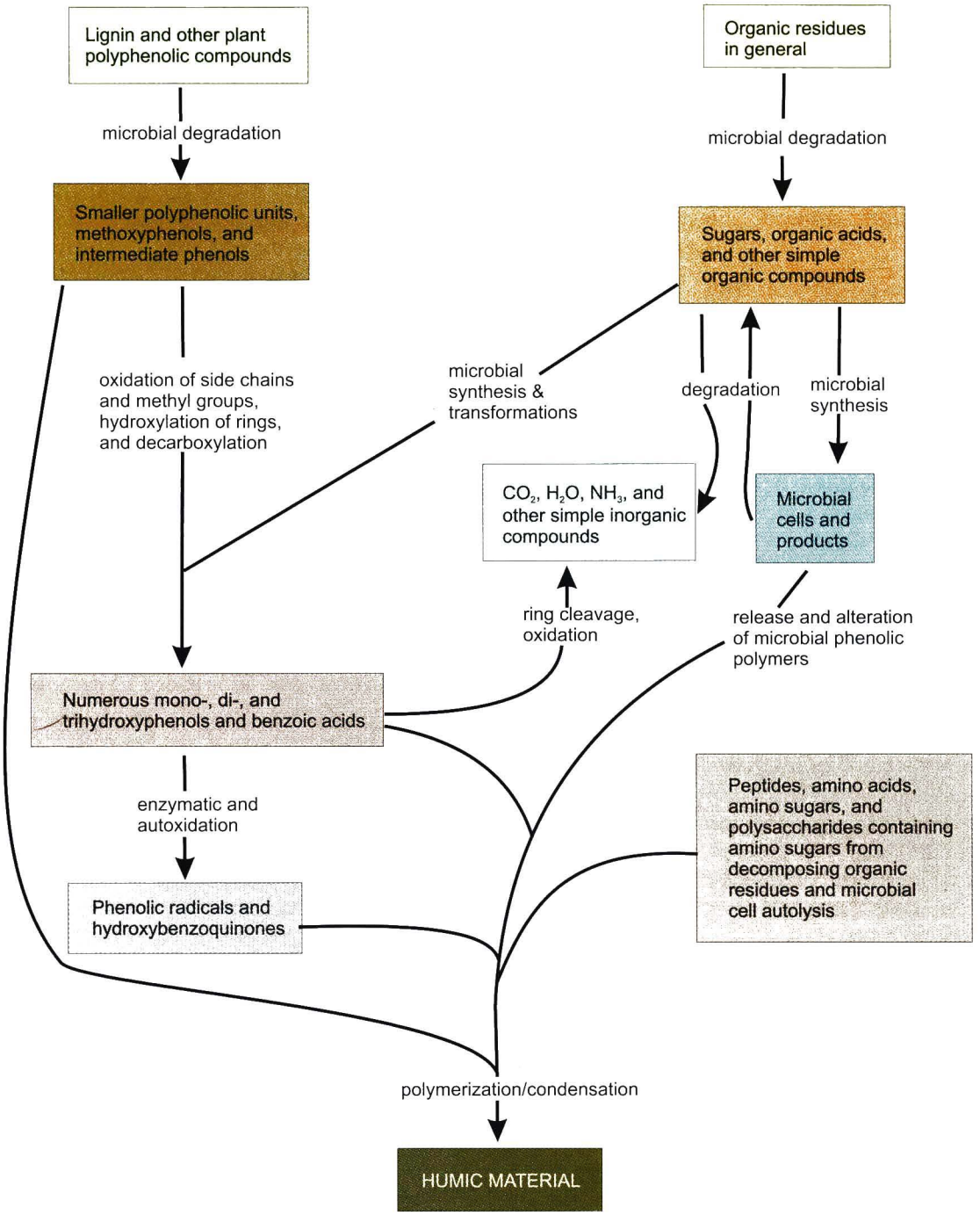


FIGURE 14.9 Possible pathways for the formation of soil humus. (Adapted with permission from Wagner and Wolf, 1998.)

ergy for the fixation of carbon dioxide. Methanogens that utilize CO_2/H_2 are therefore autotrophic. In addition to the autotrophic reaction shown in Eq. 14.1, methanogens can produce methane during heterotrophic growth on a limited number of other C_1 and C_2 substrates including acetate, methanol, and formate. Since there are very few carbon compounds that can be used by methanogens, these organisms are dependent on the production of these compounds by other microbes in the surrounding community. As such an interdependent community of microbes typically develops in anaerobic environments. In this community, the more complex organic molecules are catabolized by populations that ferment or respire anaerobically, gen-

TABLE 14.7 Chemical Properties of Humus and Lignin

Characteristic	Humic material	Lignin
Color	Black	Light Brown
Methoxyl (-OCH_3) content	Low	High
Nitrogen content	3–6%	0%
Carboxyl and phenolic hydroxyl content	High	Low
Total exchangeable acidity (cmol/kg)	≥150	≤0.5
α Amino nitrogen	Present	0
Vanillin content	<1%	15–25%

Data from Wagner and Wolf (1998).

erating C_1 and C_2 carbon substrates as well as CO_2 and H_2 that are then used by methanogens.

Methane Oxidation

Clearly, methane as the end product of anaerobiosis is found extensively in nature. As such, it is an available food source, and a group of bacteria called the **methanotrophs** have developed the ability to utilize methane as a source of carbon and energy. The methanotrophs are chemoheterotrophic and obligately aerobic. They metabolize methane as shown in Eq. 14.2.

$$\underset{\text{methane}}{CH_4} + O_2 \xrightarrow[\text{monooxygenase}]{\text{methane}} \underset{\text{methanol}}{CH_3OH} \rightarrow \underset{\text{formaldehye}}{HCHO} \rightarrow \underset{\text{formic acid}}{HCOOH} \rightarrow \underset{\text{carbon dioxide}}{CO_2} + H_2O \quad \text{(Eq. 14.2)}$$

The first enzyme in the biodegradation pathway is called **methane monooxygenase.** Oxygenases in general incorporate oxygen into a substrate and are important enzymes in the intial degradation steps for hydrocarbons (see Section 16.6.2.1.1). However, methane monooxygenase is of particular interest because it was the first of a series of enzymes isolated that can cometabolize highly chlorinated solvents such as trichloroethylene (TCE) (see Section 16.6.2.1.3). Until this discovery it was believed that biodegradation of highly chlorinated solvents could occur only under anaerobic conditions as an incomplete reaction. The application of methanogens for cometabolic degradation of TCE is a strategy under development for bioremediation of groundwater contaminated with TCE. This example is a good illustration of the way in which naturally occurring microbial activities can be harnessed to solve pollution problems.

14.2.3.4 Carbon Monoxide and Other C1 Compounds

Bacteria that can utilize C1 carbon compounds other than methane are called **methylotrophs.** There

TABLE 14.8 Estimates of Methane Released into the Atmosphere

Source	Methane emission (10^6 metric tons/year)	
Biogenic		
Ruminants	80–100	
Termites	25–150	
Paddy fields	70–120	
Natural wetlands	120–200	
Landfills	5–70	
Oceans and lakes	1–20	
Tundra	1–5	
Abiogenic		
Coal mining	10–35	
Natural gas flaring and venting	10–35	
Industrial and pipeline losses	15–45	
Biomass burning	10–40	
Methane hydrates	2–4	
Volcanoes	0.5	
Automobiles	0.5	
Total	350–820	
Total biogenic	302–665	81–86% of total
Total abiogenic	48–155	13–19% of total

Adapted from Madigan *et al.* (1997).

are a number of important C1 compounds produced from both natural and anthropogenic activities (Table 14.9). One of these is carbon monoxide. The annual global production of carbon monoxide is 3–4 $\times$ 10^9 metric tons/year (Atlas and Bartha, 1993). The two major carbon monoxide inputs are abiotic. Approximately 1.5 $\times$ 10^9 metric tons/year result from atmospheric photochemical oxidation of carbon compounds such as methane, and 1.6 $\times$ 10^9 metric tons/year results from burning of wood, forests, and fossil fuels. A small proportion, 0.2 $\times$ 10^9 metric tons/year, results from biological activity in ocean and soil environments. Carbon monoxide is a highly toxic molecule because it has a strong affinity for cytochromes, and in binding to cyctochromes, it can completely inhibit the activity of the respiratory electron transport chain.

Destruction of carbon monoxide can occur abiotically by photochemical reactions in the atmosphere. Microbial processes also contribute significantly to its destruction, even though it is a highly toxic molecule. The destruction of carbon monoxide seems to be quite efficient because the level of carbon monoxide in the atmosphere has not risen significantly since industrialization began, even though CO emissions have increased. The ocean is a net producer of carbon monoxide and releases CO to the atmosphere. In contrast, the terrestrial environment is a net sink for carbon monoxide and absorbs approximately 0.4 $\times$ 10^9 metric tons/year. Key microbes found in terrestrial environments that can metabolize carbon monoxide include both aerobic and anaerobic organisms. Under aerobic conditions *Pseudomonas carboxydoflava* is an example of an organism that oxidizes carbon monoxide to carbon dioxide:

$$CO + H_2O \rightarrow CO_2 + H_2 \qquad \text{(Eq. 14.3)}$$

$$2H_2 + O_2 \rightarrow 2H_2O \qquad \text{(Eq. 14.4)}$$

This organism is a chemoautotroph and fixes the CO_2 generated in Eq. 14.3 into organic carbon. The oxidation of the hydrogen produced provides energy for CO_2 fixation (see Eq. 14.4). Under anaerobic conditions, methanogenic bacteria can reduce carbon monoxide to methane:

$$CO + 3H_2 \rightarrow CH_4 + H_2O \qquad \text{(Eq. 14.5)}$$

A number of other C1 compounds support the growth of methylotrophic bacteria (Table 14.9). Many, but not all, methylotrophs are also methanotrophic. Both types of bacteria are widespread in the environment because these C1 compounds are ubiquitous metabolites, and in response to their presence, microbes have evolved the capacity to metabolize them under either aerobic or anaerobic conditions.

14.3 NITROGEN CYCLE

In contrast to carbon, elements such as nitrogen and sulfur are taken up in the form of mineral salts and cycle oxidoreductively. For example, nitrogen can exist in numerous oxidation states, from −3 in ammonium (NH_4^+) to +5 in nitrate (NO_3^-). These element cycles are referred to as mineral cycles. The best studied and most complex of the mineral cycles is the nitrogen cycle. There is great interest in the nitrogen cycle because nitrogen is the mineral nutrient most in demand by microorganisms and plants. It is the fourth most com-

TABLE 14.9 C1 Compounds of Major Environmental Importance

Compound	Formula	Comments
Carbon dioxide	CO_2	Combustion, respiration, and fermentation end product, a major reservoir of carbon on earth.
Carbon monoxide	CO	Combustion product, common pollutant. Product of plant, animal and microbial respiration, highly toxic.
Methane	CH_4	End product of anaerobic fermentation or respiration.
Methanol	CH_3OH	Generated during breakdown of hemicellulose, fermentation by-product.
Formaldehyde	HCHO	Combustion product, intermediate metabolite.
Formate	HCOOH	Found in plant and animal tissues, fermentation product.
Formamide	$HCONH_2$	Formed from plant cyanides.
Dimethyl ether	CH_3OCH_3	Generated from methane by methanotrophs, industrial pollutant.
Cyanide ion	CN^-	Generated by plants, fungi, and bacteria. Industrial pollutant, highly toxic.
Dimethyl sulfide	$(CH_3)_2S$	Most common organic sulfur compound found in the environment, generated by algae.
Dimethyl sulfoxide	$(CH_3)_2SO$	Generated anaerobically from dimethyl sulfide.

mon element found in cells, making up approximately 12% of cell dry weight and includes the microbially catalyzed processes of nitrogen fixation, ammonium oxidation, assimilatory and dissimilatory nitrate reduction, ammonification, and ammonium assimilation.

14.3.1 Nitrogen Reservoirs

Nitrogen in the form of the inert gas, dinitrogen (N_2), has accumulated in the earth's atmosphere since the planet was formed. Nitrogen gas is continually released into the atmosphere from volcanic and hydrothermal eruptions, and is one of the major global reservoirs of nitrogen (Table 14.10). A second major reservoir is the nitrogen that is found in the earth's crust as bound, nonexchangeable ammonium. Neither of these reservoirs is actively cycled; the nitrogen in the earth's crust is unavailable and the N_2 in the atmosphere must be fixed before it is available for biological use. Because nitrogen fixation is an energy-intensive process and is carried out by a limited number of microorganisms, it is a relatively slow process. Smaller reservoirs of nitrogen include the organic nitrogen found in living biomass and in dead organic matter and soluble inorganic nitrogen salts. These small reservoirs tend to be actively cycled, particularly because nitrogen is often a limiting nutrient in the environment. For example, soluble inorganic nitrogen salts in terrestrial environments can have turnover rates greater than one per day. Nitrogen in plant biomass turns over approximately once a year, and nitrogen in organic matter turns over once in several decades.

TABLE 14.10 Global Nitrogen Reservoirs

Nitrogen reservoir	Metric tons nitrogen	Actively cycled
Atmosphere		
N_2	3.9×10^{15}	No
Ocean		
Biomass	5.2×10^{8}	Yes
Dissolved and particulate organics	3.0×10^{11}	Yes
Soluble salts (NO_3^-, NO_2^-, NH_4^+)	6.9×10^{11}	Yes
Dissolved N_2	2.0×10^{13}	No
Land		
Biota	2.5×10^{10}	Yes
Organic matter	1.1×10^{11}	Slow
Earth's crust[a]	7.7×10^{14}	No

[a] This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Adapted from Dobrovolsky, 1994.)

TABLE 14.11 Relative Inputs of Nitrogen Fixation from Biological Sources

Source	Nitrogen fixation (metric tons/year)
Terrestrial	1.35×10^{8}
Aquatic	4.0×10^{7}
Fertilizer manufacture	3.0×10^{7}

14.3.2 Nitrogen Fixation

Ultimately, all fixed forms of nitrogen, NH_4^+, NO_3^-, and organic N, come from atmospheric N_2. The relative contributions to **nitrogen fixation** of microbes in terrestrial and aquatic environments are compared with human inputs in Table 14.11. Approximately 65% of the N_2 fixed annually is from terrestrial environments, including both natural systems and managed agricultural systems. Marine ecosystems account for a smaller proportion, 20%, of N_2 fixation. As already mentioned, nitrogen fixation is an energy-intensive process and until recently was performed only by selected bacteria and cyanobacteria. However, a substantial amount of nitrogen fixation, 15% of the total N_2 fixed, now occurs through the manufacture of fertilizers. Because this is an energy-driven process, fertilizer prices are tied to the price of fossil fuels. As fertilizers are expensive, management alternatives to fertilizer addition have become attractive. These include rotation between nitrogen-fixing crops such as soybeans and nonfixing crops such as corn. Wastewater reuse is another alternative that has become especially popular in the desert southwestern United States for nonfood crops and uses, such as cotton and golf courses, where both water and nitrogen are limiting (see Chapter 21).

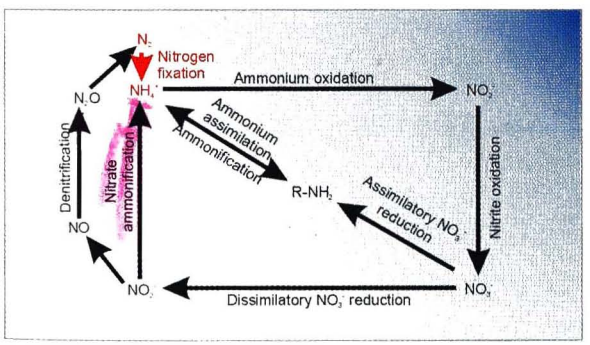
Nitrogen is fixed into ammonia (NH_3) by over 100 different free-living bacteria, both aerobic and anaerobic, as well as some actinomycetes and cyanobacteria (Table 18.2). For example, *Azotobacter* (aerobic), *Beijerinckia* (aerobic), *Azospirillum* (facultative), and *Clostridium* (anaerobic) can all fix N_2. Because fixed nitrogen is required by all biological organisms, nitrogen-fixing organisms occur in most environmental niches. The amount of N_2 fixed in each niche depends on the environment (Table 14.12). Free-living bacterial cells that

TABLE 14.12 Rates of Nitrogen Fixation

N_2-fixing system	Nitrogen fixation (kg N/hectare/year)
Rhizobium–legume	200–300
Anabaena–*Azolla*	100–120
Cyanobacteria–moss	30–40
Rhizosphere associations	2–25
Free–living	1–2

are not in the vicinity of a plant root fix small amounts of nitrogen (1 to 2 kg N/hectare/year). Bacterial cells associated with the nutrient-rich rhizosphere environment can fix larger amounts of N_2 (2 to 25 kg N/hectare/year). Cyanobacteria are the predominant N_2-fixing organisms in aquatic environments, and because they are photosynthetic, N_2 fixation rates are one to two orders of magnitude higher than for free-living nonphotosynthetic bacteria. An evolutionary strategy developed collaboratively by plants and microbes to increase N_2 fixation efficiency was to enter into a symbiotic or mutualistic relationship to maximize N_2 fixation. The best studied of these symbioses is the *Rhizobium*-legume relationship, which can increase N_2 fixation to 200 to 300 kg N/hectare/year. This symbiosis irrevocably changes both the plant and the microbe involved but is beneficial to both organisms. Both the enzymology of N_2 fixation and the development of symbiotic N_2-fixation are presented in detail in Chapter 18.2.

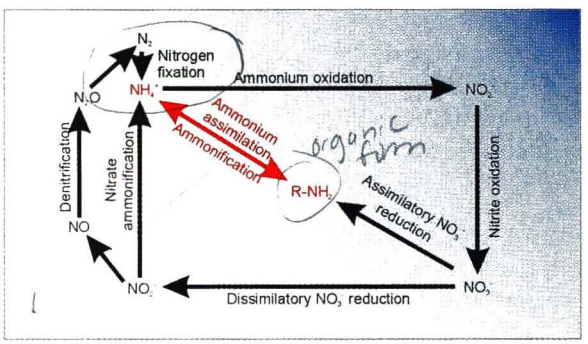
As the various transformations of nitrogen are discussed in this section, the objective is to understand how they are interconnected and controlled. As already mentioned, N_2 fixation is limited to bacteria and is an energy-intensive process. Therefore, it does not make sense for a microbe to fix N_2 if sufficient amounts are present for growth. Thus, one control on this part of the nitrogen cycle is that ammonia, the end product of N_2-fixation, is an inhibitor for the N_2-fixation reaction. A second control in some situations is the presence of oxygen. Nitrogenase is extremely oxygen sensitive, and some free-living aerobic bacteria fix N_2 only at reduced oxygen tension. Other bacteria such as *Azotobacter* and *Beijerinckia* can fix N_2 at normal oxygen tension because they have developed mechanisms to protect the nitrogenase enzyme.



Summary:
N_2 fixation is energy intensive
End product of N_2 fixation is ammonia
N_2 fixation is inhibited by ammonia
Nitrogenase is O_2 sensitive, some free-living N_2 fixers require reduced O_2 tension

14.3.3 Ammonium Assimilation (Immobilization) and Ammonification (Mineralization)

The end product of N_2 fixation is ammonium. The ammonium produced is assimiliated by cells into amino acids to form proteins, cell wall components such as *N*-acetylmuramic acid, and purines and pyrimidines to form nucleic acids. This process is known as **ammonium assimilation** or **immobilization.** Nitrogen can also be immobilized by the uptake and incorporation of nitrate into organic matter, a process known as assimilatory nitrate reduction (Section 14.3.5.1). Because nitrate must be reduced to ammonium before it is incorporated into organic molecules, most organisms prefer to take up nitrogen as ammonium if it is available. The process that reverses immobilization, the release of ammonia from dead and decaying cells, is called **ammonification** or **ammonium mineralization.** Both immobilization and mineralization of nitrogen occur under aerobic and anaerobic conditions.



Summary:
Assimilation and ammonification cycles ammonia between its organic and inorganic forms
Assimilation predominates at C:N ratios > 20
ammonification predominates at C:N ratios < 20

14.3.3.1 Ammonium Assimilation (Immobilization)

There are two pathways that microbes use to assimilate ammonium. The first is a reversible reaction that incorporates or removes ammonia from the amino acid glutamate (Fig. 14.10A). This reaction is driven by ammonium availability. At high ammonium concentrations, (>0.1 mM or >0.5 mg N/kg soil), in the presence of reducing equivalents (reduced nicotinamide adenine dinucleotide phosphate, $NADPH_2$), ammonium is incorporated into α-ketoglutarate to

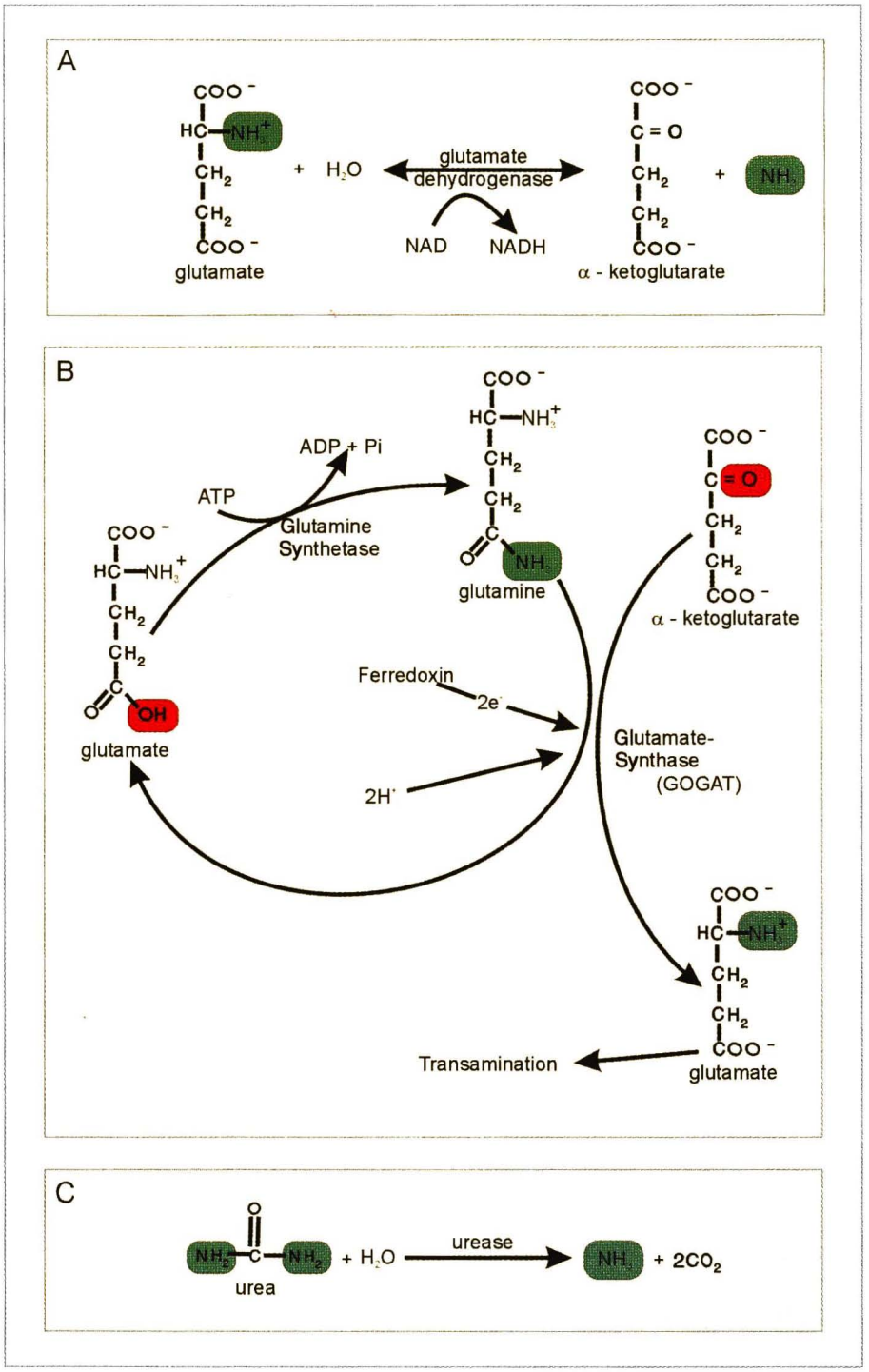


FIGURE 14.10 Pathways of ammonium assimilation and ammonification. Assimilation: (A) The enzyme glutamate dehydrogenase catalyzes a reversible reaction that immobilizes ammonium at high ammonium concentrations. (B) The enzyme system glutamine synthase–glutamate synthetase (GOGAT) that is induced at low ammonium concentrations. This ammonium uptake system requires ATP energy. Ammonification: Ammonium is released from the amino acid glutamate as shown in (A). (C) Ammonium is also released from urea, a molecule that is found in animal waste and is a common component of fertilizer. Note that the first of these reactions (A and B) occurs within the cell. In contrast, urease enzymes are extracellular enzymes resulting in release of ammonia to the environment.

form glutamate. However, in most soil and many aquatic environments, ammonium is present at low concentrations. Therefore microbes have a second ammonium uptake pathway that is energy dependent. This reaction is driven by ATP and two enzymes, glutamine synthase and glutamate synthetase (Fig. 14.10B). The first step in this reaction adds ammonium to glutamate to form glutamine, and the second step transfers the ammonium molecule from glutamine to α-ketoglutarate resulting in the formation of two glutamate molecules.

14.3.3.2 Ammonification (Mineralization)

Ammonium mineralization can occur intracellularly by the reversible reaction shown in Fig. 14.10A. Mineralization reactions can also occur extracellularly. As discussed in Section 14.2.4.1, microorganisms release a variety of extracellular enzymes that initiate degradation of plant polymers. Microorganisms also release a variety of enzymes including proteases, lysozymes, nucleases, and ureases that can initiate degradation of nitrogen-containing molecules found outside the cell such as proteins, cell walls, nucleic acids, and urea. Some of these monomers are taken up by the cell and degraded further, but some of the monomers are acted upon by extracellular enzymes to release ammonium directly to the environment, as shown in Fig. 14.10C for urea and the extracellular enzyme urease.

Which of these two processes, immobilization or mineralization, predominates in the environment? This depends on whether nitrogen is the limiting nutrient. If nitrogen is limiting, then immobilization will become the more important process. For environments where nitrogen is not limiting, mineralization will predominate. Nitrogen limitation is dictated by the carbon/nitrogen (C/N) ratio in the environment. Generally, the C/N ratio required for bacteria is 4 to 5 and for fungi is 10. So a typical average C/N ratio for soil microbial biomass is 8 (Myrold, 1998). It would then seem logical that at the C/N ratio of 8, there would be a net balance between mineralization and immobilization. However, one must take into account that only approximately 40% of the carbon in organic matter is actually incorporated into cell mass (the rest is lost as carbon dioxide). Thus, the C/N ratio must be increased by a factor of 2.5 to account for the carbon lost as carbon dioxide during respiration. Note that nitrogen is cycled more efficiently than carbon, and there are essentially no losses in its uptake. In fact, a C/N ratio of 20 is not only the theoretical balance point but also the practically observed one. When organic amendments with C/N ratios less than 20 are added to soil, net mineralization of ammonium occurs. In contrast, when organic amendments with C/N ratios greater than 20 are added, net immobilization occurs.

There are numerous possible fates for ammonium that is released into the environment as a result of ammonium mineralization. It can be taken up by plants or microorganisms and incorporated into living biomass, or it can become bound to nonliving organic matter such as soil colloids or humus. In this capacity, ammonium adds to the cation-exchange capacity (CEC) of the soil. Ammonium can become fixed inside clay minerals, which essentially traps the molecule and removes the ammonium from active cycling. Also, because ammonium is volatile, some mineralized ammonium can escape into the atmosphere. Finally, ammonium can be utilized by chemoautotrophic microbes in a process known as nitrification.

14.3.4 Nitrification

Nitrification is the microbially catalyzed conversion of ammonium to nitrate. This is predominantly an aerobic chemoautotrophic process, but some methylotrophs can use the methane monooxygenase enzyme to oxidize ammonium and a few heterotrophic fungi and bacteria can also perform this oxidation. The autotrophic nitrifiers are a closely related group of bacteria (Table 14.13). The best studied nitrifiers are from

TABLE 14.13 Chemoautotrophic Nitrifying Bacteria

Genus	Species
Ammonium oxidizers	
Nitrosomonas	*europaea* *eutrophus* *marina*
Nitrosococcus	*nitrosus* *mobilis* *oceanus*
Nitrosospira	*briensis*
Nitrosolobus	*multiformis*
Nitrosovibrio	*tenuis*
Nitrite oxidizers	
Nitrobacter	*winogradskyi* *hamburgensis* *vulgaris*
Nitrospina	*gracilis*
Nitrococcus	*mobilis*
Nitrospira	*marina*

the genus *Nitrosomonas*, which oxidizes ammonium to nitrite, and *Nitrobacter*, which oxidizes nitrite to nitrate. The oxidation of ammonium is shown in Eq. 14.6:

$$NH_4^+ + O_2 + 2H^+ \xrightarrow[\text{monooxygenase}]{\text{ammonium}} NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ \quad \Delta G = -66 \text{ kcal} \qquad \text{(Eq. 14.6)}$$

This is a two-step energy-producing reaction and the energy produced is used to fix carbon dioxide. There are two things to note about this reaction. First, it is an inefficient reaction requiring 34 moles of ammonium to fix 1 mole of carbon dioxide. Second, the first step of this reaction is catalyzed by the enzyme ammonium monooxygenase. This first step is analogous to Eq. 14.2, where the enzyme methane monooxygenase initiates oxidation of methane. Like methane monooxygenase, ammonium monooxygenase has broad substrate specificity and can be used to oxidize pollutant molecules such as TCE cometabolically (see Chapter 16.6.2.1.1).

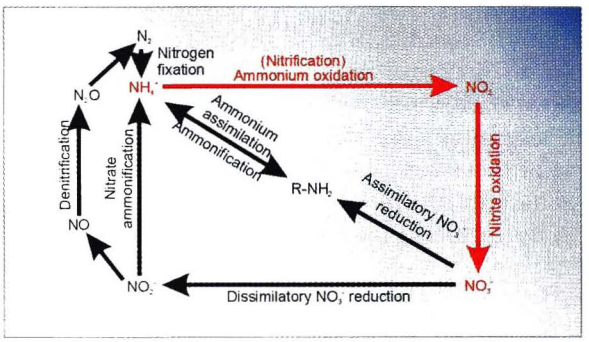
The second step in nitrification, shown in Eq. 14.7, is even less efficient than the first step, requiring approximately 100 moles of nitrite to fix 1 mole of carbon dioxide.

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad \Delta G = -18 \text{ kcal} \qquad \text{(Eq. 14.7)}$$

These two types of nitrifiers, i.e., those that carry out the reactions shown in Eqs. 14.6 and 14.7, are generally found together in the environment. As a result, nitrite does not normally accumulate in the environment. Nitrifiers are sensitive populations. The optimum pH for nitrification is 6.6 to 8.0. In environments with pH < 6.0, nitrification rates are slowed, and below pH 4.5, nitrification seems to be completely inhibited.

Heterotrophic microbes that oxidize ammonium include some fungi and some bacteria. These organisms gain no energy from nitrification, so it is unclear why they carry out the reaction. The relative importance of autotrophic and heterotrophic nitrification in the environment has not yet been clearly determined. Although the measured rates of autotrophic nitrification in the laboratory are an order of magnitude higher than those of heterotrophic nitrification, some data for acidic forest soils have indicated that heterotrophic nitrification may be more important in such environments (Myrold, 1998).

Nitrate does not normally accumulate in natural, undisturbed ecosystems. There are several reasons for this. One is that nitrifiers are sensitive to many environmental stresses. But perhaps the most important reason is that natural ecosystems do not have much excess ammonium. However, in agricultural systems that have large inputs of fertilizer, nitrification can become an important process resulting in the production of large amounts of nitrate. Other examples of managed systems that result in increased nitrogen inputs into the environment are feedlots, septic tanks, and landfills. The nitrogen released from these systems also becomes subject to nitrification processes. Because nitrate is an anion (negatively charged), it is very mobile in soil systems, which also have an overall net negative charge. Therefore, nitrate moves easily with water and this results in nitrate leaching into groundwater and surface waters. There are several health concerns related to high levels of nitrate in groundwater, including methemoglobinemia and the formation of nitrosamines. High levels of nitrate in surface waters can also lead to eutrophication and the degradation of surface aquatic systems. These consequences as well as the control of nitrification in managed systems are discussed in detail in Chapter 15.6.



Summary:
Nitrification is an aerobic process
Nitrification is sensitive to a variety of chemical inhibitors and is inhibited at low pH
Nitrification processes in managed systems can result in nitrate leaching and groundwater contamination

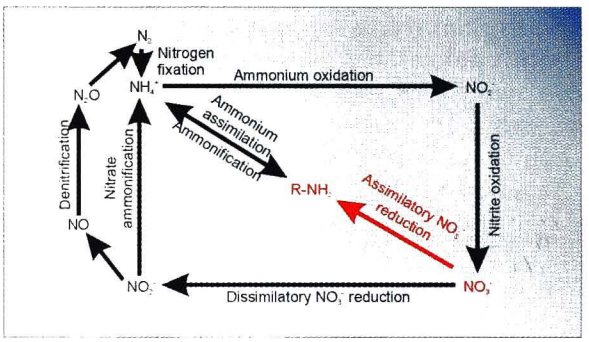
14.3.5 Nitrate Reduction

What are the possible fates of nitrate in the environment? We have just discussed nitrate leaching into groundwater and surface waters as one possible fate. In addition, nitrate can be taken up and incorporated into living biomass by plants and microorganisms. The uptake of nitrate is followed by its reduction to ammonium, which is then incorporated into biomass. This process is called **assimilatory nitrate reduction** or **nitrate immobilization.** Finally, microorganisms can utilize nitrate as a terminal electron acceptor in anaerobic respiration to drive the oxidation of organic compounds. There are two separate pathways for this dissimilatory process, one called **dissimilatory nitrate reduction to ammonium,** where ammonium is the end

product, and one called **denitrification,** where a mixture of gaseous products including N_2 and N_2O is formed.

14.3.5.1 Assimilatory Nitrate Reduction

Assimilatory nitrate reduction refers to the uptake of nitrate, its reduction to ammonium, and its incorporation into biomass (see Fig. 14.10A and B). Most microbes utilize ammonium preferentially, when it is present, to avoid having to reduce nitrate to ammonium, a process requiring energy. So if ammonium is present in the environment, assimilatory nitrate reduction is suppressed. Oxygen does not inhibit this activity. In contrast to microbes, for plants that are actively photosynthesizing and producing energy, the uptake of nitrate for assimilation is less problematic in terms of energy. In fact, because nitrate is much more mobile than ammonium, it is possible that in the vicinity of the plant roots, nitrification of ammonium to nitrate makes nitrogen more available for plant uptake. Because this process incorporates nitrate into biomass, it is also known as nitrate immobilization (see Section 14.3.3).



Summary:
Nitrate taken up must be reduced to ammonia before it is assimilated
Ammonia inhibits this process
O_2 does not inhibit this process

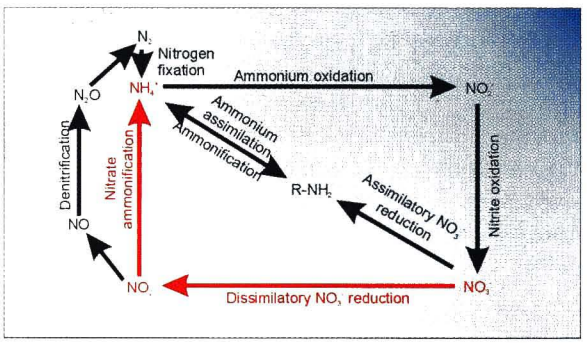
14.3.5.2 Dissimilatory Nitrate Reduction

Dissimilatory Nitrate Reduction to Ammonium

There are two separate dissimilatory nitrate reduction processes both of which are used by facultative chemoheterotrophic organisms under microaerophilic or anaerobic conditions. The first process, called dissimilatory nitrate reduction to ammonia (DNRA), uses nitrate as a terminal electron acceptor to produce energy to drive the oxidation of organic compounds. The end product of DRNA is ammonium:

$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O \quad \Delta G = -144 \text{ kcal}/8e^- \text{ transfer} \qquad \text{(Eq. 14.8)}$$

The first step in this reaction, the reduction of nitrate to nitrite, is the energy-producing step. The further reduction of nitrite to ammonium is catalyzed by an NADH-dependent reductase. This second step provides no additional energy, but it does conserve fixed nitrogen and also regenerates reducing equivalents through the reoxidation of $NADH_2$ to NAD. These reducing equivalents are then used to help in the oxidation of carbon substrates. In fact, it has been demonstrated that under carbon-limiting conditions nitrite accumulates (denitrification predominates), while under carbon-rich conditions ammonium is the major product (DNRA predominates). A second environmental factor that selects for DNRA is low levels of available electron acceptors. It is not surprising therefore that this process is found predominantly in saturated, carbon-rich environments such as stagnant water, sewage sludge, some high-organic-matter sediments, and the rumen. Table 14.14 lists a variety of bacteria that perform DNRA. It is interesting to note that most of the bacteria on this list have fermentative rather than oxidative metabolisms.



Summary:
Anaerobic respiration using nitrate as TEA
Inhibited by O_2
Not inhibited by ammonia
Found in a limited number of carbon rich, TEA poor environments
Fermentative bacteria predominate

Denitrification

The second type of dissimilatory nitrate reduction is known as **denitrification.** Denitrification refers to the microbial reduction of nitrate, through various gaseous

TABLE 14.14 Bacteria That Utilize Dissimilatory Nitrate or Nitrite to Ammonium (DRNA)

Genus	Typical habitat
Obligate anaerobes	
Clostridium	Soil, sediment
Desulfovibrio	Sediment
Selenomonas	Rumen
Veillonella	Intestinal tract
Wolinella	Rumen
Facultative anaerobes	
Citrobacter	Soil, wastewater
Enterobacter	Soil, wastewater
Erwinia	Soil
Escherichia	Soil, wastewater
Klebsiella	Soil, wastewater
Photobacterium	Seawater
Salmonella	Sewage
Serratia	Intestinal tract
Vibrio	Sediment
Microaerophiles	
Campylobacter	Oral cavity
Aerobes	
Bacillus	Soil, food
Neisseria	Mucous membranes
Pseudomonas	Soil, water

Adapted from Tiedje (1988).

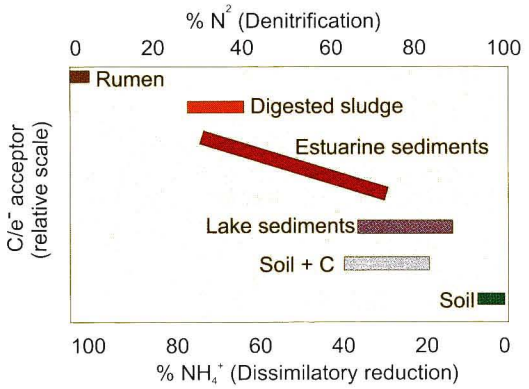


FIGURE 14.11 Partitioning of nitrate between denitrification and DNRA as a function of available carbon/electron (C/e^-) acceptor ratio. (Adapted from J. M. Tiedje in *Biology of Anaerobic Microorganisms*, A. J. B. Zehnder, ed. © 1988. Reprinted by permission of John Wiley & Sons, Inc.)

inorganic forms, to N_2. This is the primary type of dissimilatory nitrate reduction found in soil, and as such is of concern because it cycles fixed nitrogen back into N_2. This process removes a limiting nutrient from the environment. Further, some of the gaseous intermediates formed during denitrification, e.g., nitrous oxide (N_2O), can cause depletion of the ozone layer and can also act as a greenhouse gas contributing to global warming (see Section 15.5). The overall reaction for denitrification is

$$NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O \qquad \Delta G = -212 \text{ kcal}/8e^- \text{ transfer} \qquad \text{(Eq. 14.9)}$$

Denitrification, when calculated in terms of energy produced for every eight-electron transfer, provides more energy per mole of nitrate reduced than DNRA. Thus, in a carbon-limited, electron acceptor–rich environment, denitrification will be the preferred process because it provides more energy than DNRA. The relationship between denitrification and DNRA is summarized in Fig. 14.11.

The four steps involved in denitrification are shown in more detail in Fig. 14.12. The first step, reduction of nitrate to nitrite, is catalyzed by the enzyme nitrate reductase. This is a membrane-bound molybdenum–iron–sulfur protein that is found not only in denitrifiers but also in DNRA organisms. Both the synthesis and the activity of nitrate reductase are inhibited by oxygen. Thus, both denitrification and DNRA are inhibited by oxygen. The second enzyme in this pathway is nitrite reductase, which catalyzes the conversion of nitrite to nitric oxide. Nitrite reductase is unique to denitrifying organisms and is not present in the DNRA process. It is found in the periplasm and exists in two forms, a copper-containing form and a heme form, both of which are distributed widely in the environment. Synthesis of nitrite reductase is inhibited by oxygen and induced by nitrate. Nitric oxide reductase, a membrane-bound protein, is the third enzyme in the pathway, catalyzing the conversion of nitric oxide to nitrous oxide. The synthesis of this enzyme is inhibited by oxygen and induced by various nitrogen oxide forms. Nitrous oxide reductase is the last enzyme in the pathway and converts nitrous oxide to dinitrogen gas. This is a periplasmic copper-containing protein. The activity of the nitrous oxide reductase enzyme is inhibited by low pH and is even more sensitive to oxygen than the other three enzymes in the denitrification pathway. Thus, nitrous oxide is the final product of denitrification under conditions of high oxygen (in a relative sense, given a microaerophilic niche) and low pH. In summary, both the synthesis and activity of denitrification enzymes are controlled by oxygen. Enzyme activity is more sensitive to oxygen than enzyme synthesis as shown in Fig. 14.13. The amount of dissolved oxygen in equilibrium with water at 20°C and 1 atm pressure is 9.3 mg/l. However, as little as 0.5 mg/l or less inhibits the activity of denitrification enzymes. As already stated, nitrous oxide re-

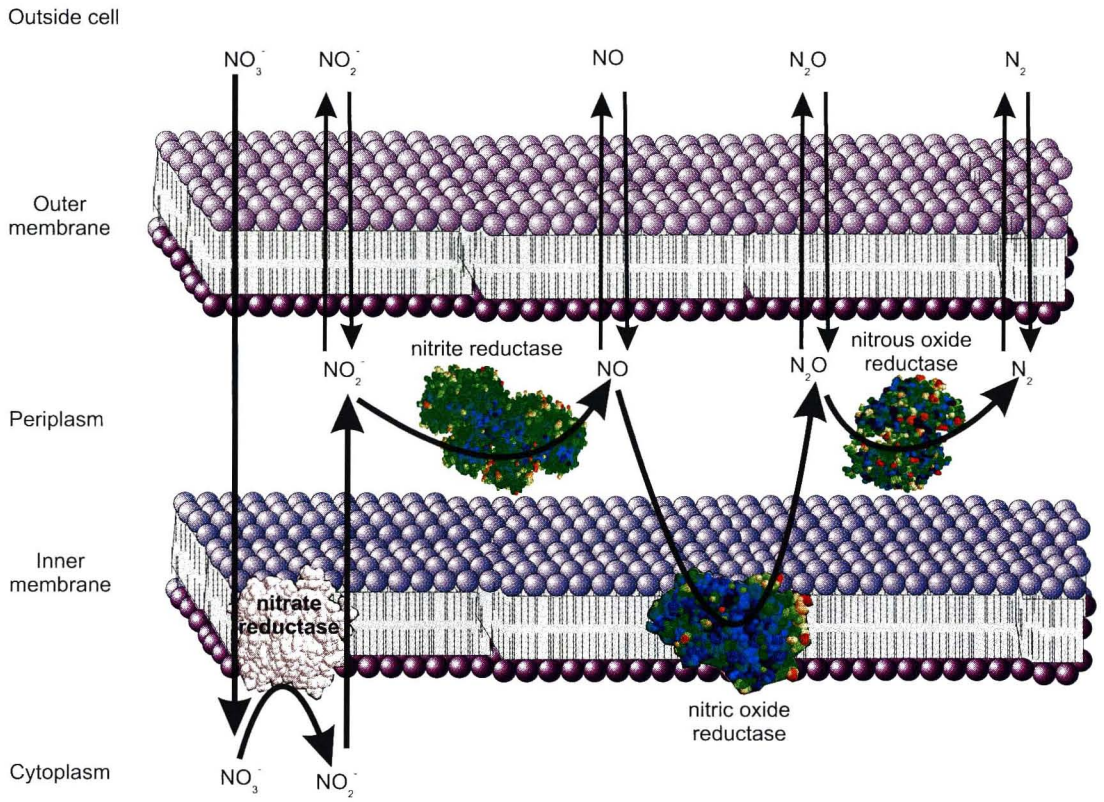


FIGURE 14.12 The denitrification pathway. (Adapted from Myrold, 1998.)

ductase is the most sensitive denitrification enzyme and it is inhibited by dissolved oxygen concentrations of less than 0.2 mg/l.

Whereas the denitrification pathway is very sensitive to oxygen, neither it nor the DNRA pathway is inhibited by ammonium as is the assimilatory nitrate reduction pathway. However, the initial nitrate level in an environmental system can help determine the extent of the denitrification pathway. Low nitrate levels tend to favor production of nitrous oxide as the end

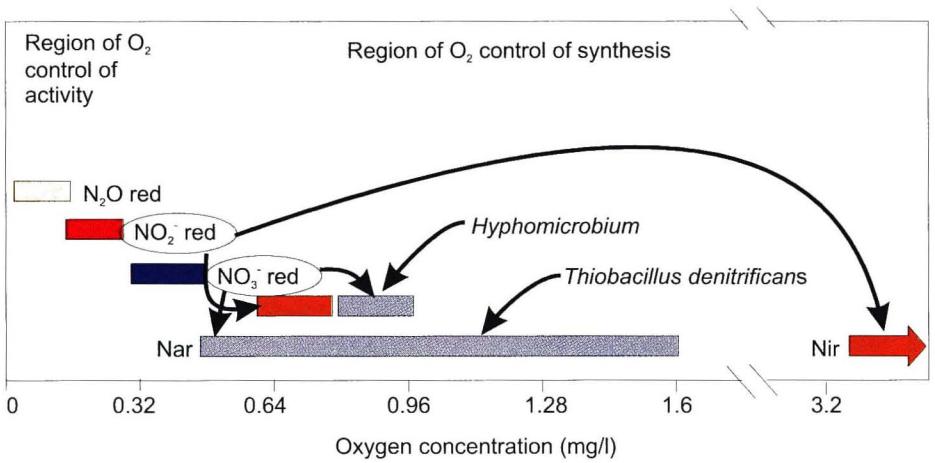
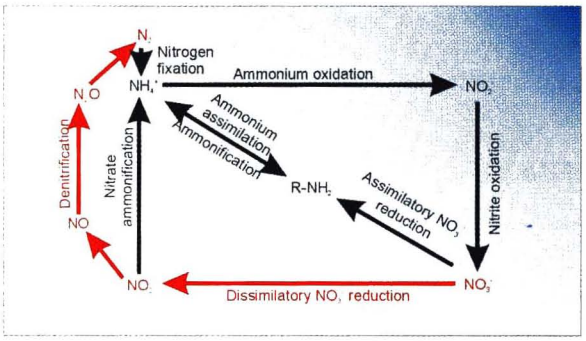


FIGURE 14.13 Approximate regions of oxygen concentration that inhibit the enzyme activity and synthesis for three steps in the denitrification pathway. (Adapted from J. M. Tiedje in *Biology of Anaerobic Microorganisms*, A. J. B. Zehnder, ed. © 1988. Reprinted by permission of John Wiley & Sons, Inc.)

product. High nitrate levels favor production of N_2 gas, a much more desirable end product.

Organisms that denitrify are found widely in the environment and display a variety of different characteristics in terms of metabolism and activities. In contrast to DNRA organisms, which are predominantly heterotrophic using fermentative metabolism, the majority of denitrifiers also are heterotrophic but use respiratory pathways of metabolism. However, as shown in Table 14.15, some denitrifiers are autotrophic, some are fermentative, and some are associated with other aspects of the nitrogen cycle; for example, they can fix N_2.



Summary:
Anaerobic respiration using nitrate as TEA
Inhibited by O_2
Not inhibited by ammonia
Produces a mix of N_2 and N_2O
Many heterotrophic bacteria are denitrifiers

14.4 SULFUR CYCLE

Sulfur is the tenth most abundant element in the crust of the earth. It is an essential element for biological organisms, making up approximately 1% of the dry weight of a bacterial cell (Table 14.1). Sulfur is not generally considered a limiting nutrient in the environment except in some intensive agricultural systems with high crop yields. Sulfur is cycled between oxidation states of +6 for sulfate (SO_4^{2-}) and −2 for sulfide (S^{2-}). In cells, sulfur is required for synthesis of the amino acids cysteine and methionine and is also required for some vitamins, hormones, and coenzymes. In proteins, the sulfur-containing amino acid cysteine is especially important because the formation of disulfide bridges between cysteine residues helps govern protein folding and hence activity. All of these compounds contain sulfur in the reduced or sulfide form. Cells also contain organic sulfur compounds in which the sulfur is in the oxidized state. Examples of such compounds are glucose sulfate, choline sulfate, phenolic sulfate, and two ATP–sulfate compounds that are required to sulfate assimilation and can also serve to store sulfur for the cell. Although the sulfur cycle is not as complex as the nitrogen cycle, the global impacts of the sulfur cycle are extremely important, including the formation of acid rain, acid mine drainage, and corrosion of concrete and metal (see Chapter 15.2 and 15.3).

14.4.1 Sulfur Reservoirs

Sulfur is outgassed from the earth's core through volcanic activity. The sulfur gases released, primarily

TABLE 14.15 Genera of Denitrifying Bacteria

Genus	Interesting characteristics
Organotrophs	
Alcaligenes	Common soil bacterium
Agrobacterium	Some species are plant pathogens
Aquaspirillum	Some are magnetotactic, oligotrophic
Azospirillum	Associative N_2 fixer, fermentative
Bacillus	Spore former, fermentative, some species thermophilic
Blastobacter	Budding bacterium, phylogenetically related to *Rhizobium*
Bradyrhizobium	Symbiotic N_2 fixer with legumes
Branhamella	Animal pathogen
Chromobacterium	Purple pigmentation
Cytophaga	Gliding bacterium; cellulose degrader
Flavobacterium	Common soil bacterium
Flexibacter	Gliding bacterium
Halobacterium	Halophilic
Hyphomicrobium	Grows on one-C substrates, oligotrophic
Kingella	Animal pathogen
Neisseria	Animal pathogen
Paracoccus	Halophilic, also lithotrophic
Propionibacterium	Fermentative
Pseudomonas	Commonly isolated from soil, very diverse genus
Rhizobium	Symbiotic N_2 fixer with legumes
Wolinella	Animal pathogen
Phototrophs	
Rhodopseudomonas	Anaerobic, sulfate reducer
Lithotrophs	
Alcaligenes	Uses H_2, also heterotrophic, common soil isolate
Bradyrhizobium	Uses H_2, also heterotrophic, symbiotic N_2 fixer with legumes
Nitrosomonas	NH_3 oxidizer
Paracoccus	Uses H_2, also heterotrophic, halophilic
Pseudomonas	Uses H_2, also heterotrophic, common soil isolate
Thiobacillus	S-oxidizer
Thiomicrospira	S-oxidizer
Thiosphaera	S-oxidizer, heterotrophic nitrifier, aerobic denitrification

(From Myrold, 1998.)

sulfur dioxide (SO_2) and hydrogen sulfide (H_2S), become dissolved in the ocean and aquifers. Here the hydrogen sulfide forms sparingly soluble metal sulfides, mainly iron sulfide (pyrite), and sulfur dioxide forms metal sulfates with calcium, barium, and strontium as shown in Eqs. 14.10 and 14.11.

$$2S^{2-} + Fe^{2+} \rightarrow FeS_2 \text{ (pyrite)} \qquad \text{(Eq. 14.10)}$$

$$SO_2^{2-} + Ca^{2+} \rightarrow CaSO_4 \text{ (gypsum)} \qquad \text{(Eq. 14.11)}$$

This results in a substantial portion of the outgassed sulfur being converted to rock. Some of the gaseous sulfur compounds find their way into the upper reaches of the ocean and the soil. In these environments, microbes take up and cycle the sulfur. Finally, the small portions of these gases that remain after precipitating and cycling find their way into the atmosphere. Here, they are oxidized to the water-soluble sulfate form, which is washed out of the atmosphere by rain. Thus, the atmosphere is a relatively small reservoir of sulfur (Table 14.16). Of the sulfur found in the atmosphere, the majority is found as sulfur dioxide. Currently, one third to one half of the sulfur dioxide emitted to the atmosphere is from industrial and automobile emissions as a result of the burning of fossil fuels. A smaller portion of the sulfur in the atmosphere is present as hydrogen sulfide and is biological in origin.

The largest reservoir of sulfur is found in the earth's crust and is composed of inert elemental sulfur deposits, sulfur–metal precipitates such as pyrite (FeS_2) and gypsum ($CaSO_4$), and sulfur associated with buried fossil fuels. A second large reservoir that is slowly cycled is the sulfate found in the ocean, where it is the second most common anion (Dobrovolsky, 1994). Smaller and more actively cycled reservoirs of sulfur include sulfur found in biomass and organic matter in the terrestrial and ocean environments. Two recent practices have caused a disturbance in the global sulfur reservoirs. The first is strip mining, which has exposed large areas of metal–sulfide ores to the atmosphere, resulting in the formation of acid mine drainage. The second is the burning of fossil fuels, a sulfur reservoir that was quite inert until recently. This has resulted in sulfur dioxide emissions into the atmosphere with the resultant formation of acid rain. These processes are discussed further in Section 15.3.

TABLE 14.16 Global Sulfur Reservoirs

Sulfur reservoir	Metric tons sulfur	Actively cycled
Atmosphere		
SO_2/H_2S	1.4×10^6	Yes
Ocean		
Biomass	1.5×10^8	Yes
Soluble inorganic ions (primarily SO_4^{2-})	1.2×10^{15}	Slow
Land		
Living biomass	8.5×10^9	Yes
Organic matter	1.6×10^{10}	Yes
Earth's crust[a]	1.8×10^{16}	No

[a] This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Adapted from Dobrovolsky, 1994.)

14.4.2 Assimilatory Sulfate Reduction and Sulfur Mineralization

The primary soluble form of inorganic sulfur found in soil is sulfate. Whereas plants and most microorganisms incorporate reduced sulfur (sulfide) into amino acids or other sulfur-requiring molecules, they take up sulfur in the oxidized sulfate form and then reduce it internally (Widdel, 1988). This is called **assimilatory sulfate reduction.** Cells assimilate sulfur in the form of sulfate because it is the most available sulfur form, and because sulfide is toxic. Sulfide toxicity occurs because inside the cell sulfide reacts with metals in cytochromes to form metal–sulfide precipitates, destroying cytochrome activity. However, under the controlled conditions of sulfate reduction inside the cell, the sulfide can be removed immediately and incorporated into an organic form (see Information Box 1). Although this process does protect the cell from harmful effects of the sulfide, it is an energy-consuming reaction. After sulfate is transported inside the cell, ATP is used to convert the sulfate into the energy-rich molecule adenosine 5′-phosphosulfate (APS) (Eq. 14.13). A second ATP molecule is used to transform APS to 3′-phosphoadenosine-5′-phosphosulfate (PAPS) (Eq. 14.14). This allows the sulfate to be reduced to sulfite and then sulfide in two steps (Eq. 14.15 and 14.16). Most commonly, the amino acid serine is used to remove sulfide as it is reduced, forming the sulfur-containing amino acid cysteine (see Eq. 14.17).

The release of sulfur from organic forms is called **sulfur mineralization.** The release of sulfur from organic molecules occurs under both aerobic and anaerobic conditions. The enzyme serine sulfhydrylase can remove sulfide from cysteine in the reverse of the reaction shown in Eq. 14.17, or a second enzyme, cysteine sulfhydrylase, can remove both sulfide and ammonia as shown in Eq. 14.18.

$$\text{cysteine} \xrightarrow{\text{cysteine sulfhydrylase}} \text{serine} + H_2S \qquad \text{(Eq. 14.18)}$$

In marine environments, one of the major products of algal metabolism is the compound dimethylsulfoniopropionate (DMSP), which is used in osmoregulation of the cell. The major degradation product of DMSP is

Information Box 1

$$\text{sulfate (outside cell)} \xrightarrow{\text{active transport}} \text{sulfate (inside cell)} \qquad \text{(Eq. 14.12)}$$

$$\text{ATP} + \text{sulfate} \xrightarrow{\text{ATP sulfurylase}} \underset{\text{adenosine phosphosulfate}}{\text{APS}} + \text{Ppi} \qquad \text{(Eq. 14.13)}$$

$$\text{ATP} + \text{APS} \xrightarrow{\text{APS phosphokinase}} \underset{\text{3-phosphoadenosine-5-phosphosulfate}}{\text{PAPS}} \qquad \text{(Eq. 14.14)}$$

$$\underset{\text{thioredoxin (reduced)}}{2\text{RSH}} + \text{PAPS} \xrightarrow{\text{PAPS reductase}} \text{sulfite} + \underset{\text{AMP-3-phosphate}}{\text{PAP}} + \underset{\text{thioredoxin (oxidized)}}{\text{RSSP}} \qquad \text{(Eq. 14.15)}$$

$$\text{sulfite} + 3\text{NADPH} \xrightarrow{\text{sulfite reductase}} \text{H}_2\text{S} + 3\text{NADP} \qquad \text{(Eq. 14.16)}$$

$$\textit{O}\text{-acetyl-L-serine} + \text{H}_2\text{S} \xrightarrow{\textit{O}\text{-acetylserine sulfhydrylase}} \text{L-cysteine} + \text{acetate} + \text{H}_2\text{O} \qquad \text{(Eq. 14.17)}$$

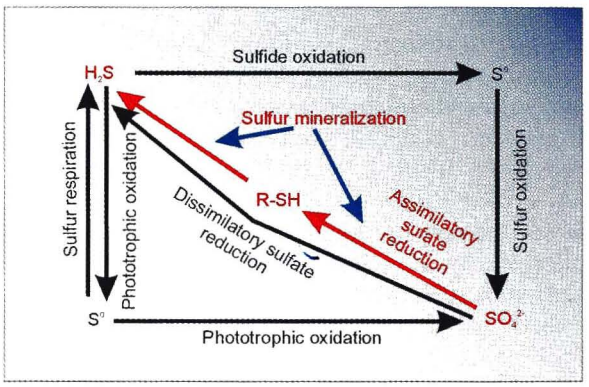
dimethylsulfide (DMS). Both H_2S and DMS are volatile compounds and therefore can be released to the atmosphere. Once in the atmosphere, these compounds are photooxidized to sulfate (Eq. 14.19).

$$\text{H}_2\text{S/DMS} \xrightarrow{\text{UV light}} \text{SO}_4^{2-} \xrightarrow{+\text{H}_2\text{O}} \underset{\text{sulfuric acid}}{\text{H}_2\text{SO}_4} \qquad \text{(Eq. 14.19)}$$

Normal biological release of reduced volatile sulfur compounds results in the formation of approximately 1 kg SO_4^{2-}/hectare/year. The use of fossil fuels, which all contain organic sulfur compounds, increases the amount of sulfur released to the atmosphere to up to 100 kg SO_4^{2-}/hectare/year in some urban areas. Exacerbating this problem is the fact that reserves of fossil fuels that are low in sulfur are shrinking, forcing the use of reserves with higher sulfur content. Burning of fossil fuels produces sulfite as shown in Eq. 14.20.

$$\text{Fossil fuel combustion} \rightarrow \text{SO}_2 \xrightarrow{+\text{H}_2\text{O}} \underset{\text{sulfurous acid}}{\text{H}_2\text{SO}_3} \qquad \text{(Eq. 14.20)}$$

Thus, increased emission of sulfur compounds to the atmosphere results in the formation of sulfur acid compounds. These acidic compounds dissolve in rainwater and can decrease the rainwater pH from neutral to as low as pH 3.5, a process also known as the formation of **acid rain**. Acid rain damages plant foliage, causes corrosion of stone and concrete building surfaces, and can affect weakly buffered soils and lakes (see Chapter 15.2).



Summary:
Sulfur is taken up as sulfate due to H_2S toxicity
Assimilation and mineralization cycles sulfur between its organic and inorganic forms
Mineralization predominates at C:S ratios < 200, assimilation predominates at C:S > 400

14.4.3 Sulfur Oxidation

In the presence of oxygen, reduced sulfur compounds can support the growth of a group of chemoautotrophic bacteria under strictly aerobic conditions and a group of photoautrophic bacteria under strictly anaerobic conditions (Table 14.17). In addition, a number of aerobic heterotrophic microbes, including both bacteria and fungi, oxidize sulfur to thiosulfate or to

TABLE 14.17 Sulfur-Oxidizing Bacteria

Group	Sulfur conversion	Habitat requirements	Habitat	Genera
Obligate or facultative chemoautotrophs	$H_2S \rightarrow S^\circ$ $S^\circ \rightarrow SO_4^{2-}$ $S_2O_3^{2-} \rightarrow SO_4^{2-}$	H_2S-O_2 interface	Mud, hot springs, mining surfaces, soil	*Thiobacillus* *Thiomicrospira* *Achromatium* *Beggiatoa* *Thermothrix*
Anaerobic phototrophs	$H_2S \rightarrow S^\circ$ $S^\circ \rightarrow SO_4^{2-}$	Anaerobic, H_2S, light	Shallow water, anaerobic sediments meta- or hypolimnion, anaerobic water	*Chlorobium* *Chromatium* *Ectothiorhodospira* *Thiopedia* *Rhodopseudomonas*

Adapted from Germida (1998).

sulfate. The heterotrophic sulfur oxidation pathway is still unclear, but apparently no energy is obtained in this process. Chemoautotrophs are considered the predominant sulfur oxidizers in most environments. However, because many chemoautotrophic sulfur oxidizers require a low pH for optimal activity, heterotrophs may be more important in some aerobic, neutral to alkaline soils. Further, heterotrophs may initiate sulfur oxidation, resulting in a lowered pH that is more amenable for chemoautotrophic activity.

14.4.3.1 Chemoautotrophic Sulfur Oxidation

Of the chemoautotrophs, most oxidize sulfide to elemental sulfur, which is then deposited inside the cell as characteristic granules (Eq. 14.21).

$$H_2S + \tfrac{1}{2}\,O_2 \rightarrow S^\circ + H_2O \qquad \Delta G = -50 \text{ kcal} \qquad \text{(Eq. 14.21)}$$

The energy provided by this oxidation is used to fix CO_2 for cell growth. In examining Eq. 14.21, it is apparent that these organisms require both oxygen and sulfide. However, reduced compounds are generally abundant in areas that contain little or no oxygen. So these microbes are **microaerophilic;** they grow best under conditions of low oxygen tension (Fig. 14.14). Characteristics of marsh sediments that contain these organisms are their black color due to sulfur deposits and their "rotten egg" smell due to the presence of H_2S. Most of these organisms are filamentous and can easily be observed by examining a marsh sediment under the microscope and looking for small white filaments.

Some chemoautotrophs, most notably *Thiobacillus thiooxidans*, can oxidize elemental sulfur as shown in Eq. 14.22.

$$S^\circ + 1.5O_2 + H_2O \rightarrow H_2SO_4 \qquad \Delta G = -150 \text{ kcal} \qquad \text{(Eq. 14.22)}$$

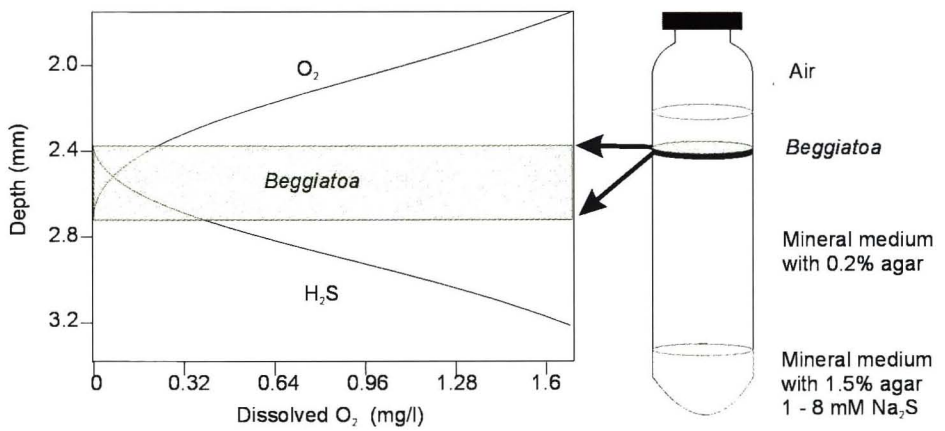


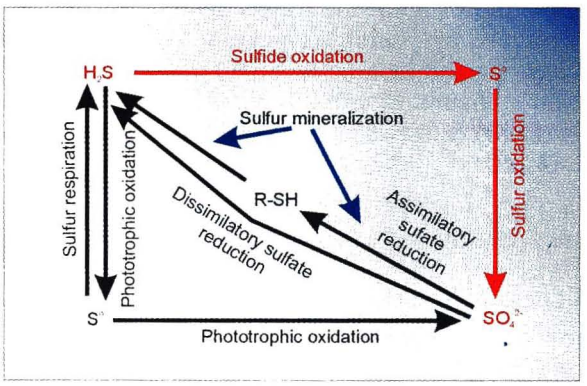
FIGURE 14.14 Cultivation of the sulfur-oxidizing chemolithotroph *Beggiatoa*. At the right is a culture tube with sulfide agar overlaid with initially sulfide-free soft mineral agar. The airspace in the closed tube is the source of oxygen. Stab-inoculated *Beggiatoa* grows in a narrowly defined gradient of H_2S and oxygen as shown. (Adapted from *Microbial Ecology* by R. M. Atlas and R. Bartha. © 1993 by Benjamin Cummings. Adapted by permission.)

This reaction produces acid, and as a result, *T. thiooxidans* is extremely acid tolerant with an optimal growth pH of 2. It should be noted that there are various *Thiobacillus* species, and these vary widely in their acid tolerance. However, the activity of *T. thiooxidans* in conjunction with the iron-oxidizing, acid-tolerant, chemoautotroph *T. ferrooxidans* is responsible for the formation of acid mine drainage, an undesirable consequence of sulfur cycle activity (see Chapter 15.3). It should be noted that the same organisms can be harnessed for the acid leaching and recovery of precious metals from low-grade ore, also known as **biometallurgy** (see Chapter 17). Thus, depending on one's perspective, these organisms can be very harmful or very helpful.

Although most of the sulfur-oxidizing chemoautotrophs are obligate aerobes, there is one exception, *Thiobacillus denitrificans*, a facultative anaerobic organism that can substitute nitrate as a terminal electron acceptor for oxygen (Eq. 14.23).

$$S^\circ + NO_3^- + CaCO_3 \rightarrow CaSO_4 + N_2 \qquad \text{(Eq. 14.23)}$$

In the above equation, the sulfate formed is shown as precipitating with calcium to form gypsum. *T. denitrificans* is not acid tolerant but has an optimal pH for growth of 7.0.



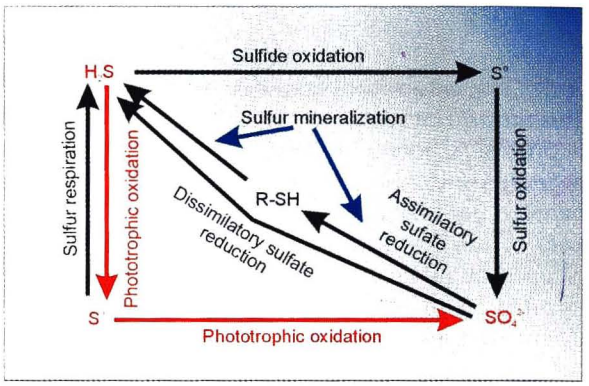
Summary:
Sulfur oxidation is a chemoautotrophic, aerobic process
Most sulfur oxidizers are microaerophilic
Some heterotrophs oxidize sulfur but obtain no energy
This process can result in the formation of acid mine drainage
This process can be used in metal recovery

14.4.3.2 Photoautotrophic Sulfur Oxidation

Photoautotrophic oxidation of sulfur is limited to green and purple sulfur bacteria (Table 14.17). This group of bacteria evolved on early Earth when the atmosphere contained no oxygen. These microbes fix carbon using light energy, but instead of oxidizing water to oxygen, they use an analogous oxidization of sulfide to sulfur.

$$CO_2 + H_2S \rightarrow S^\circ + \text{fixed carbon} \qquad \text{(Eq. 14.24)}$$

These organisms are found in mud and stagnant water, sulfur springs, and saline lakes. In each of these environments, both sulfide and light must be present. Although the contribution to primary productivity is small in comparison with aerobic photosynthesis, these organisms are important in the sulfur cycle. They serve to remove sulfide from the surrounding environment, effectively preventing its movement into the atmosphere and its precipitation as metal sulfide.



Summary:
Photoautotrophic, anaerobic process
Limited to purple and green sulfur bacteria
Responsible for a very small portion of photosynthetic activity

14.4.4 Sulfur Reduction

There are three types of sulfur reduction. The first, already discussed in Section 14.4.2, is performed to assimilate sulfur into cell components (Widdel, 1988). Assimilatory sulfate reduction occurs under either aerobic or anaerobic conditions. In contrast, there are two dissimilatory pathways, both of which use an inorganic form of sulfur as a terminal electron acceptor. In this case sulfur reduction occurs only under anaerobic conditions. The two types of sulfur that can be used as terminal electron acceptors are elemental sulfur and sulfate. These two types of metabolism are differentiated as **sulfur respiration** and **dissimilatory sulfate reduction.** *Desulfuromonas acetooxidans* is an example of a bacterium that grows on small carbon compounds such as acetate, ethanol, and propanol using elemental sulfur as the terminal electron acceptor (Eq. 14.25).

$$\underset{\text{acetate}}{CH_3COOH} + 2H_2O + 4S^\circ \rightarrow 2CO_2 + 4S^{2-} + 8H^+ \qquad \text{(Eq. 14.25)}$$

However, the use of sulfate as a terminal electron acceptor seems to be the more important environmental process. The following genera, all of which utilize sulfate as a terminal electron acceptor, are found widely distributed in the environment, especially in anaerobic sediments of aquatic environments, water-saturated soils, and animal intestines: *Desulfobacter, Desulfobulbus, Desulfococcus, Desulfonema, Desulfosarcina, Desulfotomaculum,* and *Desulfovibrio.* Together these organisms are known as the **sulfate-reducing bacteria (SRB).** These organisms can utilize H_2 as an electron donor to drive the reduction of sulfate (Eq. 14.26):

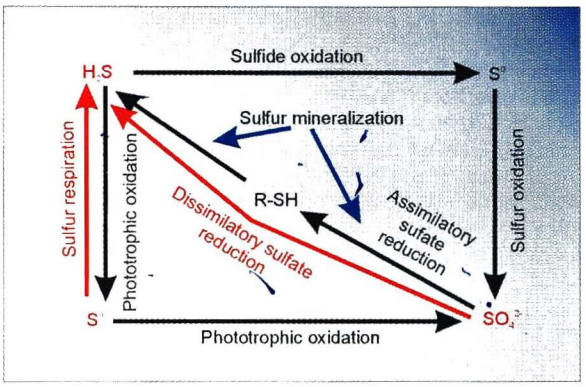
$$4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4H_2O \qquad \text{(Eq. 14.26)}$$

Thus, SRB compete for available H_2 in the environment, as H_2 is also the electron donor required by methanogens. It should be noted that this is not usually a chemoautotrophic process because most SRB cannot fix carbon dioxide. Instead they obtain carbon from low-molecular-weight compounds such as acetate or methanol. The overall reaction for utilization of methanol is shown in Eq. 14.27.

$$\underset{\text{methanol}}{4CH_3OH} + 3SO_4^{2-} \rightarrow 4CO_2 + 3S^{2-} + 8H_2O \qquad \text{(Eq. 14.27)}$$

Both sulfur and sulfate reducers are strict anaerobic chemoheterotrophic organisms that prefer small carbon substrates such as acetate, lactate, pyruvate, and low-molecular-weight alcohols. Where do these small carbon compounds come from in the environment? They are by-products of fermentation of plant and microbial biomass that occurs in anaerobic regions. Thus, the sulfate reducers are part of an anaerobic consortium of bacteria including fermenters, sulfate reducers, and methanogens that act together to completely mineralize organic compounds to carbon dioxide and methane (see Chapter 3). More recently, it has been found that some SRB can also metabolize more complex carbon compounds including some aromatic compounds and some longer chain fatty acids. These organisms are being looked at closely to determine whether they can be used in remediation of contaminated sites that are highly anaerobic and that would be difficult to oxygenate.

The end product of sulfate reduction is hydrogen sulfide. What are the fates of this compound? It can be taken up by chemoautotrophs or photoautotrophs and reoxidized, it can be volatilized into the atmosphere, or it can react with metals to form metal sulfides. In fact, the activity of sulfate reducers and the production of hydrogen sulfide are responsible for the corrosion of underground metal pipes. In this process, the hydrogen sulfide produced reacts with ferrous iron metal to more iron sulfide (see Chapter 15).



Summary:
Anaerobic respiration using SO_4^{2-} or S^0 as a TEA
Completely inhibited by O_2
Produces H_2S which can cause metal corrosion

QUESTIONS AND PROBLEMS

1. Give an example of
   a. a small actively cycled reservoir
   b. a large actively cycled reservoir
   c. a large inactively cycled reservoir
2. Describe how the ocean has reduced the expected rate of increase of CO_2 in the atmosphere since industrialization began.
3. What strategy is used by microbes to initiate degradation of large plant polymers such as cellulose?
4. Define what is meant by a greenhouse gas and give 2 examples. For each example describe how microorganisms mediate generation of the gas, and then describe how human activity influence generation of the gas.
5. Both autotrophic and heterotrophic activities are important in element cycling. For each cycle discussed in this chapter (carbon, nitrogen, and sulfur), name the most important heterotrophic and autotrophic activity. Justify your answer.
6. What would happen if microbial nitrogen fixation suddenly ceased?

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