5
Microbial Metabolism: Importance for Environmental Biotechnology

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CONTENTS

Abstract Microorganisms are the main agents responsible for biogeochemical transformations of carbon, nitrogen, sulfur, iron, and other elements. The prokaryotic world (domains *Archaea* and *Bacteria*) presents us with a far larger variety of metabolic types than are found among the eukaryotes (fungi, higher plants, protozoa and animals). The range of substrates used by prokaryotes as carbon sources for growth (assimilatory metabolism) is far greater than in the eukaryotic world. In addition, many groups of prokaryotes perform types of energy generation (dissimilatory reactions) that are altogether unknown among the eukaryotes. This chapter provides a general overview of the metabolism of microorganisms, with special emphasis on the prokaryotic world. Processes such as oxygenic and anoxygenic

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photosynthesis, aerobic and anaerobic respiration, and chemolithotrophic metabolism are discussed. Finally, it is shown how these processes together enable the functioning of the biogeochemical cycles of the elements on Earth.

Key Words Prokaryotes • metabolic diversity • energy generation • assimilatory metabolism • oxygenic photosynthesis • anoxygenic photosynthesis • respiration • anaerobic respiration • fermentation • methanogenesis • chemoautotrophs • biogeochemical cycles.

1. INTRODUCTION: THE METABOLIC DIVERSITY OF PROKARYOTIC AND EUKARYOTIC MICROORGANISMS

Microorganisms – prokaryotic as well as eukaryotic – are the main agents responsible for biogeochemical transformations of carbon, nitrogen, sulfur, iron, and other elements. Therefore, they are of utmost importance to the environmental engineer: in processes such as bioremediation, water purification, and many other applications of environmental biotechnology the microbes do the work. A thorough understanding of the ways in which microorganisms function is therefore essential in all aspects of environmental engineering.

The prokaryotic world (domains *Archaea* [Archaebacteria] and *Bacteria* [Eubacteria]) presents us with a far larger variety of metabolic types than are found among the eukaryotes (fungi, higher plants, protozoa, and animals) (1–6). First of all, the range of substrates used by prokaryotes as carbon sources for growth (assimilatory metabolism) is far greater than in the eukaryotic world. Secondly, many groups of prokaryotes perform types of energy generation (dissimilatory reactions) that are altogether unknown among the eukaryotes. Such dissimilatory processes are of great interest in environmental biotechnology as the amounts of substrates transformed in the course of the energy-generating process are generally much larger than the amounts of substrates necessary for assimilatory purposes, i.e., cell growth and multiplication. Processes, such as denitrification (dissimilatory reduction of nitrate to dinitrogen and other gaseous nitrogen compounds), nitrification (oxidation of ammonium ions to nitrate with nitrite as intermediate), dissimilatory reduction of sulfate to badly smelling and corrosive hydrogen sulfide, the formation of acidic wastewater loaded with yellow–brown iron hydroxides in the neighborhood of coal mines and other mining operations, and formation of the greenhouse gas methane in the course of anaerobic degradation of organic matter are just a few examples of such microbial processes that the environmental biotechnologist has to understand before he/she can try to prevent such processes from occurring, or stimulate desirable processes. Another reason why microorganisms are so important in the environment, both natural and manipulated by the environmental engineer, is their rapid growth and their high metabolic rates. As a result, the processes they perform are often extremely rapid. We will also encounter many cases in which a small community of microorganisms has a huge impact on the properties of the ecosystem – terrestrial as well as aquatic. An understanding of these processes and of the role of the different types of microorganisms that mediate them is therefore essential for the environmental engineer.

The following sections provide a general overview of the metabolism of microorganisms, with special emphasis on the prokaryotic world. They aim at an understanding of why the different processes occur, and of what use they are to the organisms that perform them.

2. DISSIMILATORY METABOLISM OF MICROORGANISMS: THERMODYNAMIC AND MECHANISTIC PRINCIPLES

2.1. General Overview of the Metabolic Properties of Microorganisms: A Thermodynamic Approach

If we want to gain insight into the ways in which microorganisms function and the nature of the transformations they perform in nature, we need to understand both the assimilatory and the dissimilatory processes they perform. Each cell has to obtain building blocks to produce new cellular components necessary for growth and multiplication. These building blocks need to contain all the elements of which the cell is built: carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, iron, and many others that are required in small quantities ("trace elements"). Some cells can build all their complex molecules, such as proteins, nucleic acids, cell walls, etc. from simple inorganic components such as carbon dioxide as carbon source, nitrate or ammonium ions as nitrogen source, and sulfate and phosphate as sources of sulfur and phosphorus, respectively. Such cells that do not require any organic carbon compounds for growth are designated autotrophic (Fig. 5.1). Green plants and eukaryotic algae are such autotrophs, but many more types of autotrophic microorganisms are known, such as phototrophic purple and green sulfur bacteria that use sulfide rather than water as electron

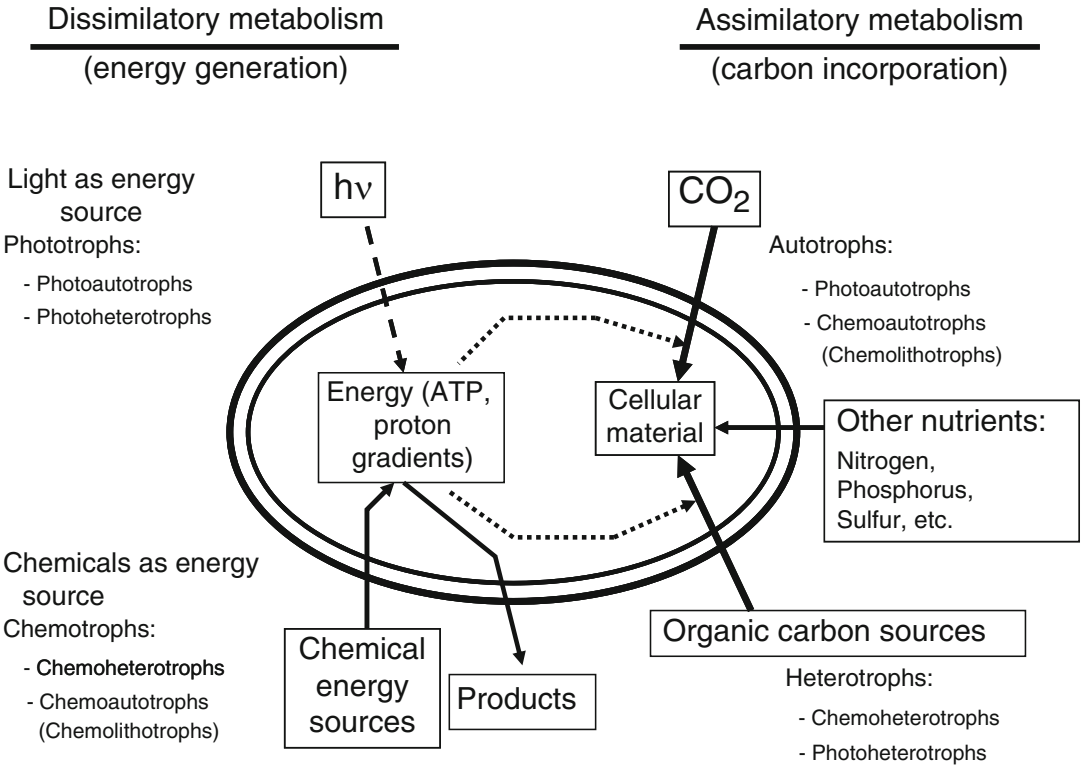

Fig. 5.1. Types of microorganisms, classified according to their energy and carbon sources.

donor to fix CO_2, aerobic chemoautotrophic (chemolithotrophic) bacteria that obtain their energy from the oxidation of inorganic compounds such as ammonium or nitrite ions or sulfide (see Sect. 6.1), and anaerobic prokaryotes such as those methanogenic *Archaea* that obtain their energy from the reduction of CO_2 to methane using hydrogen as electron donor and also use CO_2 as their sole carbon source for growth. Other microorganisms – many bacteria, the fungi, the protozoa, and also all higher animals – are heterotrophs that require organic carbon compounds, and often organic sources of nitrogen and sulfur as well, as building blocks for the production of more cell material.

Biosynthesis of proteins, nucleic acids, and other cellular macromolecules from simple precursors is an energetically expensive process. An autotrophic organism that has to produce all its chemical components from CO_2 and other simple, generally oxidized components will need far more energy to produce the same amount of cell material than a heterotrophic organism that takes up sugars, amino acids, etc. from the medium and needs only to assemble these components into proteins, nucleic acids, and cell wall polysaccharides. The amount of new biomass that can be formed, thus, depends both on the availability of building blocks – organic and/or inorganic – and on the generation of sufficient amounts of energy by the cells to enable the biosynthesis and assembly processes to take place.

A basic understanding of the principles of chemical thermodynamics is required to obtain insight into the metabolism of microorganisms and into the transformations these microorganisms perform in nature. For our purpose, the most relevant parameter of the different reactions involved in the energy metabolism of cells is the change in free energy (Gibbs free energy, ΔG) that accompanies any reaction. This free energy change represents the amount of energy that can be used to perform useful work, this in contrast to energy released as heat that cannot be further used by the organism. When the change in free energy is positive, i.e., the amount of free energy in the reaction products exceeds the amount of free energy in the reagents, the reaction is called endergonic ($\Delta G > 0$); when the total free energy in the products is lower than in the reagents, the reaction is exergonic ($\Delta G < 0$). Such reactions can be used by the cell to generate energy, and they form the core of the dissimilatory metabolism of the cell. Reactions associated with the assimilatory metabolism of the cell are typically endergonic, and they are driven by the energy obtained in the dissimilatory processes.

The change in free energy of any reaction or process determines whether it can (at least theoretically) be used for energy generation. The amount of free energy released in the course of the dissimilatory process performed is therefore the most important bioenergetic parameter for any living organism (7, 8).

The amount of free energy released or required in the course of chemical reactions, expressed in kilojoules (kJ), can be calculated according to the equation:

$$\Delta G^{o} = \Sigma \Delta G_{f}^{o}(\text{products}) - \Sigma \Delta G_{f}^{o}(\text{reagents}) \quad (1)$$

where

ΔG^{o} = the free energy change associated with the reaction, when molar amounts of the reagents and products are converted according to the reaction stoichiometry. The sign ^{o} refers to standard conditions, i.e., concentrations of all compounds involved

in the reaction being 1 M or 1 atmosphere in the case of gases, and at a temperature of 25°C.

ΔG_f = the free energy (in kJ/mol) required for synthesis of the reagents or the reaction products from the elements of which they are composed.

Based on the known ΔG_f^o values of common substrates and metabolic products such as those found in Table 5.1, the free energy yield or demand of different reactions performed by microorganisms can easily be calculated.

For example, the free energy change associated with the alcoholic fermentation of yeast:

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2CH_3CH_2OH \text{ (ethanol)} + 2CO_2 \text{ (carbon dioxide)} \quad (2)$$

under standard conditions can be calculated according to:

$$-917.2 - (2 \times -181.8) + (2 \times -386)\text{kJ} = -218.4\,\text{kJ per mol glucose fermented} \quad (3)$$

Many reactions, which occur during cellular metabolism involve the participation of protons (H^+). For such reactions, calculation of the ΔG values under standard conditions would imply an H^+ concentration of 1 mol/l, i.e., a pH of zero. Such conditions are of little relevance to the metabolism of the cell, in which reactions proceed under near-neutral to slightly alkaline conditions. It is therefore customary to calculate all ΔG^o values at neutral pH (H^+ concentration of 10^{-7} M). The free energy change is then indicated as $\Delta G^{o\prime}$. The $\Delta G'_f$ for protons is 39.8 kJ/mol (see Table 5.1).

Another important parameter to be taken into account when estimating the energy yield or energy requirement of metabolic reactions is the actual concentrations of the reactants and the reaction products. The $\Delta G^{o\prime}$ value defined above refers to standard conditions of

Table 5.1
Free energy of formation (ΔG_f^o) for some substances relevant to microbial metabolism. For more extensive tables see (1) and (8)

CO_2 (aqueous)	−386.0	N_2	0
HCO_3^-	−586.9	NO	+86.6
CH_4	−50.8	NO_2^-	−37.2
CO	−137.2	NO_3^-	−111.3
Formate $^-$	−351.0	NH_4^+	−79.4
Acetate $^-$	−369.4	N_2O	+104.2
Propionate $^-$	−361.1		
Butyrate $^-$	−352.6		
Ethanol	−181.8	S^o	0
Lactate $^-$	−517.8	SO_4^{2-}	−744.6
Succinate $^{-2}$	−690.2	H_2S (aqueous)	−27.9
Glucose	−917.2	HS^-	+12.1
H_2O	−237.2		
H^+	−39.8 (pH 7)	Fe^{2+}	−78.9
OH^-	−198.8 (pH 7)	Fe^{3+}	−4.6
O_2	0		

concentrations and temperature. The true ΔG value of the reaction is determined both by the $\Delta G^{o\prime}$ and by the concentrations at which the reagents and the end products of the reaction are present, according to:

$$\Delta G' = \Delta G^{0'} + RT \ln \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b} \tag{4}$$

where $\Delta G'$ is the free energy change associated with the reaction (in kJ/mol), $\Delta G^{o'}$ is the free energy change associated with the reaction under standard conditions (in kJ/mol), R is the gas constant (8.29 J/mol.K), T is the temperature in K,

for a reaction between reagents A and B to yield products C and D with stoichiometries of a, b, c, and d, respectively.

Equation (4) implies that the true free energy change of a reaction may change from exergonic to endergonic and vice versa according to the concentrations of the reactants and the products. This is also predicted from Le Chatelier's principle ("If some stress is brought to bear upon a system in equilibrium, a change occurs, such that the equilibrium is displaced in a direction which tends to undo the effect of the stress"). As discussed in Sect. 5.7, such effects may have a profound impact on the progress of degradation of organic compounds and other metabolic pathways, especially under anaerobic conditions.

To mediate between energy-yielding (exergonic, dissimilatory) reactions and energy-consuming (endergonic, assimilatory) reactions, all cells use adenosine triphosphate (ATP), a compound that contains two energy-rich anhydride bonds linking the phosphate groups. Synthesis of ATP from ADP (adenosine diphosphate) and inorganic phosphate requires a large amount of energy:

$$\text{ADP} + \text{phosphate} \rightarrow \text{ATP} + \text{H}_2\text{O} \quad (\Delta G^{o'} = +33\,\text{kJ mol}) \tag{5}$$

$$(\Delta G' = \text{approximately} + 44\,\text{kJ/mol})$$

In practice, the amount of free energy needed to drive formation of ATP is approximately 70 kJ/mol (8, 9). This value takes into account both the true concentrations of ATP, ADP and phosphate typically found within the cell and the inevitable amount of energy lost as heat during nonequilibrium situations. Hydrolysis of ATP in the cell is coupled with energy-requiring reactions, thus driving thermodynamically unfavorable reactions.

ATP can be formed in biological systems in either or both of two ways:

1. ATP can be synthesized by "substrate-level phosphorylation," in which the formation of ATP is directly coupled to a strongly exergonic reaction in which an intermediate that carries a "high-energy" phosphate group, (i.e., a phosphate group whose hydrolytic cleavage is associated with a highly negative $\Delta G^{o\prime}$), transfers the phosphate group to ADP. Examples are the formation of ATP and 3-phosphoglycerate from 1,3-bisphosphoglycerate and the formation of ATP from phosphoenolpyruvate to yield pyruvate in the glycolytic Embden–Meyerhof pathway (Fig. 5.2, upper part). An analogous reaction is the formation of GTP (guanosine triphosphate) from GDP (guanosine diphosphate) and inorganic phosphate coupled to the formation of succinate from the high-energy compound succinyl-CoA in the tricarboxylic acid cycle (Krebs cycle) (Fig. 5.2, lower part).

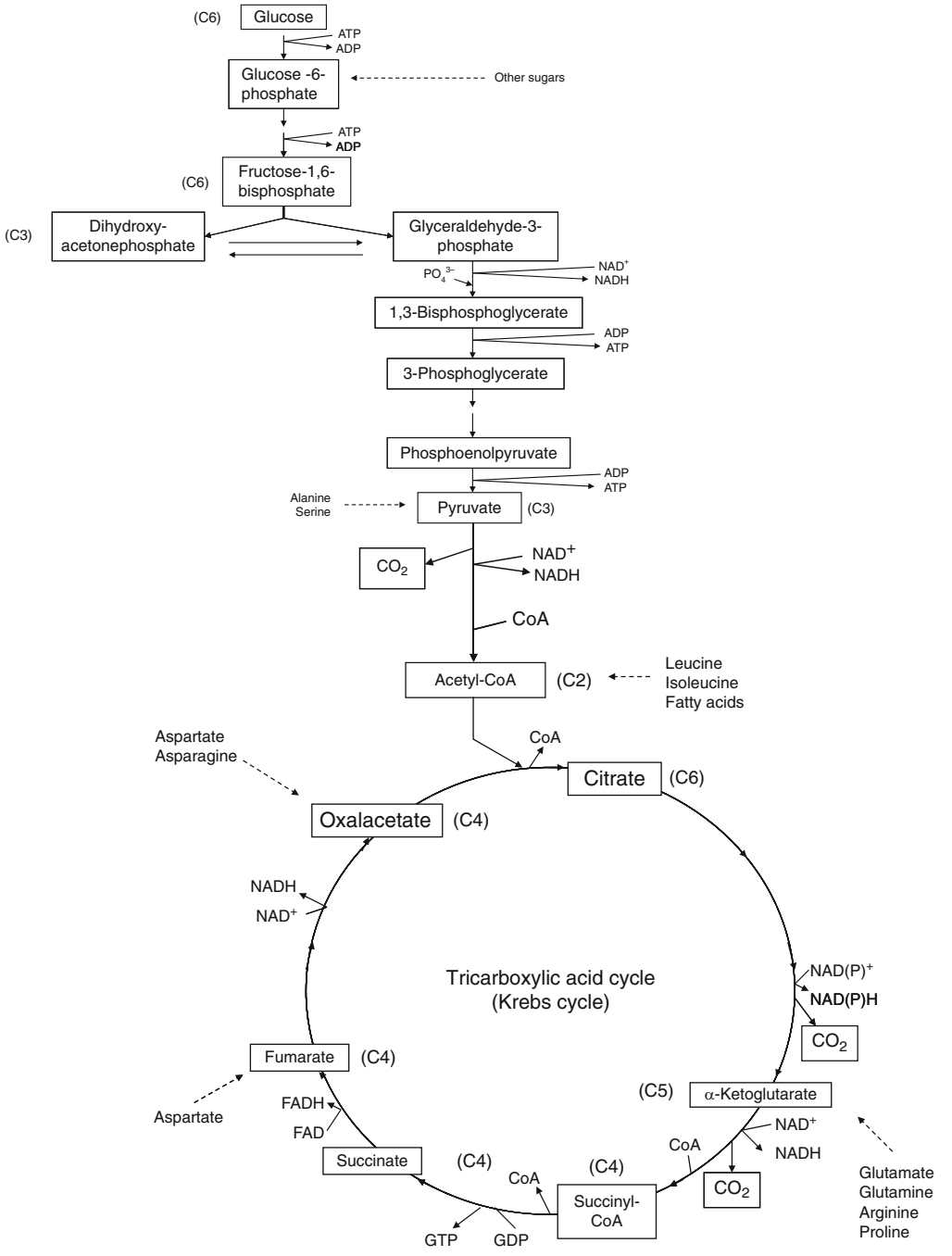

Fig. 5.2. Glycolysis (the Embden–Meyerhof pathway) and the tricarboxylic acid cycle as the backbone of dissimilatory metabolism in heterotrophic bacteria.

Table 5.2
The most important high-energy compounds involved in substrate-level phosphorylation and their $\Delta G^{o\prime}$ of hydrolysis (1, 8)

High-energy compound	$\Delta G^{o\prime}$ of hydrolysis (kJ/mol)
1,3-Bisphosphoglycerate	−51.9
Phosphoenolpyruvate	−51.6
Acetyl-CoA	−35.7
Succinyl-CoA	−35.1
Acetyl phosphate	−44.8
Carbamyl phosphate	−39.3
Adenosine-5′-phosphosulfate	−88.0

The number of high-energy compounds that can be used for ATP production by substrate-level phosphorylation is limited (1, 5, 8, 10). The most important of these compounds are listed in Table 5.2.

2. Energy is also available to living systems in the form of gradients of protons (H^+) across biological membranes. In many dissimilatory (energy-yielding) processes in prokaryotes, energy is conserved in the form of proton gradients generated by transport of protons from the cytoplasmic side of the membrane to the extracellular environment. Such electrochemical gradients of protons involve both a pH difference (alkaline inside, acidic outside) and a membrane potential (negative inside, positive outside). In eukaryotes, similar processes take place across the membranes of mitochondria and chloroplasts. Controlled entry of protons through the enzyme ATP synthase ("ATPase") located within the membrane (which otherwise is highly impermeable to protons) is coupled with the synthesis of ATP from ADP and inorganic phosphate. The generation of a proton electrochemical gradient at the expense of ATP can occur as well. ATP and proton electrochemical gradients across membranes may therefore be considered as fully interconvertible forms of energy, to be used to drive energy-requiring processes within the cell, including biosynthesis of new cellular components enabling growth.

Many reactions performed by the cell, both in dissimilatory and assimilatory metabolism, are electron transfer processes in which electrons flow from an electron donor to an electron acceptor. Aerobic respiration in which organic substrates are oxidized, coupled to the transfer of the electrons to molecular oxygen with the formation of water is just one example. The tendency of different compounds to gain electrons by reduction or to donate electrons and become oxidized can be expressed in terms of the standard reduction potential of the redox couples. Figure 5.3 presents the standard redox potentials of the most important compounds that become oxidized and/or reduced in the course of cellular metabolism. The more negative the standard reduction potential, the stronger the tendency of the reduced form to donate electrons to an oxidized compound with a higher reduction potential. The amount of energy to be invested or to be gained during such redox reactions is directly proportional to the difference in the standard reduction potential of the reductant and the oxidant involved according to:

$$\Delta G^{o\prime} = -n\,F\,\Delta E'_{o} \tag{6}$$

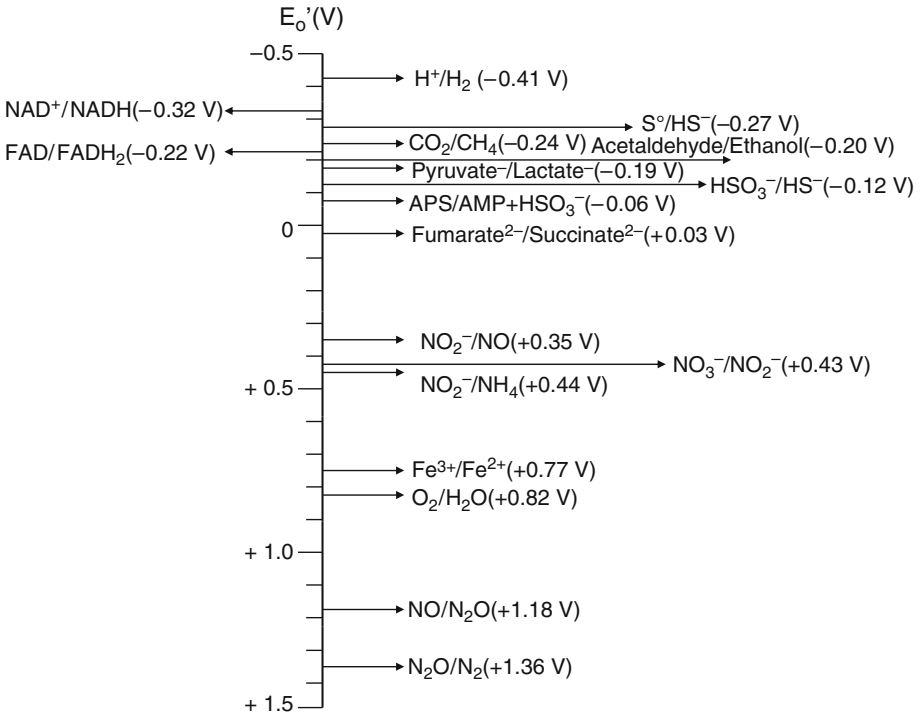

Fig. 5.3. Standard reduction potentials of selected redox couples relevant to microbial metabolism.

where $\Delta G^{o\prime}$ = the free energy change associated with the reaction under standard conditions (in kJ/mol), n = the number of electrons transferred in the reaction, F = the Faraday constant (96.5 kJ/V), and $\Delta E_o{}'$ = the difference in standard reduction potential of the redox couples participating in the reaction.

Flow of electrons from a reductant with a low reduction potential to an oxidant with a high reduction potential gives rise to an exergonic reaction; "uphill" flow of electrons to form a stronger reductant from a weak reductant is an endergonic process that can only proceed with the expenditure of energy. From the data presented in Fig. 5.3, it can for example be concluded that oxidation of ammonium ions to nitrite with transfer of the electrons to molecular oxygen is an energy-yielding process (the process that *Nitrosomonas* and other nitrifying bacteria use to gain energy for growth), while the same bacteria have to invest energy in order to use electrons from ammonium ions to reduce $NADP^+$ to NADPH, which is required by the cell to serve as the electron donor for autotrophic fixation of CO_2 in the Calvin cycle, the major carbon assimilation pathway used by nitrifying bacteria (see also Sect. 6.1).

2.2. *Modes of Energy Generation of Prokaryotic and Eukaryotic Microorganisms*

Microorganisms display a tremendous diversity in the modes of energy generation that can support their growth. In this respect, their abilities greatly exceed those of the eukaryotic microorganisms as well as the macroorganisms.

The metabolic diversity of the prokaryotes, especially as far as their dissimilatory processes are concerned, will form the topic of most of the following sections in this chapter. An in-depth insight in to the diversity in dissimilatory metabolism is essential when we need to understand the conversions performed by microorganisms in the natural environment. Here again, the nitrifying bacteria provide an excellent example. Nitrification (see also Sects. 2.1 and 6.1) consists of two steps, the first being the aerobic oxidation of ammonium ions (NH_4^+) to nitrite (NO_2^-) by organisms such as *Nitrosomonas* (a six-electron transfer in which N^{3-} is oxidized to N^{3+}), followed by oxidation of the nitrite to nitrate (NO_3^-, N^{5+}) by organisms such as *Nitrobacter*, again using molecular oxygen as electron acceptor. Due to the fact that the electron donors (ammonium and nitrite, respectively) are relatively weak reductants (see Fig. 5.3), the amount of energy gained per mol of substrate oxidized is relatively small, as can be calculated from Eqs. (1) and (6):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2 + 2H^+ + H_2O \quad \Delta G^{o'} = -274.6\,\text{kJ} \tag{7}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad \Delta G^{o'} = -74.1\,\text{kJ} \tag{8}$$

Moreover, for the autotrophic fixation of CO_2, both groups of nitrifying bacteria need NADPH as electron donor, a strong reductant that can only be formed at the expenditure of much energy from the weak reductants available, i.e., ammonium and nitrite respectively. The result is that to fix one molecule of CO_2, *Nitrosomonas* has to oxidize in the order of 30–40 ammonium ions, while *Nitrobacter* needs around 100 ions of nitrite to provide both the energy and the electrons necessary for the fixation of one CO_2 molecule. If we further assume that the ratio of carbon to nitrogen in cell material is 6.6:1, *Nitrosomonas* will need to assimilate (based on the general empirical formula $C_{106}H_{263}O_{110}N_{16}P$ for [phytoplankton] cell material, known as the Redfield ratios) 4.5 mmol of ammonium per gram of dry cell material, while as much as 0.9–1.2 mol ammonium are needed in the dissimilatory reaction used to provide the energy and the electrons for the autotrophic carbon fixation. A similar calculation for *Nitrobacter* shows that the ratio between the amount of inorganic nitrogen (as nitrite) converted in the dissimilatory reaction and the amount of inorganic nitrogen assimilated into proteins and other nitrogen-containing cellular components is more than 600. Such calculations clearly show that the dissimilatory processes that the bacteria perform have the most profound impact on the environment, and that small numbers of bacteria and accordingly small amounts of biomass may influence the chemical composition of the environment in the most dramatic way. This, together with their generally rapid growth and their accordingly short generation times makes the prokaryotic microorganisms (*Bacteria* and certain *Archaea*, notably the methanogenic species) responsible for the greatest part of the biogeochemical transformations of matter, quantitatively spoken, and the often very rapid turnover rates in the biogeochemical cycles.

A general overview of the different modes of life, as based on the diversity of dissimilatory and assimilatory pathways, was presented in Fig. 5.1. According to the energy source used, we can divide the organisms living on Earth into phototrophs – organisms that use photons of light in the visible and sometimes the near-infrared range as their source of energy, and chemotrophs – organisms that use chemical energy to produce ATP. The phototrophic organisms can further be divided into photoautotrophs, i.e., organisms that use CO_2 as their carbon source with light providing the energy for autotrophic carbon fixation, and photoheterotrophs, which derive their energy from light, but obtain their cellular carbon from organic compounds rather than from carbon dioxide (11).

Most phototrophic microorganisms use chlorophyll, a tetrapyrrole derivative with a central bound magnesium atom, as the central molecule responsible for the photochemical processes. Excitation of the chlorophyll in the reaction center liberates an electron at a low reduction potential. This electron can return to the reaction center through a chain of electron carriers, including quinones and cytochromes ("cyclic electron flow"), or reduce an electron acceptor such as NAD^+ or $NADP^+$. An external electron donor is then required in order to replenish the missing electron in the reaction center.

Among the prokaryotic photoautotrophs, there is considerable diversity with respect to the electron donors used. Eukaryotic phototrophs (green plants, macro- and microalgae) invariably use water as electron donor, and they excrete molecular oxygen as a waste product. Therefore, this process is called oxygenic ("oxygen-forming") photosynthesis. In the eukaryotic phototrophs, the photosynthetic machinery is localized in intracellular organelles, named chloroplasts. The same kind of metabolism is also used by one group of photoautotrophic prokaryotes, the cyanobacteria. A wide range of alternative electron donors are available for photosynthetic CO_2 fixation by other prokaryotes. These include reduced sulfur compounds (sulfide, elemental sulfur, thiosulfate), molecular hydrogen, and others. As no oxygen is evolved in these cases, the process is termed anoxygenic photosynthesis. In both types of phototrophic life, energy is conserved as a proton electrochemical gradient, which may serve for the generation of ATP.

Figure 5.4 presents a schematic overview of the metabolism of oxygenic photoautotrophs (a), anoxygenic photoautotrophs (b), and anoxygenic photoheterotrophs (c). The metabolism of the different types of phototrophs, the habitats in which they are found, and the impact they have on their environment, are discussed in further depth in Sects. 4.1 and 4.2.

The chemoorganotrophic microorganisms use organic compounds, both as energy source and as a source of carbon to be taken up and incorporated into cell material. There are many different ways in which energy can be derived from conversion of organic compounds. One mode of metabolism is aerobic respiration, i.e., oxidation of the organic carbon while using molecular oxygen as terminal electron acceptor. In most cases, this oxidation proceeds all the way to form CO_2 and H_2O as only products. The amount of free energy released is large, as may be expected based on the high standard reduction potential of the couple O_2/H_2O (Fig. 5.3). The principles of aerobic respiration are summarized in Fig. 5.5a. Aerobic respiration is by far the most common way of energy generation in the animal world. It is also used by plants to obtain energy during the night when light is not available as energy source, thus gaining energy by aerobic oxidation of storage polymers (starch and others) that had

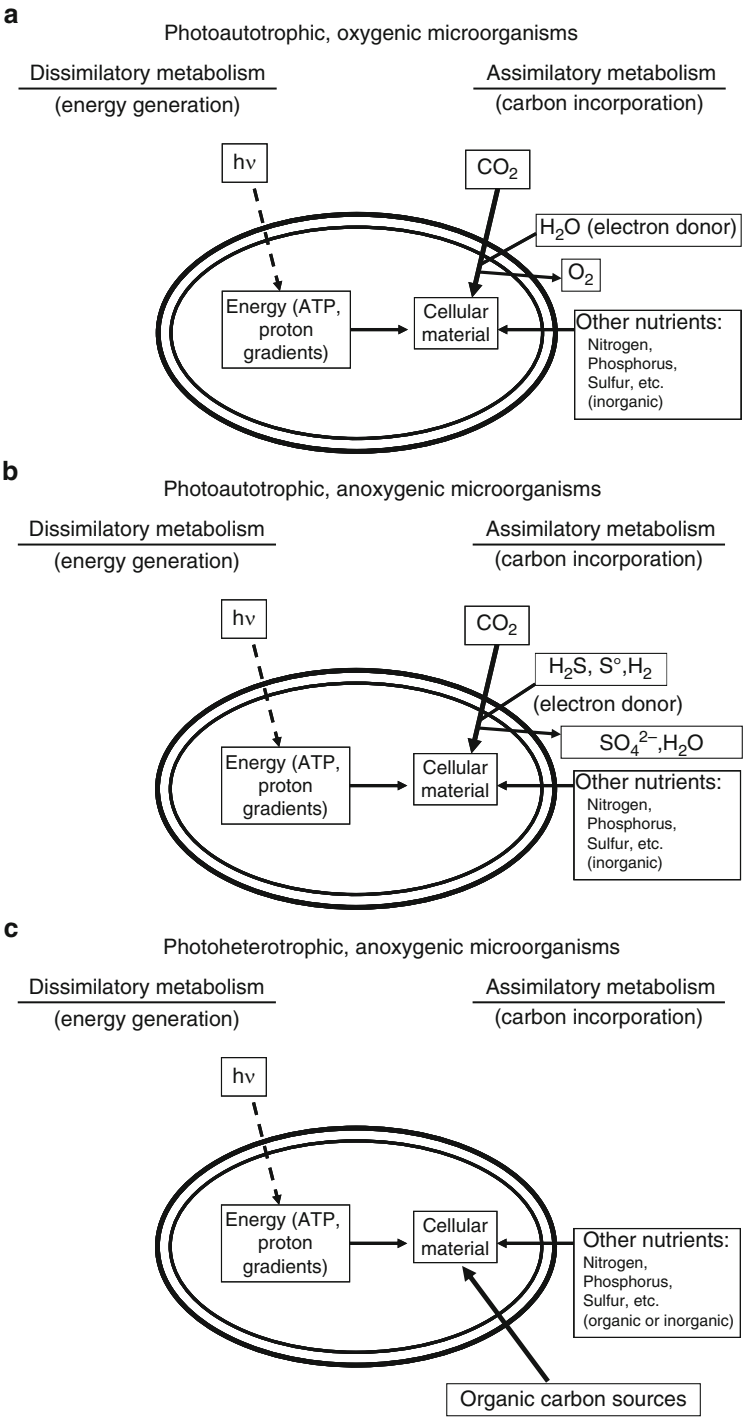

Fig. 5.4. The principles of oxygenic, anoxygenic photoautotrophic, and anoxygenic photoheterotrophic life.

been accumulated in the course of the photoautotrophic metabolism during daytime. Fungi use aerobic respiration as their major type of metabolism. However, the ability to proliferate anaerobically by fermentation is also widespread in the fungi, and especially in the yeasts. Also, many bacteria obtain their energy from the aerobic oxidation of an often tremendously wide variety of organic substrates. Among the prokaryotes we find many obligate aerobes, while others use aerobic respiration only when oxygen is available. In the absence of oxygen they shift to alternative modes of energy generation, such as anaerobic respiration (see below), fermentation, and even photoheterotrophic growth, in some cases.

Aerobic breakdown of complex organic compounds generally proceeds through the reactions of the central cellular metabolic pathways such as the glycolytic Embden–Meyerhof pathway and the tricarboxylic acid cycle (Krebs cycle) (Fig. 5.2). Large polymeric biodegradable compounds (polysaccharides, proteins, lipids) are first split outside the cell into monomers by extracellular depolymerizing enzymes. The resulting small molecules are taken up by the cells and converted in one or more enzymatic steps to intermediates of these nearly universal central metabolic pathways, enabling complete oxidation to CO_2. It should be stated here that not all aerobic chemoorganotrophic microorganisms use the Embden–Meyerhof pathway for sugar degradation. Alternative pathways exist, such as the Entner–Doudoroff pathway in which 6-phosphogluconate and 2-keto-3-deoxy-6-phosphogluconate are key intermediates, or the oxidative pentosephosphate cycle, in which sugar conversions by the enzymes transaldolase and transketolase play a great role.

In some organisms, aerobic respiration does not lead to complete oxidation of the organic substrates to CO_2 but rather to other, more reduced products. Well-known examples are the acetic acid bacteria *Acetobacter* and *Gluconobacter*, which oxidize ethanol while using oxygen as electron acceptor. The acetic acid formed is excreted into the medium. *Acetobacter* can oxidize the acetic acid to CO_2 under suitable conditions, while *Gluconobacter* cannot further metabolize the acetic acid formed.

The electrons released during the oxidation of organic material to CO_2 are transferred to molecular oxygen through a chain of cytochromes and other electron carriers located in the cytoplasmic membrane in prokaryotes, leading to the formation of proton electrochemical gradients that can be converted to ATP. In eukaryotes, this respiratory electron transport is localized in the inner membranes of the mitochondria, specialized intracellular organelles that are used for energy generation.

When oxygen is not available as electron acceptor, respiration may still be possible when other potential electron acceptors are present in the medium. Such processes of "anaerobic respiration" are known to occur in the prokaryotic world only. Depending on the organism, electron acceptors that can be used for the purpose are oxidized nitrogen compounds (nitrate, nitrite), sulfur compounds such as sulfate and elemental sulfur, trivalent iron, tetravalent manganese, and others. Such alternative electron acceptors are generally more reduced than molecular oxygen, and the amount of energy gained by respiratory electron transport from NADH generated during oxidation of the organic electron donor to the acceptor molecule is therefore less than what can be obtained during aerobic respiration (see also Fig. 5.2 and Eq. (6)). Fig. 5.5b explains the principles of anaerobic respiration, principles that are explained in further depth in Sects. 5.2, 5.4, and 5.5. Also in organisms performing anaerobic respiration,

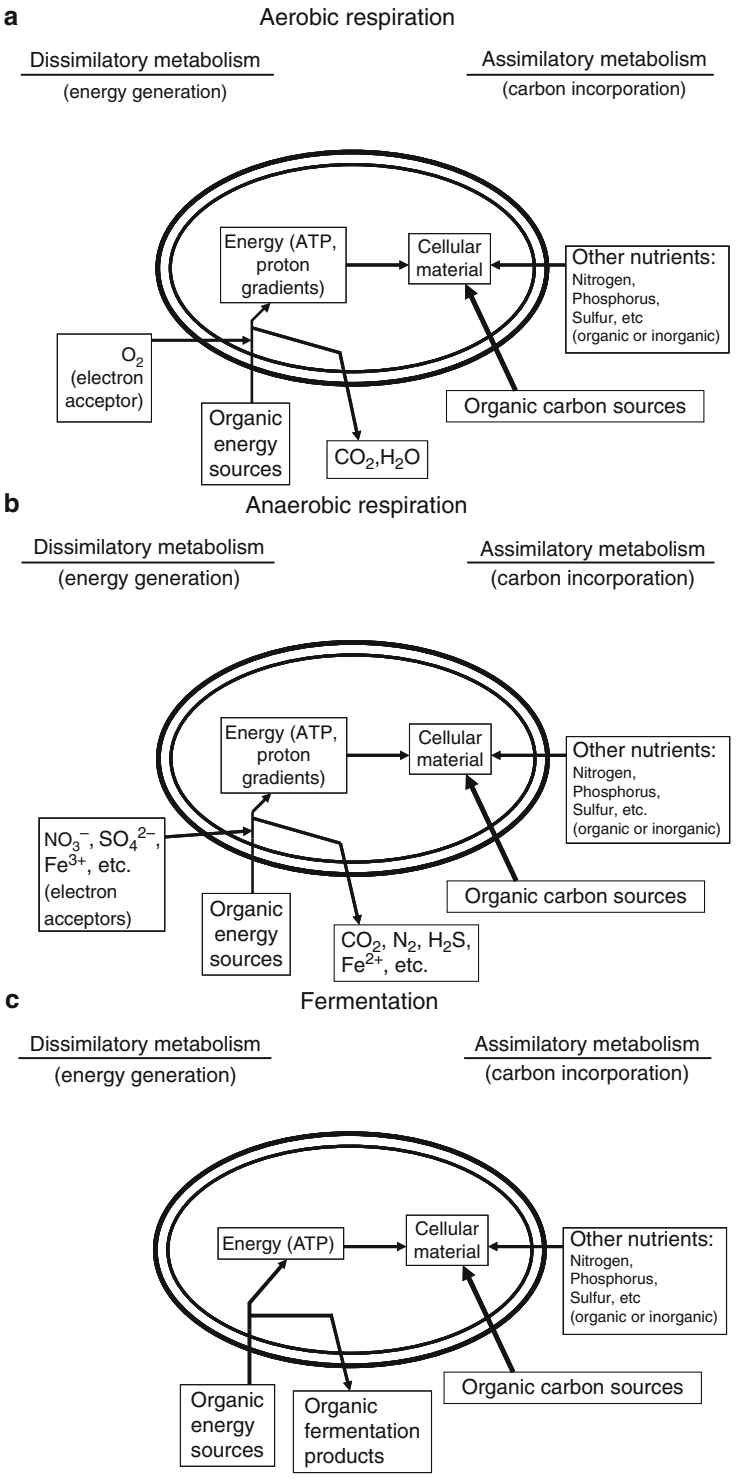

Fig. 5.5. The principles of respiratory and fermentative life.

incomplete oxidation processes may occur, such as shown by the case of the sulfate-reducing bacteria of the genus *Desulfovibrio*, which oxidize lactic acid to acetic acid $+CO_2$ while using sulfate as the electron acceptor.

When no potential electron acceptors are available, energy may still be gained by fermentation. In fermentative processes, organic substrates are degraded to other, generally smaller, organic products. Energy generation proceeds via substrate-level phosphorylation only, and is based on ATP formation from those high-energy intermediates of the metabolic pathways presented in Table 5.2. No respiratory electron transport from NADH to an acceptor occurs in the cell membrane during fermentation that would enable the generation of a transmembrane proton gradient. A schematic representation of the principle of fermentative life is given in Fig. 5.5c (12).

Fermentation is seldom found in the eukaryotes. Higher animals, including man, have a limited potential of energy generation by fermentation; muscles that do not receive a sufficient supply of oxygen, e.g., as a result of excessively high body activity, can still form two molecules of ATP per molecule of glucose taken up from the blood by fermenting it to two molecules of lactic acid ("homolactic fermentation"), in a reaction that is similar to that performed by lactic acid bacteria (*Lactobacillus*, *Streptococcus*) that produce lactic acid from sugars during the fabrication of cheese, yogurt, sauerkraut, etc., and during the formation of silage (see Sect. 5.3). One well-known group of fermentative eukaryotes is that of the yeasts (which generally can live by aerobic respiration as well when oxygen is supplied). Yeasts involved in the production of bread, wine, beer, and similar products ferment sugars to ethanol and CO_2, with the formation of two molecules of ATP per hexose molecule. Both the homolactic fermentation and the alcohol fermentation proceed via the reactions of the glycolytic pathway (see also Fig. 5.2). The intermediate pyruvate is in the first case reduced by NADH to lactic acid, and in the second it is decarboxylated to acetaldehyde which is then reduced to ethanol.

The third mode of energy generation in nature is chemoautotrophy or chemolithotrophy (13, 14). Chemoautotrophs are organisms that use inorganic compounds as energy sources for the generation of ATP as well as electron donors for assimilatory metabolism – the autotrophic fixation of CO_2. Chemoautotrophs, thus, depend on inorganic compounds only for growth and are independent of light energy. No chemoautotrophs are known among the eukaryotes, but a wide diversity of chemoautotrophic types is found in both the *Bacteria* and the *Archaea*. A variety of electron donors are used by different chemoautotrophs. Thus, aerobic chemoautotrophs can be found that use ammonium or nitrite as energy sources, producing nitrite and nitrate, respectively (the nitrifying bacteria, see also Sect. 6.1), and there are colorless sulfur bacteria that use sulfide, elemental sulfur, and other reduced sulfur compounds as energy source and electron donor, producing sulfate in the process (see Sect. 6.2). Other types make a living by oxidizing molecular hydrogen to water (Sect. 6.4), divalent iron to trivalent iron, or divalent manganese to tetravalent manganese (Sect. 6.3). The amount of energy to be gained by the transfer of electrons from the respective electron donors to molecular oxygen, the electron acceptor in all these cases, depends on the difference in standard reduction potential of the electron donating reactions involved and the reduction potential of the O_2/H_2O couple (+0.82 V), in accordance with Eq. (6) (see also Fig. 5.3). As explained above for the nitrifying

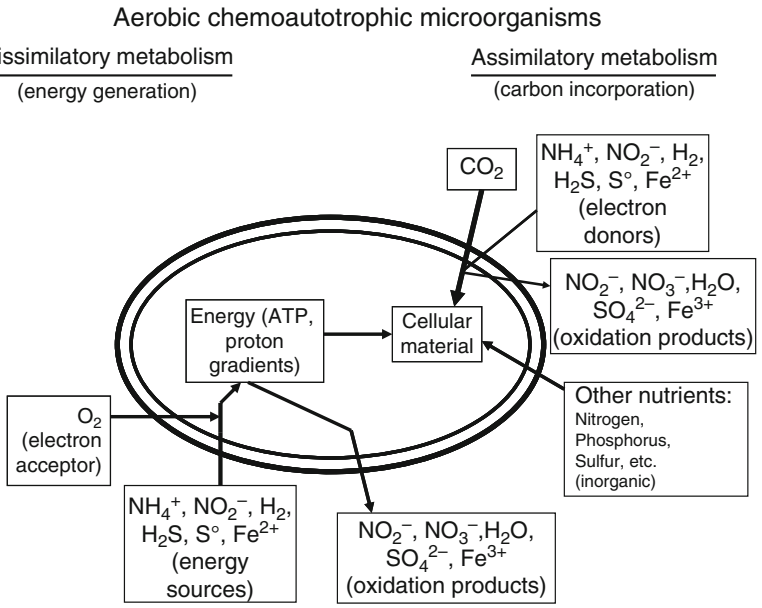

Fig. 5.6. The principles of aerobic chemoautotrophic (chemolithotrophic) life.

bacteria, chemoautotrophic prokaryotes that use relatively oxidized electron donors will have to invest considerable amounts of energy to produce reducing equivalents (as NADPH) for the autotrophic fixation of CO_2, and large amounts of substrate have therefore to be oxidized for the production of only a small amount of new cell material. Figure 5.6 provides an overview of the principles behind the metabolism of aerobic chemoautotrophs.

Chemoautotrophic life is possible under anaerobic conditions as well. As long as the electron transport occurs "downhill" from an electron donor with a redox potential lower than that of the electron acceptor (see Fig. 5.3), energy can be gained. We thus know bacteria that couple the oxidation of sulfide or elemental sulfur to sulfate with the reduction of nitrate (e.g., *Thiobacillus denitrificans*, an organism that can also grow aerobically using oxygen as electron acceptor). Many thermophilic and hyperthermophilic *Archaea* grow autotrophically by coupling the oxidation of molecular hydrogen with the reduction of elemental sulfur. Oxidation of hydrogen can also be coupled with the reduction of sulfate to sulfide in some sulfate-reducing bacteria.

Hydrogen can be used as electron donor for the dissimilatory reduction of CO_2 under anaerobic conditions in two types of metabolism. One yields methane as the product, the other acetic acid. All known methanogens are *Archaea*, and most are able to obtain their energy from the oxidation of hydrogen with CO_2 as electron acceptor. Such methanogens also use CO_2 as assimilatory carbon source, and they can therefore be termed chemoautotrophs. A schematic representation of the reactions performed by the hydrogen-oxidizing methanogens can be found in Fig. 5.7a. It should be noted that the details of the energy-yielding reactions involved differ greatly from those of the other chemoautotrophs. In the aerobic nitrifying and sulfur-oxidizing prokaryotes, energy is gained by the generation of a transmembrane proton

gradient during transport of electrons through a respiratory chain consisting of cytochromes, quinones, and other components, from the inorganic electron donor to molecular oxygen (or to nitrate in special cases, as stated above). The proton gradient is then used for the production of ATP. Also in the case of the methanogens, a proton gradient is generated, but here the formation of the proton gradient (and in some cases a sodium ion gradient as well) is directly coupled to some of the reactions that occur during the stepwise reduction of CO_2 to CH_4. Such hydrogen-oxidizing methanogenic *Archaea* even do not possess cytochromes. It should be stated here that not all methanogens are autotrophs. Some types, for example *Methanosarcina* and *Methanosaeta*, use acetate both as energy source and as their main carbon source (Fig. 5.7b), and accordingly they should be classified as heterotrophs. Such methanogens do possess cytochromes in their membranes, and their mode of energy generation thus differs quite significantly from that of the autotrophic methanogens.

A second type of metabolism that exploits the reduction of CO_2 with hydrogen as electron donor to generate energy exists in homoacetogenic bacteria. These organisms produce no methane but acetic acid, formed from two molecules of CO_2. More in-depth information about the function of methanogenic and the homoacetogenic prokaryotes in nature is given in Sect. 5.6.

A highly intriguing novel mode of autotrophic growth under anaerobic conditions was discovered a few years ago, when it was shown that ammonium ions can be oxidized with nitrite serving as the electron acceptor with the formation of molecular nitrogen as the product (15). In fact, the possible existence of such a process had already been predicted by Engelbert Broda in 1977 on purely thermodynamic grounds (16), as it was calculated that such a reaction is exergonic:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad \Delta G^{o\prime} = -357.8\,\text{kJ} \tag{9}$$

The process, now called the "anammox reaction" (*an*aerobic *amm*onium *ox*idation), was first found to occur in a laboratory-scale bioreactor for wastewater treatment, but it is now becoming clear that it commonly occurs in nature in anaerobic environments in which both nitrite (or nitrate) and ammonium ions are available. The organisms responsible for the anaerobic oxidation of ammonium appear to be representatives of the *Planctomyces* group (*Bacteria*). They use CO_2 as carbon source, which is reduced using nitrite as electron donor with the production of nitrate. The discovery of the anammox process proves once more that the prokaryotic world can use (nearly) any reaction that is thermodynamically feasible for energy generation, and that the appropriate enzymatic mechanisms can be developed to exploit such reactions. In the case of the anammox bacteria, some of these reactions are very unusual indeed; intermediates in the process of ammonium oxidation are exotic compounds as nitric oxide (NO) and even hydrazine (N_2H_2), a compound better known as a rocket propulsion fuel than as an intermediate in biochemical pathways. It is yet to be seen whether two other autotrophic processes calculated as thermodynamically feasible by Broda are also realized in the microbial world. One is the anaerobic oxidation of ammonium ions with nitrate as electron acceptor:

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \quad \Delta G^{o\prime} = -1483.5\,\text{kJ} \tag{10}$$

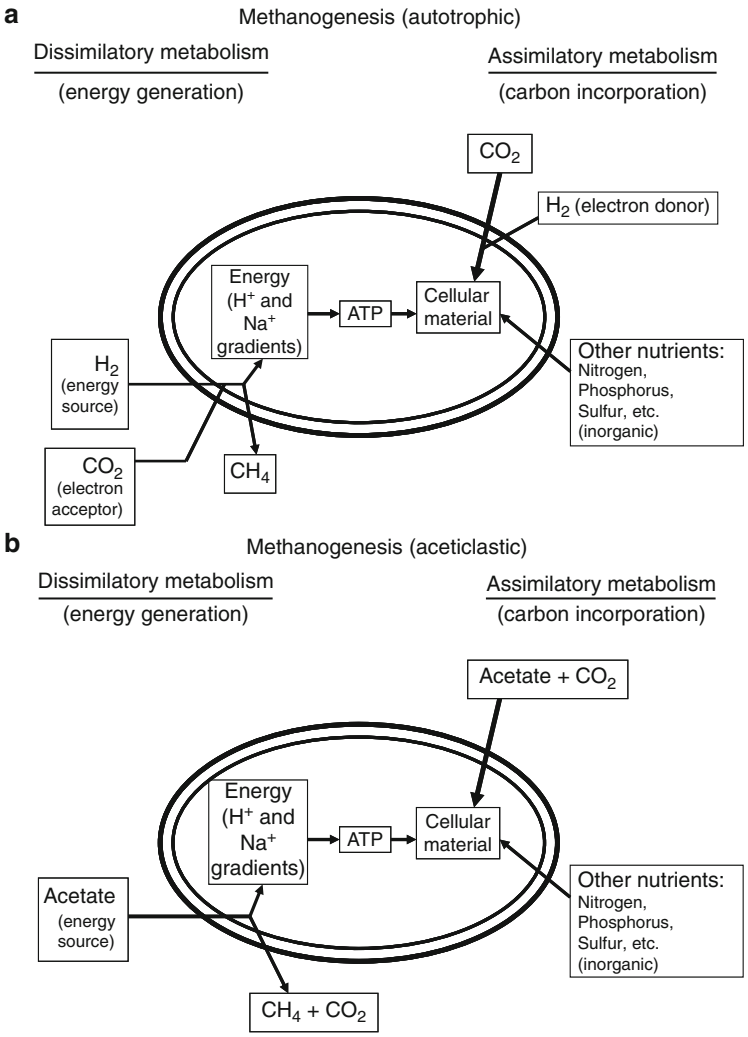

Fig. 5.7. The principles of the metabolism of methanogenic bacteria.

As far as known, this reaction is only realized in two steps, with "conventional" nitrate-reducing bacteria reducing the nitrate to nitrite, and the anammox organisms then performing the remaining part of the reaction as given in Eq. (9). The second process envisaged by Broda (16) is the use of ammonium ions as electron donor in anoxygenic photosynthesis and photoautotrophic growth, analogous to the use of reduced sulfur compounds by the purple and green sulfur bacteria (see also Sect. 4.2). No organisms have been discovered thus far that perform such a type of metabolism.

3. ASSIMILATORY METABOLISM OF MICROORGANISMS

Each cell that grows and multiplies needs a sufficient supply of building blocks for the synthesis of all components found in the cell. Molecules such as amino acids, sugars, and nucleotides must be available, and these have to be polymerized to form biological macromolecules at the expense of energy. In addition to carbon, many other elements are needed to fulfill the nutritional demands of the cell. They include nitrogen, sulfur, phosphorus, and a large number of other elements that are required in small amounts, such as iron, manganese, nickel, cobalt, and other metals that are needed in trace concentrations.

The prokaryotes display not only a large diversity in their dissimilatory metabolism, exploiting many different ways of generating energy, but they are also extremely resourceful in finding different modes of obtaining and exploiting different sources of nutrients to supply their assimilatory demands and to synthesize all those compounds needed for the proper functioning of the cell. Like in the dissimilatory metabolism, the prokaryotes are considerably more diverse than the eukaryotic micro- and macroorganisms when it comes to the variety of building blocks that can be used and the ways these are incorporated by the cells.

This section provides a brief overview of the ways prokaryotic microorganisms fulfill their assimilatory demands for the different elements of which the living cell is composed.

3.1. Carbon Assimilation

As explained in Sect. 2.1, we can divide the microorganisms into two groups with respect to the nature of their assimilatory carbon source; autotrophs that use CO_2 and heterotrophs that depend on organic carbon for growth.

Among the heterotrophic microorganisms, there is a wide diversity in the demands for organic carbon compounds. Some bacteria have very limited biosynthetic abilities and can only grow when supplied with a complex mixture of amino acids, nucleotides, and vitamins. The lactic acid bacteria (*Lactobacillus* and relatives) are well-known such highly fastidious organisms. For many other heterotrophs, a single compound such as glucose is sufficient to provide the cellular carbon. An organism such as *Escherichia coli* can grow aerobically on a simple medium that contains glucose as carbon source with inorganic nitrogen being supplied as ammonium or nitrate ions, and other essential elements, such as sulfur and phosphorus, being present as inorganic salts. Certain bacteria can use a tremendous variety of different carbon compounds for growth. Some representatives of the genus *Pseudomonas* can use no less than 70–80 different organic compounds to provide both the cellular carbon and the energy for biosynthesis. The list of such single carbon sources for growth includes carbohydrates, fatty acids, dicarboxylic acids, amino acids, alcohols, and more exotic substrates such as aromatic compounds (benzoate, phenol), including many that are toxic and that are degraded by a few microorganisms only. Between the extreme cases of the lactic acid bacteria with their extremely limited biosynthetic potential and *Pseudomonas* species that can grow on nearly any bioavailable carbon source are many intermediate cases of microorganisms that require the presence of a number of different organic compounds (e.g., amino acids, vitamins) for growth as their biosynthetic machinery is unable to synthesize these from simpler components.

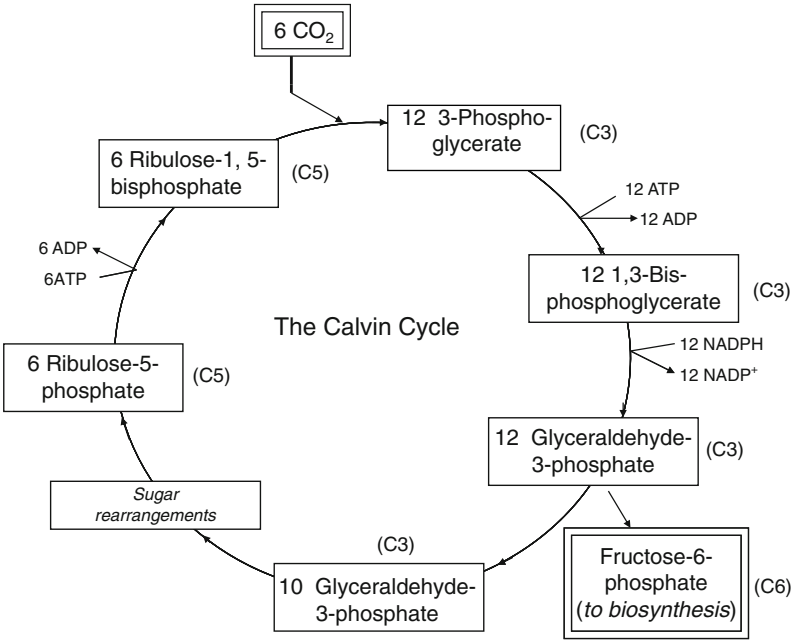

Fig. 5.8. Autotrophic CO_2 fixation by the Calvin cycle.

As shown in the earlier sections, the autotrophic way of life is widespread in the prokaryotic world. We know oxygenic phototrophs (the cyanobacteria) (see Sect. 4.1), anoxygenic phototrophic bacteria (see also Sect. 4.2), aerobic chemoautotrophs such as the nitrifying and the colorless sulfur bacteria (see Sects. 6.1 and 6.2), and anaerobic chemoautotrophs such as many methanogenic and homoacetogenic bacteria (see Sects. 5.6 and 6.4). These groups differ in the nature of their energy sources and the electron donors used for the reduction of CO_2.

Considerable diversity exists in the biochemical pathways enabling autotrophic CO_2 fixation. Green plants use the Calvin cycle, in which ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the key enzyme responsible for the conversion of inorganic to organic carbon (Fig. 5.8). The same pathway is used to drive autotrophic growth in eukaryotic algae, in cyanobacteria, in purple sulfur bacteria, and in aerobic chemoautotrophic bacteria such as the nitrifiers (*Nitrosomonas*, *Nitrobacter* and relatives) and the colorless sulfur bacteria (*Thiobacillus*, *Beggiatoa*, and related organisms). However, the Calvin cycle is by no means the only way carbon dioxide can be fixed by autotrophic organisms. For example, the green sulfur bacteria (*Chlorobium*, *Prosthecochloris*) fix CO_2 by reversing the reactions of the tricarboxylic acid cycle Krebs cycle, see also the lower part of Fig. 5.2). This, combined with the reductive carboxylation of acetyl-CoA to pyruvate, enables the biosynthesis of sugars and other cellular components. Another strategy for autotrophic carbon fixation, found both in certain representatives of the *Bacteria*, e.g., a number of autotrophic sulfate-reducing bacteria and homoacetogens, as well as in many of the methanogens, starts with the reduction of carbon

dioxide to carbon monoxide, which is then coupled with methyl groups (bound to coenzymes such as coenzyme B_{12}) to form acetyl-CoA.

A limited number of prokaryotes can grow on methane as sole carbon and energy source. Strictly spoken such methanotrophs cannot be considered autotrophs their substrate, CH_4, is an organic compound. However, the use of a one-carbon compound, such as methane as their carbon source, presents the cells with similar problems as autotrophic growth on CO_2. Two different pathways lead to the goal. One (the "ribulose monophosphate cycle") uses formaldehyde, formed by partial oxidation of the methane, as precursor of cellular carbon, with ribulose 5′-monophosphate serving as the acceptor of the one-carbon units. The second (the "serine pathway") uses two reactions in which one-carbon compounds enter the metabolism, one being the formation of serine from glycine and formaldehyde (bound to the coenzyme tetrahydrofolate), the second binding CO_2 to phosphoenolpyruvate to form oxalacetate. A full discussion of the details of these unique pathways is outside the scope of the present review; more details can be found in textbooks (1, 4, 5) and in specialized review articles.

3.2. Nitrogen Assimilation

After carbon, nitrogen is the most important nutrient to be incorporated by any cell to produce proteins, nucleic acids, and other compounds. Many heterotrophic microorganisms (and all heterotrophic macroorganisms as well) are unable to obtain their cell nitrogen from inorganic sources, and they have to take up organic forms of nitrogen such as amino acids that had been produced by the autotrophs in their ecosystem. Extracellular proteases are often excreted by such heterotrophs to degrade proteins to amino acids that can be taken up.

Inorganic nitrogen sources that can be used by many groups of prokaryotes, both autotrophic and heterotrophic, include nitrate (NO_3^-), ammonium ions (NH_4^+), and molecular nitrogen (N_2). Nitrate and ammonium are also suitable nitrogen sources for higher plants and eukaryotic algae. However, the ability of using dinitrogen as assimilatory nitrogen source is restricted to the prokaryotic world. The nitrogen in proteins and other cellular components mainly exists in the form of $–NH_2$ groups, i.e., in the same oxidation state as ammonium (N^{3-}). The incorporation of ammonium ions into amino acids is therefore relatively simple, and ammonium is generally available both under aerobic and under anaerobic conditions. One way of ammonium incorporation is by reductive amination of α-ketoglutarate, an intermediate of the tricarboxylic acid cycle, (see Fig. 5.2) to form the amino acid L-glutamate. This reaction is mediated by the enzyme glutamate dehydrogenase. The disadvantage of this pathway is the low affinity of the enzyme for ammonium ions, making the reaction little effective when ammonium concentrations in the medium are low, as they often are. A quantitatively much more important pathway starts with the amidation of glutamate to form glutamine by glutamine synthase, a reaction that proceeds under expenditure of ATP by an enzyme that has a high affinity for ammonium ions. The amino group is subsequently transferred to α-ketoglutarate (α-oxoglutarate) to form glutamate in a reaction mediated by an enzyme known as GOGAT (glutamine–oxoglutarate aminotransferase). The overall result of both pathways is that glutamate is formed from α-ketoglutarate and ammonium, in the second case at the cost of ATP, allowing nitrogen incorporation also at low ammonium concentrations. From

glutamate, the newly formed amino group can be transferred to other carbon compounds to form all other amino acids, purine and pyrimidine bases of DNA and RNA, and other organic nitrogen compounds present in the cell.

Nitrate is another widely used source of nitrogen for assimilation. It is generally available in aerobic environments only, as anaerobically it is preferentially used as an electron acceptor for anaerobic respiration (denitrification, see Sect. 5.2). To serve as assimilatory nitrogen source, nitrate first has to be reduced to ammonium, whereafter it is incorporated into amino acids by the reactions outlined in the previous paragraph. When both ammonium and nitrate-nitrogen are available in the environment, most microorganisms that can use both preferentially take up the ammonium and repress the assimilatory reduction of nitrate at the expense of reducing power and energy.

The ability to use molecular nitrogen (dinitrogen; N_2), the most abundant form of nitrogen in the biosphere, as a source of cellular nitrogen is limited to the prokaryote world, and only a limited number of prokaryotes possess nitrogenase, the enzyme complex needed to fix nitrogen. Most nitrogen-fixing microorganisms are found in the domain *Bacteria*. Many photosynthetic prokaryotes have nitrogenase, both oxygenic species (many, but not all cyanobacteria) and anoxygenic phototrophs. Among the free-living chemoheterotrophs that can use dinitrogen as N-source are aerobes (*Azotobacter*, *Beijerinckia*, and others) and anaerobes (some members of the genus *Clostridium*, many sulfate-reducing bacteria). The finding of nitrogenase activity in some methanogens proves that nitrogen fixation is also possible in representatives of the *Archaea*. There are many known cases of symbiotic associations between nitrogen-fixing prokaryotes and higher organisms. The best-known such association is that of the aerobic bacterium *Rhizobium* that lives in the root nodules of leguminous plants and supplies the plant with nitrogen in exchange for organic nutrients. Other examples are the association of *Azospirillum* with the roots of many plants, and the symbiosis of filamentous cyanobacteria related with the genus *Anabaena* within the leaves of the water fern *Azolla*.

The nitrogenase complex reduces dinitrogen to ammonium ions, which are then available for assimilation into amino acids as outlined above. From the point of view of bioenergetics, nitrogen fixation is a very expensive process. The triple bond between the two atoms in the nitrogen molecule is very stable, and the activation energy needed to break this bond is accordingly high. It has been estimated that between 6 and 15 molecules of ATP are required to provide the energy for the fixation of one molecule of nitrogen. It can therefore be expected that when other forms of nitrogen are available (ammonium, nitrate), nitrogen-fixing microorganisms shut down the synthesis and the activity of nitrogenase, and use energetically more favorable nitrogen sources instead.

Nitrogenase is a complex enzyme that contains both iron and molybdenum. A common property of all bacterial nitrogenases, both from aerobic and from anaerobic microorganisms, is their high sensitivity to molecular oxygen. This is not a problem for the many anaerobic prokaryotes that fix nitrogen, but microorganisms that lead an aerobic life and at the same time need to fix nitrogen will need special arrangements to protect the nitrogen-fixing machinery against damage by oxygen. Many (but not all) filamentous nitrogen-fixing cyanobacteria produce differentiated cells called heterocysts. These cells lack activity of photosystem II, so that no photosynthetic oxygen evolution takes place intracellularly, but they do contain

photosystem I, enabling the production of ATP at the expense of light. Moreover, the cell wall of the heterocyst is particularly thick, restricting entry of oxygen from outside. Some other nitrogen-fixing cyanobacteria protect their nitrogenase by separating the processes of photosynthesis and nitrogen assimilation in time, evolving oxygen during daytime and fixing nitrogen during the night driven by energy released during the oxidation of storage polymers (starch and others) that had been accumulated during the light periods. *Azotobacter*, a free-living heterotrophic bacterium common in the soil, and responsible for a significant flux of nitrogen from the atmosphere into the soil, has among other adaptations an extremely high rate of respiration, efficiently removing oxygen before it can damage the sensitive nitrogenase complex. Finally, complex mechanisms exist in the symbiotic association of *Rhizobium* in the root nodules of leguminous plants to regulate the oxygen in the surroundings of these aerobic bacteria to levels optimal for both survival and nitrogen fixation, mechanisms to which both the plant and the bacterium contribute. A hemoglobin-like protein is present in the root nodules, which binds oxygen and enables its controlled release when needed.

An interesting storage polymer named cyanophycin can be accumulated by many cyanobacteria. Cyanophycin is a polymer in which two amino acids, arginine (which contains four nitrogen atoms) and aspartate (containing one nitrogen atom) alternate. Cyanophycin is formed when suitable nitrogen sources are abundantly available, and it can be degraded later when nitrogen is in short supply. Another possible function of cyanophycin is energy storage; conversion of arginine to the amino acid ornithine releases carbamyl phosphate, a high-energy compound (see Table 5.2) that can be split to ammonium ions and CO_2 with the formation of ATP.

3.3. Phosphorus Assimilation

Phosphorus is almost exclusively found in nature in its most oxidized state (P^{5+}), either as inorganic phosphate ($PO_4{}^3-$) or as organically bound phosphate. Uptake of phosphate occurs in the inorganic form, and organic phosphorus compounds present in the environment can be used by microorganisms as assimilatory source of phosphorus only, following the release of the phosphate groups catalyzed by extracellular phosphatases.

Many microorganisms, including heterotrophic bacteria and cyanobacteria, can accumulate phosphorus intracellularly as storage granules ("volutin granules") in the form of inorganic polyphosphate when phosphorus is abundantly present in their medium. Such polyphosphates can later serve as source of phosphorus.

3.4. Sulfur Assimilation

Sulfur is found in the cell mainly in two amino acids: cysteine and methionine. The sulfur is here present in its reduced form. Reduced sulfur is present in the cell in other forms as well, in organic molecules such as coenzyme A, in iron–sulfur centers that make part of the respiratory electron transport chain, etc.

Many heterotrophic bacteria (as well as most heterotrophic higher organisms, including animals) can obtain sulfur only in the form of amino acids. Autotrophs (higher plants, photosynthetic and chemosynthetic bacteria, as well as many heterotrophic bacteria) use inorganic

sources of sulfur. The direct precursor of the organic sulfur in the sulfur-containing amino acids is H_2S. In anaerobic environments, sulfide is generally abundantly found, derived both from degradation of proteins and from dissimilatory reduction of sulfate during anaerobic respiration by sulfate-reducing bacteria (sees Sect. 5.5). Therefore, sulfide is generally directly available for incorporation into amino acids. In the aerobic world, sulfate (SO_4^{2-}) is the only abundant form of available sulfur. Assimilatory use of sulfate to be incorporated into amino acids requires its prior reduction to sulfide. Assimilatory reduction of sulfate is an energy requiring process, in which the sulfate is coupled to ATP with the formation of adenosine-5′-phosphosulfate (APS) at the expense of two high-energy bonds of ATP, following by the phosphorylation of APS to PAPS (adenosine-3′-phosphate-5′-phosphosulfate). PAPS is then reduced to form sulfite (SO_3^{2-}), which is subsequently further reduced to sulfide in a single enzymatic step, followed by its incorporation into sulfur-containing amino acids.

3.5. Iron Assimilation

Iron is needed by almost all cells as components of cytochromes, ferredoxin (a low potential mediator of redox reactions in photosynthesis, nitrogen fixation, and other cellular functions), and other enzymes. The lactic acid bacteria, a group of fastidious bacteria that lack respiration and have very limited biosynthetic potential, are probably the only organisms that do not require any iron for growth.

Iron can be taken up from the medium by different mechanisms. These often involve special carrier molecules (“siderophores”) that act as chelators and thus keep trivalent iron in solution, preventing its precipitation as iron oxides or other poorly soluble compounds.

A special case of an exceptionally high requirement for iron to be assimilated by the cells is that of the magnetotactic bacteria. Certain groups of bacteria, generally microaerophilic types that thrive best at low oxygen concentrations, synthesize small magnetic particles consisting of either magnetite (Fe_3O_4) or greigite (Fe_3S_4). These are accumulated in the cytoplasm, and are arranged in one or more well-ordered rows. These rows of “magnetosomes” enable the cells to orient themselves in the Earth magnetic field and to direct their swimming movement along the downward oriented magnetic field lines to reach optimal oxygen concentrations near the surface of the sediment.

4. THE PHOTOTROPHIC WAY OF LIFE

The previous pages have provided a general introduction, describing the principles of the dissimilatory and the assimilatory metabolism of different groups of microorganisms. We have outlined the tremendous metabolic diversity that is found especially in the prokaryote world (*Bacteria* and *Archaea*). In the sections below a more in-depth picture will be given of the different types of microorganisms found in nature, of the ways they exploit the resources present in their environment, and of the modes they act together to enable the functioning of the major biogeochemical cycles in nature: the cycles of carbon, nitrogen, and sulfur. First the phototrophic way of life will be discussed.

4.1. Oxygenic Photosynthesis

We owe the presence of molecular oxygen in the atmosphere to the process of oxygenic photosynthesis (photosynthesis that uses water as electron donor with the evolution of oxygen, see Sect. 2.2). This type of photosynthesis is found not only in eukaryotic microalgae (as well as in macroalgae and in higher plants), but also in the cyanobacteria, a branch of the *Bacteria*.

Nearly, all oxygenic phototrophs all use chlorophyll *a* as the major pigment in the photosynthetic reaction centers, generally accompanied by other forms of chlorophyll such as chlorophyll *b* in the green algae. In most representatives of the cyanobacteria, we find chlorophyll *a* as the only chlorophyll. The prochlorophytes, which phylogenetically are affiliated with the cyanobacteria, are exceptional among the prokaryotes as they contain chlorophyll *b* derivatives as well. An ecologically highly important member of this group is the small unicellular phototroph *Prochlorococcus*, one of the most abundant photosynthetic organisms in the ocean (17). Accessory pigments are generally present to aid in the harvesting of light. These include carotenoid pigments and the phycobiliproteins – the blue phycocyanin and the red phycoerythrin of the cyanobacteria, the latter also being found in the red algae. The range of wavelengths used by the oxygenic phototrophs is from 400 nm (blue light) to 700 nm (red light), spanning whole range of the visible light. We find oxygenic phototrophic microorganisms anywhere in nature where light and water are available and aerobic conditions prevail. The extent of development of oxygenic phototrophic planktonic organisms in water bodies generally depends mainly on the availability of essential inorganic nutrients, such as nitrogen (see Sect. 3.2), phosphorus (Sect. 3.3), and in certain cases such in the central parts of the Pacific Ocean, iron (see Sect. 3.5).

4.2. Anoxygenic Photosynthesis

The world of phototrophs is far much diverse than appears from the abundance of oxygenic phototrophs only. Use of light energy mediated by chlorophyll derivatives (bacteriochlorophylls) not coupled to the evolution of oxygen is possible in many ways, as shown by a variety of groups of prokaryotes, all belonging to the domain *Bacteria*, but phylogenetically affiliated with very different phyla within the *Bacteria* (11, 18) With very few exceptions these anoxygenic phototrophs develop under anaerobic conditions only. Due to the high standard reduction potential of the couple H_2O/O_2 (+0.82 V, see Fig. 5.3), the reduction of $NADP^+$ needed for autotrophic CO_2 fixation with electrons from water during oxygenic photosynthesis is an energetically expensive process. Two photons are required per electron transferred to $NADP^+$, and oxygenic photosynthesis therefore involves two photosynthetic reaction centers acting in series: photosystem II that oxidizes water and is responsible for oxygen evolution, transferring the electrons to photosystem I which enables the reduction of $NADP^+$. Alternative electron donors used by the different types of anoxygenic phototrophs discussed below are more reduced than water. Such organisms use a single photosystem to drive the photosynthetic processes.

Purple sulfur bacteria phylogenetically belong to the *Proteobacteria* branch of the *Bacteria*. They use sulfide and other reduced sulfur compounds as electron donor for autotrophic CO_2 fixation. They are found in environments in which both light and sulfide are present.

Such habitats include stratified lakes: when sufficient light penetrates through the upper, oxygenated water layer and reaches the anaerobic, sulfide-containing deeper water, dense blooms of purple sulfur bacteria such as *Chromatium*, *Thiocapsa*, and others often develop just below the oxic-anoxic boundary. We also find such organisms in a thin layer below the surface of many shallow marine sediments in which sulfide diffuses upward and meets light coming from above. Such a layer of purple bacteria is often found just below a blue-green layer of cyanobacteria (oxygenic phototrophs) that inhabit the aerobic surface layer of the sediment. A third type of environment in which dense development of purple sulfur bacteria are often observed is in sulfur springs in which geothermal sulfide that emerges from the spring comes in contact with light. The main photosynthetically active pigment in most purple sulfur bacteria is bacteriochlorophyll *a*, a pigment that absorbs light not only in the blue range of the spectrum but also in the infrared range (800–860 nm), Other members of the group have bacteriochlorophyll *b*, a pigment that in vivo shows an absorption peak in the far infrared at 1,020 nm. These are wavelengths not used by oxygenic phototrophs, as these do not absorb light above 700 nm. As a result, anoxygenic and oxygenic phototrophs can coexist in stratified systems such as marine sediments as each group selectively absorbs wavelengths not used by the others. In deep water bodies the ability to use infrared light to drive anoxygenic photosynthesis is of little practical value, as such long-wavelength radiation does not penetrate deeply into the water column, which preferentially transmits light of from around 480 nm (clear "blue" waters) to about 550 nm (turbid "green" waters). The sulfur used as electron donor is oxidized to sulfate. Elemental sulfur formed as intermediate is stored as intracellular sulfur granules in many species (*Chromatium*, *Thiocapsa*, and others), but other members of the group such as *Ectothiorhodospira* and relatives – purple sulfur bacteria that are often found in saline and in hypersaline environments, excrete the sulfur into the medium, to be taken up later to be further oxidized to sulfate.

The green sulfur bacteria (*Chlorobium*, *Prosthecochloris*, and related genera) resemble the purple sulfur bacteria in many aspects. They also oxidize sulfur to sulfate and produce elemental sulfur as intermediate, which is excreted from the cells. However, phylogenetically they are unrelated to the Proteobacteria, and they form a separate lineage within the *Bacteria*. Their absorption spectrum is different with an in vivo absorption maximum at 750–760 nm attributable to bacteriochlorophyll *c*, and the structure of the photosynthetic system differs from that of the purple bacteria in many features. Moreover, they do not use the Calvin cycle for autotrophic fixation of CO_2, but use an alternative pathway, based on reversal of the reactions of the tricarboxylic acid cycle (see Sect. 3.1). Like the purple sulfur bacteria, the green sulfur bacteria are often found to accumulate in stratified lakes at those depths just below the aerobic-anaerobic boundary where sulfide is available and sufficient light is present. Some species are very efficient at growing at extremely low light intensities, such as the case of the Black Sea shows: a species of *Chlorobium* was found developing there at depths between 80 and 100 m, at light intensities as low as 1/500,000 of full sunlight.

Not all anoxygenic photosynthetic bacteria are autotrophs. Photoheterotrophic growth is found in purple nonsulfur bacteria such as *Rhodobacter* and *Rhodospirillum* (Proteobacteria) and green nonsulfur bacteria such as *Chloroflexus* (which forms a separate lineage within the domain *Bacteria*). In such organisms, light provides the energy, while most of the carbon

needed for growth is derived from organic carbon sources (see Sect. 2.2). *Rhodobacter* and relatives are among the metabolically most versatile of all prokaryotes. They can live as photoheterotrophs under anaerobic conditions, as photoautotrophs using hydrogen as electron donor, or even (in spite of their designation as purple "nonsulfur" bacteria) on sulfide when present in very low concentrations. Photoautotrophic growth using divalent iron as electron donor, which is oxidized in the process to trivalent iron, has also been documented in some representatives of the group. Furthermore, they can live aerobically as chemoheterotrophs by aerobic respiration on a variety of organic compounds or as chemoautotrophs on a mixture of hydrogen, oxygen, and carbon dioxide. During aerobic growth, these cells do not synthesize bacteriochlorophyll and other components of the photosynthetic machinery, and the cells do not obtain the red color of light-grown cells. Dark anaerobic growth by fermentation is possible as well. In spite of this metabolic versatility, the purple nonsulfur bacteria are not among the most abundant organisms in nature, and they are never seen to accumulate in high density in any ecosystem. The green nonsulfur bacteria are often found in coastal microbial mats in shallow sediments, and also in microbial biofilms that cover the bottom of many thermal springs. Recently, the presence of photoheterotrophic representatives of the Gram-positive branch of the *Bacteria* (e.g., the genus *Heliobacterium*) has also been documented. Such organisms appear to be common in soil.

Generally spoken, it is true that oxygenic phototrophs (plants, algae, cyanobacteria) inhabit the aerobic world, and the anoxygenic phototrophs are restricted to anaerobic environments. However, exceptions exist. Certain species of cyanobacteria can shift from their oxygenic type of photosynthesis to an anoxygenic photoautotrophic metabolism, using sulfide as electron donor. In anoxic sulfide-containing habitats, such cyanobacteria repress the activity of the water-splitting photosystem II. Electrons from sulfide are donated to photosystem I, and the sulfide is oxidized to elemental sulfur in the process.

It has also become clear that anoxygenic, bacteriochlorophyll *a* containing photoheterotrophic bacteria exist that inhabit aerobic environments. Occurrence of such bacteria (genera *Erythrobacter*, *Roseobacter*, and others) in the oxic marine environment was first documented in the early 1990s. It was recently documented that such photoheterotrophic organisms are very abundant in the ocean, and there are even indications that they may be responsible for a significant part of the photosynthetic electron transport occurring in the marine environment (19, 20).

4.3. Retinal-Based Phototrophic Life

A completely different way of using light as energy source for photo(hetero)trophic growth, not depending on chlorophyll derivatives as the photoactive pigments, is by using retinal-containing proteins. The best known case of retinal-based photoheterotrophic growth is documented in *Halobacterium salinarum*, a representative of the extremely halophilic *Archaea*. *Halobacterium* is an aerobic heterotrophic archaeon that requires at least 150 g/l salt for growth, and grows at salt concentrations up to saturation. *Halobacterium* and related genera develop in hypersaline lakes, saltern evaporation and crystallizer ponds, and on heavily salted meat, fish, and hides. These extreme halophiles are colored red due to a high content of carotenoid pigments. Under the proper conditions (availability of light, low

oxygen concentrations), *Halobacterium* produces in addition to the red carotenoids a purple pigment named bacteriorhodopsin, which is located in patches in the cytoplasmic membrane ("purple membrane"). Bacteriorhodopsin is a 25 kDa protein that carries retinal as a prosthetic group. It absorbs light with an absorbance maximum at 570 nm. In many of its properties, it resembles rhodopsin, the visual pigment of the human eye. When bacteriorhodopsin is excited, protons are transported from the cytoplasmic side of the membrane to the outer medium, thus establishing a transmembrane proton gradient that can be used for the generation of ATP mediated by the membranal ATP synthase. Bacteriorhodopsin-containing cells can use light energy to drive photoheterotrophic growth in the absence of oxygen. Photoautotrophic growth has never yet been demonstrated in the halophilic *Archaea*. A second retinal pigment present in *Halobacterium* and in many other halophilic *Archaea* is halorhodopsin (absorbance maximum 580 nm), which uses light energy to pump chloride ions from the medium into the cells.

Until recently, it was assumed that photoheterotrophy based on retinal proteins is restricted to *Halobacterium* and a few other extremely halophilic *Archaea*. However, genes that encode bacteriorhodopsin-like proteins were first detected in DNA extracted from marine bacterioplankon in 2000 (21). The presence of a 16S rRNA gene in the same genome fragment showed that the DNA fragment carrying the bacteriorhodopsin-like gene belongs to a yet uncultured representative of the Proteobacteria. Cloning and expression of this gene in *Escherichia coli* led to the formation of a functional light-driven proton pump, now termed proteorhodopsin, when the prosthetic group retinal was supplied in the medium. Subsequently, it was shown that functional proteorhodopsin in present in large amounts in the membranes of marine bacterioplankton (22). Different varieties of proteorhodopsin are now known, with absorbance maxima from around 530 nm – abundant in ocean (surface water) to around 490 nm – in the deeper waters (75 m) in the oligotrophic ocean where light of 480 nm penetrates deepest. Genes for proteorhodopsin have now been found in different representatives of the α- and the γ-branch of the Proteobacteria, all yet uncultured. It is now estimated that light energy absorbed by proteorhodopsin contributes significantly to the energy household of the bacterial community in the sea.

5. CHEMOHETEROTROPHIC LIFE: DEGRADATION OF ORGANIC COMPOUNDS IN AEROBIC AND ANAEROBIC ENVIRONMENTS

Chemoheterotrophic bacteria are by far the most abundant among the known species of prokaryotes. They display a tremendous diversity in the range of organic compounds they degrade (see also Sect. 2.2) and in their way of life: aerobic, anaerobic, or facultative aerobic, degrading organic compounds by fermentation or by respiration using a range of electron acceptors. The following sections provide an overview of the ways organic compounds are degraded in nature. Complete aerobic degradation of commonly found organic compounds to carbon dioxide can generally be accomplished by a single type of microorganism. However, complete degradation under anaerobic conditions (to CO_2 when external electron acceptors are present, to a mixture of CO_2 and methane when no suitable electron acceptors are supplied), generally requires a collaborative effort involving a variety of metabolic types of

microorganisms that often maintain complex interactions that have to be closely coordinated to enable anaerobic mineralization of organic carbon in nature.

5.1. Aerobic Degradation

As long as oxygen is available as electron acceptor, nearly any naturally occurring organic compound can be degraded to CO_2. As explained in Sect. 2.2, the strategy of aerobic heterotrophic microorganisms – bacteria as well as fungi and other microorganisms – is to convert the available compounds to intermediates of the central metabolic pathways of the cell such as the glycolytic pathway and the tricarboxylic acid cycle (see Fig. 5.3). These intermediates can then be oxidized to CO_2 through the major metabolic pathways. The electrons released in the process (mostly in the form of NADH) are transferred to oxygen through the cytochromes and other components of the electron transport chain present in the cytoplasmic membrane (in the case of prokaryotes) or in the mitochondria (in the case of eukaryotic microorganisms), coupled with the formation of transmembrane proton gradients that are subsequently used to generate ATP.

As explained before (see Sect. 3.1), the number of different carbon compound that can be degraded by prokaryotes, aerobically as well as anaerobically, is extremely great. The list contains many compounds of importance in environmental engineering such as pesticides and other toxic or harmful chemicals. Biodegradation and bioremediation processes are based on the existence of degradation pathways for such compounds. An overview of 190 metabolic pathways identified to be involved in the breakdown of 1206 compounds (as of November 18, 2009), can be found in the University of Minnesota Biocatalysis/Biodegradation Database (http://umbbd.msi.umn.edu). An in-depth discussion of all these pathways is outside the scope of the present chapter.

The degradation of certain organic molecules, such as hydrocarbons and many aromatic compounds, also involves a direct participation of molecular oxygen. Such molecules can only enter the central metabolic pathways after oxygen molecules have been introduced derived from O_2 by enzymes such as dioxygenases (enzymes that introduce both oxygen atoms of O_2 into the organic compound to be degraded) and monooxygenases (enzymes that add one of the two atoms of O_2 to the organic carbon chain, and reduce the second oxygen atom to water with electrons derived from NADH). A well-known case of the activity of monooxygenases in the initial stage of microbial degradation of organic compounds is the aerobic breakdown of oil hydrocarbons. The first reaction toward the degradation of straight-chain aliphatic hydrocarbons is generally the introduction of an –OH group at one end of the carbon chain, mediated by a monooxygenase. The alcohol group is further oxidized to a carboxyl group, whereafter the long-chain fatty acid formed is degraded stepwise to release two-carbon units in the form of acetyl-CoA, and these are further degraded through the reactions of the tricarboxylic acid cycle. Aerobic oxidation of methane is also initiated by a monooxygenase reaction, in which methane is oxidized to methanol. Such a requirement for molecular oxygen in the initial steps of the aerobic breakdown of aliphatic and aromatic hydrocarbons and derivatives does not imply that such compounds cannot be broken down anaerobically as well. Alternative pathways often exist (not all of them understood in detail) that enable mineralization of such compounds also under anaerobic conditions (23, 24).

5.2. *Anaerobic Respiration: Denitrification*

It commonly occurs in nature that oxygen is not available as electron acceptor to allow aerobic degradation of biodegradable organic material. Examples are plentiful: deeper layers in marine and lake sediments, swamps, the hypolimnion of permanently stratified lakes and other water bodies such as the Black Sea, the digestive tract of animals, sewage treatment systems, etc. Even in well-aerated soils, anaerobic microenvironments are commonly found in which the local rate of oxygen consumption exceeds its supply by diffusion.

When oxygen becomes depleted, one of the first processes to occur is anaerobic respiration using nitrate as electron acceptor (25). The main product is generally gaseous nitrogen (N_2). When nitrate respiration leads to the loss of gaseous nitrogen from the system in the form of N_2 or other gases such as nitrous oxide (N_2O) and nitric oxide (NO), the process is known as denitrification. Dissimilatory reduction of nitrate proceeds in a number of steps in which the nitrate (NO_3^-, N^{5+}) is reduced through nitrite (NO_2^-, N^{3+}), nitric oxide (NO, N^{2+}) and nitrous oxide (N_2O, N^{1+}) to gaseous nitrogen (N_2, N^0). There are also cases known in which nitrate is reduced in a dissimilatory process to ammonium (NH_4^+, N^{3-}) ("nitrate ammonification"). From the point of view of bioenergetics, the amount of free energy released when organic material is oxidized with nitrate as electron acceptor is only little, less than what is obtained during aerobic oxidation with molecular oxygen as electron acceptor, for example:

$$\text{Glucose} + 4.8NO_3^- + 4.8H^+ \rightarrow 6CO_2 + 2.4N_2 + 8.4H_2O \quad \Delta G^{o\prime} = -2666\,\text{kJ} \qquad (11)$$

as compared to:

$$\text{Glucose} + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad \Delta G^{o\prime} = -2822\,\text{kJ} \qquad (12)$$

Such a high energy yield can also be predicted from the relatively high standard reduction potentials of the different redox couples involved (NO_3^-/NO_2^-; NO_2^-/NO; NO/N_2O; N_2O/N_2, see Fig. 5.3). However, the amount of ATP gained by the cells during denitrification is significantly lower than during aerobic oxidation of the same substrates due to mechanistic constraints of the cellular metabolism.

Many bacterial species, belonging to highly diverse phylogenetic groups (including the *Archaea*), can live anaerobically by denitrification. They are nearly all facultative aerobes that use aerobic respiration as long as molecular oxygen is present. Only when oxygen is depleted and nitrate is available do the cells induce the special enzymatic machinery necessary for dissimilatory nitrate reduction. The number of organic compounds that can be degraded while using nitrate as electron acceptor is also very large. However, many compounds that require prior activation by molecular oxygen through the action of monooxygenases and dioxygenases cannot be broken down by denitrification, unless alternative pathways exist for their degradation that bypass the need for molecular oxygen.

Dissimilatory reduction of nitrate is found widespread in nature, and the process is responsible for great losses of biologically available nitrogen with the formation of nitrogen gas. Sometimes the occurrence of denitrification is favorable from the point of view of the environmental engineer, for example when denitrifying bacteria decrease the amount of nitrate-nitrogen during wastewater treatment. In other cases, denitrification causes economic losses,

such as the loss of nitrogen fertilizer (nitrate or ammonium that had been oxidized to nitrate by nitrifying bacteria), from poorly aerated soils (see Sect. 6.1).

5.3. Fermentation

When neither molecular oxygen nor oxidized nitrogen compounds are available as electron acceptors, degradation of organic material cannot generally proceed through anaerobic respiration processes. Potential alternative electron acceptors may still be available; sulfate is present in most environments, and abundantly so in the marine ecosystem. Other potential electron acceptors that are generally present in small amounts only are oxidized forms of iron (Fe^{3+}) and manganese (Mn^{4+}). However, sulfate-reducing bacteria do not use sugars, amino acids, and most other common organic compounds available in nature as electron donors, and the range of compounds that can serve as their electron donors/energy sources is extremely restricted. The list includes short-chain aliphatic acids (acetate, propionate, butyrate, and longer-chain fatty acids of up to 16–18 carbon atoms), other acids such as lactate, and a few other compounds such as ethanol and molecular hydrogen. Also, those bacterial species known to grown anaerobically while reducing trivalent to divalent iron (*Geobacter*, for example) prefer energy sources such as acetate over more complex compounds. As a result, the most important process responsible for further degradation of organic material after the possibilities of aerobic respiration and denitrification had been exhausted is fermentation, a process that does not depend on the presence of external electron acceptors.

Energy generation in fermentative organism (see also Sect. 2.2 and Fig. 5.5c) is based on substrate-level phosphorylation performed by cytoplasmic enzymes. No electron transport through membrane-bound electron carriers such as cytochromes is involved. A common characteristic of nearly all fermentation processes is that in the course of the reactions leading to the formation of ATP (often but not always reactions of the glycolytic Embden–Meyerhof pathway, see the upper part of Fig. 5.2), oxidation reactions occur that generate NADH or other reduced coenzymes. To obtain a sustainable process, these reduced coenzymes need to be reoxidized. The great diversity in fermentative pathways and the accordingly great diversity in fermentation end products are the result of the many different ways in which the cell disposes of this reducing power with the generation of reduced fermentation products (1, 4, 5, 8).

Many microorganisms combine the ability to grow anaerobically as fermenters with other modes of energy formation, including aerobic respiratory metabolism. Well-known examples are the enteric bacteria such as *Escherichia* and *Salmonella*, and yeasts that perform alcoholic fermentation. *Escherichia coli* is commonly grown in the laboratory under aerobic conditions. Organic substrates are then completely oxidized to CO_2. However, the natural habitat of *E. coli* is the human intestine, an environment devoid of oxygen, and there the organism leads an anaerobic life, fermenting carbohydrates to a mixture of products that include ethanol, lactate, acetate, hydrogen, succinate, CO_2, and also formate ("mixed acid fermentation"). In addition, *E. coli* is able of anaerobic respiration using electron acceptors such as nitrate (which is reduced to nitrite), fumarate (which is reduced to succinate), and dimethylsulfoxide (which is reduced to dimethylsulfide). Many fermentative bacteria are sensitive to oxygen. Others are oxygen-tolerant, and can these not only survive but also grow in an aerobic atmosphere, even when they do not use oxygen as electron acceptor. The lactic acid bacteria

(*Lactobacillus*, *Streptococcus*) are well-known examples of such aerotolerant obligatory fermentative bacteria.

The list of products excreted by fermentative microorganisms is long indeed, as the following examples show:

- One-carbon compounds: CO_2, formate
- Two-carbon compounds: ethanol, acetic acid
- Three-carbon compounds: lactic acid, propionic acid, isopropanol, acetone
- Four-carbon compounds: butyric acid, *n*-butanol, 2,3-butanediol, succinic acid
- Molecular hydrogen

No single fermentative microorganism produces all the above products. Some types produce only a small number of products in a fixed stoichiometry. One example is the homolactic fermentation, in which sugars such as glucose are fermented by *Lactobacillus* (some species) and by *Streptococcus* and related organisms to lactic acid as the sole product:

$$\text{Glucose} \rightarrow 2\,\text{Lactate}^- + 2\text{H}^+ \quad \Delta G^{o\prime} = -198\,\text{kJ} \tag{13}$$

These bacteria play important roles in the production of cheese, yogurt, and other milk products, as well as sauerkraut and other fermented vegetables. A second example is the ethanol fermentation of yeasts:

$$\text{Glucose} \rightarrow 2\,\text{Ethanol} + 2\text{CO}_2 \quad \Delta G^{o\prime} = -218.4\,\text{kJ} \tag{14}$$

A third example is the heterolactic fermentation of *Leuconostoc* and certain species of *Lactobacillus*:

$$\text{Glucose} \rightarrow \text{Lactate}^- + \text{Ethanol} + \text{CO}_2 + \text{H}^+ \quad \Delta G^{o\prime} = -208.2\,\text{kJ} \tag{15}$$

In other cases, the stoichiometry of the products formed depends to a large extent on the environmental conditions, among others on the pH and on the concentrations of different fermentation products that have accumulated in the medium. This can be exemplified by the case of the butytic acid forming members of the genus *Clostridium* such as *Clostridium acetobutylicum*, *Butyrivibrio* in the rumen of the cow and *Eubacterium* in the human intestinal flora show a similar type of metabolism. Typical fermentation products excreted are acids such as acetate and butyrate, together with neutral products such as *n*-butanol, acetone, and isopropanol, and the gases hydrogen and CO_2. Fig. 5.9 presents a schematic overview of the reactions occurring during this type of fermentation. This fermentation scheme illustrates a number of principles important for the understanding of the nature of anaerobic degradation processes in nature, and therefore it deserves to be discussed here in some depth.

In the initial stages of the process (Fig. 5.9, part A), sugars are degraded through the glycolytic pathway to pyruvate with the net formation of 2 molecules of ATP and the release of 4 electrons in the form of NADH (compare the upper part of Fig. 5.2). The pyruvate is then further oxidized to acetyl-CoA and CO_2. In contrast to the situation during aerobic respiration (Fig. 5.2) where the electrons are released as NADH as well, here molecular hydrogen is excreted from the cell.

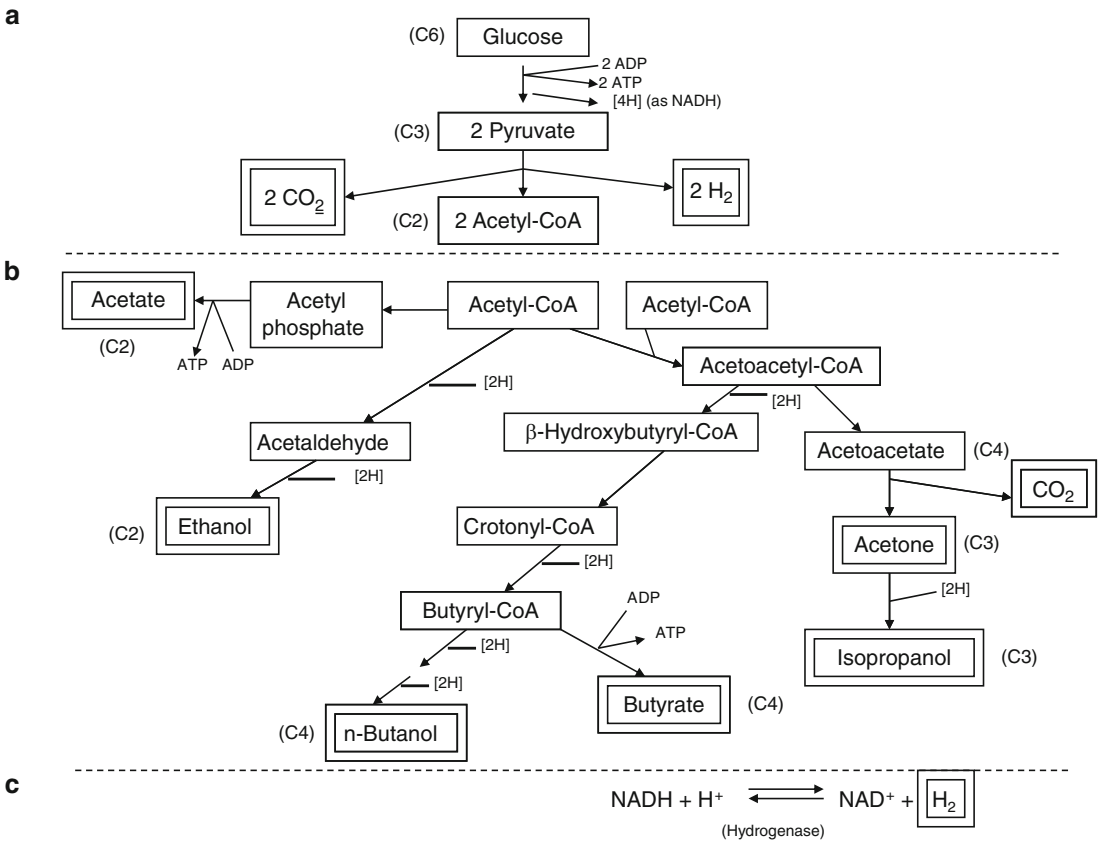

Fig. 5.9. The butyric acid fermentation of *Clostridium acetobutylicum.*

Starting from acetyl-CoA, there are several possibilities as shown in part B of Fig. 5.9. The different pathways presented are used by the cells according to the environmental conditions and the thermodynamic possibilities. Acetyl-CoA is a high-energy compound (see Table 5.2), and the energy of the bond between the acetate group and the coenzyme A moiety is sufficient to enable the production of ATP. The cells exploit this possibility by first exchanging the CoA for a phosphate group with the formation of acetyl phosphate, another high-energy compound (Table 5.2). The phosphate group is then transferred to ADP to form ATP, and acetate is released as the end product. The result is that for each acetate formed in this pathway, the cell gains an additional molecule of ATP. ATP is also formed when butyrate is produced from butyryl-CoA in a similar mechanism, but as each butyryl-CoA is derived from two molecules of acetyl-CoA, the amount of energy to be gained in this way is half of that could have been obtained when only acetate was formed. It is therefore in the interest of the cells to form as much acetate as possible. In poorly buffered environments, the acetic acid excreted causes acidification of the medium to pH values too low for growth. Under such conditions, the cells will shift their metabolism toward the formation of neutral fermentation products such as

n-butanol, ethanol, acetone and isopropanol. There is, however, a much more compelling reason why *Clostridium acetobutylicum* and other bacteria with a similar type of metabolism have to divert part of the acetyl-CoA toward the formation of reduced end products rather than producing only acetic acid; the NADH formed during the first steps of the fermentation has to be reoxidized. For that purpose, the different reaction chains leading to ethanol, isopropanol, and butanol/butyric acid all contain reduction reactions that reoxidize NADH. The aim of the cells is thus to find a balance between maximizing ATP production, i.e., excreting as much acetate as possible, while getting rid of excess electrons (in the form of NADH) by diverting part of the acetyl-CoA toward reactions that lead to the formation of more reduced end products.

Clostridium and many other fermentative anaerobes have an additional mechanism to get rid of excess electrons, and that is by producing molecular hydrogen. The enzyme hydrogenase catalyzes the following reaction (Fig. 5.9, part C):

$$\mathrm{NADH} + \mathrm{H^+} \rightleftarrows \quad \mathrm{NAD^+} + \mathrm{H_2} \tag{16}$$

If this option exists, then theoretically the following fermentation reaction should be possible:

$$\mathrm{Glucose} + 2\mathrm{H_2O} \rightarrow 2\,\mathrm{Acetate^-} + 4\mathrm{H_2} + 2\mathrm{CO_2} + 2\mathrm{H^+} \quad \Delta G^{o\prime} = -198.8\,\mathrm{kJ} \tag{17}$$

with the formation of no less than four molecules of ATP per glucose fermented – two during the degradation of glucose to two molecules of pyruvate, and one more for each acetate produced. However, as explained above (see Sect. 2.1), the true amount of free energy needed by the cell to produce a mol of ATP under, including allowance for the unavoidable loss of energy as heat, is around 70 kJ. Accordingly, Eq. (17) under standard conditions does not provide sufficient energy to drive the synthesis of 4 ATP per glucose. Another way to understand the problem is by considering the hydrogenase reaction (Eq. (16)). As the standard reduction potential of $\mathrm{H^+/H_2}$ is more negative than that of $\mathrm{NAD^+/NADH}$ (see Fig. 5.3), the reaction in the direction of hydrogen evolution is endergonic:

$$\mathrm{NADH} + \mathrm{H^+} \rightarrow \mathrm{NAD^+} + \mathrm{H_2} \quad \Delta G^{o\prime} = +18.1\,\mathrm{kJ} \tag{18}$$

Accumulating molecular hydrogen will tend to reverse the reaction, forcing the cell to find alternative ways of disposing of electrons, i.e., formation of reduced fermentation products at the expense of potential gain of ATP while excreting acetic acid. However, it must be realized that all these calculations were made under standard conditions, and that the true free energy yield or requirement of reactions depends not only on the $\Delta G^{o\prime}$ values that can be calculated from Table 5.1, but also on the concentrations of the reactants and the reaction products, all according to Eq. (4). The implications of this fact will be made clear later in this chapter (see Sect. 5.7).

Some of the compounds formed as end products of the fermentative processes described above can be fermented further by other anaerobes with the gain of additional energy. For example, lactate can be further fermented to propionate, acetate, and $\mathrm{CO_2}$, according to the equation:

$$3\,\mathrm{Lactate^-} \rightarrow 2\,\mathrm{Propionate^-} + \mathrm{Acetate^-} + \mathrm{CO_2} + \mathrm{H_2O} \quad \Delta G^{o\prime} = -161.4\,\mathrm{kJ} \tag{19}$$

Such a reaction is performed by *Propionibacterium* in the course of the manufacturing of Emmenthaler and similar cheeses; the acids provide the characteristic taste, and the CO_2 evolved causes the formation of the big holes in the cheese. A similar fermentation, albeit the biochemical pathway involved is different, is catalyzed by bacteria such as *Megasphaera elsdenii* in the bovine rumen. Propionate can also be formed by fermentation of succinate, a compound formed, e.g., during the mixed acid fermentation of *Escherichia coli* and other enteric bacteria:

$$\text{Succinate}^{2-} + \text{H}^+ \rightarrow \text{Propionate}^- + \text{CO}_2 \quad \Delta G^{o\prime} = -17.1\,\text{kJ} \tag{20}$$

This type of fermentation is of considerable theoretical interest, not only because of the very low gain in free energy associated with the reaction, but also from a mechanistic point of view. None of the compounds involved in the reactions contains any high-energy bonds that can give rise to the formation of ATP by substrate-level phosphorylation (compare Table 5.2). It is now known that the decarboxylation reaction of succinate to yield propionate is coupled with the extrusion of sodium ions from the cell. The sodium gradient thus established can be converted to ATP by a Na^+-driven membranal ATP synthase.

Sugars are not the only compounds that can be anaerobically degraded by fermentation. Amino acids can be fermented as well. Some representatives of the genus *Clostridium* grow anaerobically on glutamate in a type of reaction analogous to the butyrate fermentation of *Clostridium acetobutylicum* (Fig. 5.9); glutamate is converted in a complex series of reactions to acetate, pyruvate, and ammonium ions. The pyruvate is then further fermented to products including acetate, butyrate, CO_2, and H_2.

Another amino acid that can be fermented by many anaerobes is arginine:

$$\text{Arginine}^+ + 2\text{H}_2\text{O} + 2\text{H}^+ \rightarrow \text{Ornithine}^+ + \text{CO}_2 + 2\text{NH}_4^+ \quad \Delta G^{o\prime} = -70\,\text{kJ} \tag{21}$$

Carbamyl phosphate, a high-energy compound (see Table 5.2) is an intermediate in this reaction, and its hydrolysis to carbon dioxide and ammonium ions is coupled with the formation of ATP.

Many other amino acids are anaerobically degraded in an interesting type of fermentation in which some amino acids serve as the electron donor (e.g., alanine, tryptophan, proline, arginine) and others as the electron acceptor (e.g., glycine, valine, histidine, leucine, isoleucine) (1, 10). This process is known as the Stickland reaction. An example of such a pair-wise fermentation of amino acids is:

$$\text{Arginine} + 2\,\text{Glycine} + 2\text{H}_2\text{O} \rightarrow 3\,\text{Acetate}^- + 3\text{NH}_4^+ + \text{CO}_2 \quad \Delta G^{o\prime} = -144.9\,\text{kJ} \tag{22}$$

5.4. Anaerobic Respiration: Dissimilatory Iron and Manganese Reduction

The short fatty acids, alcohols, and other compounds formed as the end products of fermentation, as explained in the previous section, can be further degraded by anaerobic respiration with a variety of electron acceptors. They can be broken down by denitrification (see Sect. 5.2), as well as by respiration with electron donors such as sulfate (see Sect. 5.5), trivalent iron, or tetravalent manganese (26).

Trivalent iron is seldom abundantly found in anaerobic environments, and it is generally assumed that anaerobic respiration by reducing Fe^{3+} to Fe^{2+} is not of great quantitative importance in nature. Still, specialized iron-reducing anaerobes exist, and the understanding of their metabolism has greatly increased in recent years. Members of the genera *Geobacter* and *Shewanella* oxidize acetate and other short fatty acids, alcohols, hydrogen, and even some aromatic compounds to CO_2 with the reduction of trivalent to divalent iron or tetravalent manganese to divalent manganese.

5.5. Anaerobic Respiration: Dissimilatory Sulfate Reduction

A quantitatively much more important process than iron reduction is dissimilatory reduction of sulfate to sulfide. Sulfate is found at a concentration of around 23 mM in the world oceans, and is generally available as potential electron acceptor in freshwater environments as well.

Most known sulfate-reducing bacteria belong to the δ-Proteobacteria, but we also know species such as *Desulfotomaculum* (which produces heat-resistant endospores) that phylogenetically cluster with the Gram-positive bacteria. In addition, dissimilatory sulfate reduction also occurs in a few thermophilic *Archaea* such as *Archaeoglobus*. Most sulfate-reducing bacteria are obligate anaerobes, although some species are to some extent aerotolerant. The range of electron donors that can be used by sulfate reducers is quite limited. Complex organic material cannot be degraded, and only a few types can use a few sugars or amino acids, and they do so at a low rate. The substrates preferred as electron donors for dissimilatory sulfate reduction are fatty acids from formate and acetate up to a chain length of 16–18 carbon atoms, lactate, pyruvate, alcohols with 2–5 carbon atoms, and hydrogen. A few species can even use aromatic compounds such as benzoate.

We can divide the sulfate-reducing bacteria into incomplete oxidizers and complete oxidizers. The former only partially oxidize their substrates, and they excrete acetate as the main product, accompanied by CO_2 when the substrate had an odd number of carbons. Examples of such incomplete oxidizers are *Desulfovibrio*, some species of *Desulfotomaculum*, and *Desulfobulbus*. The first two preferentially oxidize lactate, ethanol, and H_2, the latter degrades propionate to acetate $+CO_2$. The acetate formed may become available as substrate for complete oxidizers such as *Desulfobacter*, *Desulfobacterium*, *Desulfococcus*, *Desulfonema*, *Desulfosarcina*, and *Desulfotomaculum acetoxidans*. *Desulfococcus*, *Desulfonema*, and *Desulfosarcina* can also oxidize longer fatty acids up to a chain length of 12–14 carbons. The thermophilic archaeon *Archaeoglobus* oxidizes lactate completely to CO_2 in a unique biochemical pathway that shares many unusual coenzymes with the methanogenic *Archaea* (see Sect. 5.6).

Before sulfate can be reduced, it has to be activated in an ATP-dependent reaction to form adenosine-5′-phosphosulfate (APS). As a result, the amount of energy that can be gained by dissimilatory sulfate reduction is limited. The APS is directly reduced to sulfite, without prior additional activation to PAPS, as is the case during assimilatory reduction of sulfate (see Sect. 3.4). Sulfate is not a strong oxidant as appears from the relatively low standard reduction potentials of the redox couples APS/AMP + HSO_3^- and HSO_3^-/HS^- (see Fig. 5.3). The free energy change associated with the dissimilatory reactions performed by the sulfate reducers

is accordingly small, as the following examples prove:

$$4H_2 + SO_4^{2-} + H^+ \rightarrow 4H_2O + HS^- \quad \Delta G^{o\prime} = -152.3\,\text{kJ} \tag{23}$$

as compared to:

$$4H_2 + 2O_2 \rightarrow 4H_2O \quad \Delta G^{o\prime} = -948.8\,\text{kJ} \tag{24}$$

$$4H_2 + 1.6NO_3^- + 1.6H^+ \rightarrow 0.8N_2 + 4.8H_2O \quad \Delta G^{o\prime} = -896.8\,\text{kJ} \tag{25}$$

or:

$$\text{Acetate}^- + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + 2H_2O + HS^- \quad \Delta G^{o\prime} = -40.7\,\text{kJ} \tag{26}$$

to be compared with:

$$\text{Acetate}^- + 2O_2 + H^+ \rightarrow 2CO_2 + 2H_2O \quad \Delta G^{o\prime} = -837.2\,\text{kJ} \tag{27}$$

or:

$$\text{Acetate}^- + 1.6NO_3^- + 2.6H^+ \rightarrow 2CO_2 + 0.8N_2 + 2.8H_2O \quad \Delta G^{o\prime} = -785.2\,\text{kJ} \tag{28}$$

Another type of reaction performed by some species of sulfate reducers is energy generation by disproportionation of thiosulfate or sulfite. In this process, a part of the substrate is oxidized to sulfate, and the electrons released are used to reduce the remainder to sulfide:

$$S_2O_3{}^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad \Delta G^{o\prime} = -21.7\,\text{kJ} \tag{29}$$

$$4SO_3^{2-} + 2H^+ \rightarrow 3SO_4^{2-} + HS^- \quad \Delta G^{o\prime} = -235.5\,\text{kJ} \tag{30}$$

The process of dissimilatory sulfate reduction has great environmental and also economic impact. Massive production of sulfide under anaerobic conditions leads to bad smelling waters and sediments. Sulfide-containing sediments are generally colored black due to the reaction of sulfide with divalent iron to yield FeS. Not only is sulfide, the end product of sulfate reduction, highly corrosive to metals, the activity of sulfate-reducing bacteria may also directly contribute to corrosion of metal pipe lines and other metal structures. Spontaneous oxidation of metals establishes a thin layer of molecular hydrogen ($Fe + 2H^+ \rightarrow Fe^{2+} + H_2$) that protects the metal surface from further oxidation. Sulfate-reducing bacteria effectively oxidize the hydrogen as expressed in Eq. (23), a process known as "cathodic depolarization," opening the way to further oxidation of the metal.

Elemental sulfur can also be used an electron acceptor in anaerobic respiration. For example, the obligatory anaerobic *Desulfuromonas acetoxidans* is unable to use sulfate as electron acceptor, but grows on acetate as electron donor and sulfur as electron acceptor:

$$\text{Acetate}^- + 2H_2O + 4S^o \rightarrow 2CO_{2(\text{gaseous})} + 4HS^- + 3H^+ \quad \Delta G^{o\prime} = -16.7\,\text{kJ} \tag{31}$$

5.6. Methanogenesis

As shown above, all common fermentation products can be mineralized to CO_2 when sulfate is present as electron acceptor, either by a one-step complete oxidation or by a collaboration of incomplete oxidizers, which produce acetate and complete oxidizers that take

care of the further oxidation to CO_2. When also sulfate is not available, the only remaining way for the further anaerobic degradation is by methanogenesis.

Methane production is known to occur only in a few groups of obligatory anaerobes, all belonging to the *Euryarchaeota* branch of the domain *Archaea*. We find methanogens in a wide range of anaerobic environments such as sediments and marshes, as well as in the digestive tracts of many animals, notably in ruminants. They are also important components of the microbial community of anaerobic digestion systems in water purification plants and in other systems in which organic material is degraded under anaerobic conditions.

The range of substrates used for energy generation by methanogens is even more limited than that available to the sulfate-reducing bacteria (see Sect. 5.5). In fact, most biologically formed methane is produced by the following two reactions:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^{o\prime} = -139.2\,\text{kJ} \tag{32}$$

$$\text{Acetate}^- + H^+ \rightarrow CH_4 + CO_2 \quad \Delta G^{o\prime} = -27.6\,\text{kJ} \tag{33}$$

Reactions expressed by Eqs. (32) and (33) thus remove hydrogen and acetate, two of the common fermentation products formed in many fermentative pathways (see Sect. 5.3). The first reaction is performed by many methanogenic species (genera *Methanobacterium*, *Methanococcus*, and others). As these bacteria derive both their energy and their cellular carbon from inorganic compounds, they should be classified as autotrophs (see also Sect. 3.1). The second reaction provides little energy, and we know only two genera of methanogens able to grow on acetate as carbon and energy source: *Methanosarcina* and *Methanosaeta*. The acetate-using methanogens are notoriously slow growers, with doubling times in the order of days. In spite of this slow growth they are responsible for more than half of the biologically produced methane in the biosphere. The unusual biochemical pathways and the principles of energy conservation in the methanogens were discussed earlier in Sect. 2.2.

There is a second group of obligate anaerobes that grow by reducing CO_2 with electrons derived from molecular hydrogen. These are the homoacetogens, and they produce acetate instead of methane:

$$4H_2 + 2CO_2 \rightarrow \text{Acetate}^- + H^+ + 2H_2O \quad \Delta G^{o\prime} = -111.6\,\text{kJ} \tag{34}$$

Examples of bacteria that perform this process are *Acetobacterium*, *Acetogenium*, and certain members of the genus *Clostridium*. The acetate formed can be further converted to methane and carbon dioxide, according to Eq. (33).

A third reaction performed by a number of methanogens that also can grow autotrophically on hydrogen $+CO_2$ is the disproportionation of formate:

$$4\text{Formate}^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O \quad \Delta G^{o\prime} = -120\,\text{kJ} \tag{35}$$

As we have seen above, formate is one of the products of the mixed-acid fermentation of *E. coli* and other enteric bacteria.

There are only a few additional reactions that can provide the energy for growth of methanogenic *Archaea*. These include the degradation of methylated amines such as trimethylamine – $(CH_3)_3N$ – to methane, carbon dioxide and ammonium ions, and the

formation of methane, carbon dioxide and sulfide from dimethylsulfide (CH_3–S–CH_3):

$$4\text{Trimethylamine} + 6H_2O + 4H^+ \rightarrow 9CH_4 + 3CO_2 + 4NH_4^+ \quad \Delta G^{o\prime} = -668.7\,\text{kJ} \tag{36}$$

$$2\text{Dimethylsulfide} + 2H_2O \rightarrow 3CH_4 + CO_2 + 2H_2S \quad \Delta G^{o\prime} = -221.4\,\text{kJ} \tag{37}$$

Trimethylamine and other methylated amines are not among the major products formed during degradation of biomass. These unpleasantly smelling substances can expected to be formed when compounds that contain tertiary amine groups, such as choline and notably glycine betaine, are degraded in anoxic environments. Glycine betaine (trimethylglycine) is accumulated as an osmotic stabilizer by many microorganisms (cyanobacteria, other photosynthetic, and nonphotosynthetic prokaryotes) that grow in highly saline environments. Dimethylsulfide (DMS) is formed for example during the degradation of dimethylsulfoniopropionate (DMSP), accumulated by marine micro- and macroalgae as an osmotic stabilizer (27). DMS at low concentrations is responsible for the characteristic smell of marine algae; at high concentrations, it is one of the worst smelling substances known.

5.7. Proton-Reducing Acetogens and Interspecies Hydrogen Transfer

As shown in Sect. 5.6, the variety of substrates used by methanogens is extremely limited. Hydrogen and acetate, two of the end products of fermentative processes, can be metabolized with the formation of methane, but many other compounds formed as fermentation end products cannot. The question, thus, remains how other fermentation products such as ethanol, propionate, and butyrate, are further metabolized in the absence of electron acceptors such as nitrate and sulfate.

Further degradation of the above-mentioned fermentation end products and others is possible by their oxidation to acetate and molecular hydrogen, which both can further be metabolized by methanogens. A calculation of the standard free energy change associated with the formation of acetate and hydrogen from ethanol, propionate, butyrate, and similar compounds shows that these reactions are endergonic:

$$\text{Ethanol} + H_2O \rightarrow 2H_2 + \text{Acetate}^- + H^+ \quad \Delta G^{o\prime} = +9.8\,\text{kJ} \tag{38}$$

$$\text{Propionate}^- + 2H_2O \rightarrow \text{Acetate}^- + CO_2 + 3H_2 \quad \Delta G^{o\prime} = +80.1\,\text{kJ} \tag{39}$$

$$\text{Butyrate}^- + 2H_2O \rightarrow 2\text{Acetate}^- + H^+ + 2H_2 \quad \Delta G^{o\prime} = +48.4\,\text{kJ} \tag{40}$$

Therefore, bacteria cannot make a living by performing these reactions under standard conditions (all reagents and products being present in concentrations of 1 M or 1 atmosphere, see also Sect. 2.1), as the equilibrium of the reactions is to the left, at the side of the reagents rather than of the products. However, it must be remembered that the true free energy change associated with chemical reactions depends not only on the standard free energy change, but also on the actual concentrations of the reagents and the products, in accordance with Eq. (4). When the products of the reaction (i.e., hydrogen and acetate) are effectively removed by the activity of methanogenic bacteria in the ecosystem or by other bacteria, the equilibrium of the reactions (38–40) shifts to the right in accordance with Le Chatelier's principle. When the concentrations of the end products are kept at very low levels, the $\Delta G'$ may become

sufficiently negative to allow the formation of ATP. It should be realized that the total amount of free energy that becomes available during anaerobic degradation does not increase by removal of hydrogen and acetate, as the methanogens will have to cope with low substrate concentrations, lowering the $\Delta G'$ of their dissimilatory reactions below the standard values given in Eqs. (32) and (33). For example, the energy available during the oxidation of ethanol to acetate coupled to the reduction of carbon dioxide to methane:

$$2\text{Ethanol} + CO_2 \rightarrow CH_4 + 2\text{Acetate}^- + 2H^+ \quad \Delta G^{o\prime} = -119.6\,\text{kJ} \tag{41}$$

is now shared between the ethanol-oxidizing bacterium (often referred to as the "S-organism) (Eqs. (38) and (42)) and the methanogenic *Archaea* that reduce CO_2 with hydrogen as electron donor (Eq. (32)).

$$2\text{Ethanol} + 2H_2O \rightarrow 2\text{Acetate}^- + 2H^+ + 4H_2 \quad \Delta G^{o\prime} = +19.6\,\text{kJ} \tag{42}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^{o\prime} = -139.2\,\text{kJ} \tag{32}$$

The cooperation between the two types of microorganisms is based on "interspecies hydrogen transfer," and organisms, such as the "S-organism" (Eq. (38)), *Syntrophobacter* (Eq. (39)), and *Syntrophomonas* (Eq. (40)), are often designated "proton-reducing acetogens," i.e., organisms that produce acetate while excreting molecular hydrogen (9, 10, 28, 29). We even know proton-reducing acetogens such as the species of the genus *Syntrophus* that degrade benzoate and other aromatic compounds to acetate and molecular hydrogen in reactions that are endergonic under standard conditions, but become feasible for energy production when the end products are efficiently removed:

$$\text{Benzoate}^- + 6H_2O \rightarrow 3\text{Acetate}^- + CO_2 + 2H^+ + 3H_2 \quad \Delta G^{o\prime} = +49.5\,\text{kJ} \tag{43}$$

There are many more cases, based on the same principle, of reactions that under standard conditions are endergonic, but participate in the anaerobic breakdown of organic matter when the concentrations of the products are kept low thanks to the action of methanogenic *Archaea*. To name just one more interesting example: methanogenesis from acetate yields very little energy (see Eq. (33)), and those methanogens that perform the reaction grow very slowly. It is therefore surprising that the reaction may also be performed by a thermophilic consortium of microorganisms that divide the little energy available among each other; one organism that oxidizes acetate to CO_2 with the evolution of hydrogen in a reaction that is highly endergonic, and a methanogen that reduces CO_2 with hydrogen as electron donor (30).

The above-documented principle of interspecies hydrogen transfer that drives otherwise energetically unfavorable reactions is not restricted to cases of obligatory syntrophic associations such as those involving bacteria such as *Syntrophomonas* and *Syntrophobacter*. As discussed in Sect. 5.7, many clostridia and other fermentative anaerobes can choose between gaining more energy by excreting acetate and producing more reduced fermentation products while disposing of excess reducing power that had accumulated in the form of intracellular NADH. The balance between these two options is determined by the ability of hydrogenase to excrete the excess electrons as molecular hydrogen. The hydrogenase reaction is endergonic in the direction of hydrogen production (Eq. (18)), but removal of the accumulating

hydrogen, e.g., by methanogenic or by sulfate-reducing bacteria will shift the equilibrium so that the reaction becomes favorable. The theoretical yield of four molecules of ATP per glucose fermented now becomes feasible as the true $\Delta G'$ of the reaction presented in Eq. (17) will be more negative than the –215.6 kJ calculated under standard conditions, so that the requirements for the production of the maximum amount of ATP can be met. This also means that in anaerobic ecosystems in which fermentative microorganisms live in close association with hydrogen oxidizers (sulfate reducers, methanogens, homoacetogens), and in which acetate is removed as well (both by sulfate reducers and methanogens), the need for the production of reduced fermentation products such as alcohols is largely abolished. The mixture of fermentation products excreted by an organism such as *Clostridium acetobutylicum* in pure culture (Fig. 5.9) is therefore very different from that produced in mixed cultures in which a hydrogen-oxidizing anaerobe is present as well.

The cooperation of fermentative bacteria and methanogens, if necessary with the assistance of syntrophic proton-reducing acetogens, enables the anaerobic mineralization of organic material to carbon dioxide and methane in the absence of any external electron acceptors:

$$\text{Glucose} \rightarrow 3CO_2 + 3CH_4 \quad \Delta G^{o\prime} = -393.2\,\text{kJ} \tag{44}$$

The yield in free energy is only a small fraction of the amount obtained during aerobic oxidation of glucose (−2822 kJ/mol, see Eq. (12)). It should also be noted that during aerobic respiration, all the energy becomes available to a single organism, while anaerobic breakdown to methane and carbon dioxide involves a number of partners, who thus have to share the relatively small amount of free energy released in the process. The remainder of the energy is stored in the methane, and can become available when the methane reaches an aerobic environment and is there oxidized by methanotrophic bacteria:

$$3CH_4 + 6O_2 \rightarrow 3CO_2 + 6H_2O \quad \Delta G^{o\prime} = -2428.8\,\text{kJ} \tag{45}$$

Methane is generally considered to be the end product of anaerobic degradation of organic material. However, the concept that methane cannot be further metabolized under anaerobic conditions needs revision. In recent years, it has become clear that anaerobic oxidation of methane is possible with sulfate serving as the electron acceptor (31):

$$CH_4 + SO_4^{2-} + H^+ \rightarrow CO_2 + HS^- + 2H_2O \quad \Delta G^{o\prime} = -13.1\,\text{kJ} \tag{46}$$

This reaction is performed by a consortium of two organisms, one being a sulfate-reducing eubacterium, and the other an archaeon, which is responsible for oxidation of the methane. No full information is yet available on the mechanism of the anaerobic methane oxidation or on the nature of the reaction intermediate that is transferred between the partners (15, 32) and couples the two partial reactions that lead to the products shown in Eq. (46).

From the information provided earlier, it is obvious that the two molecules that take a central place in the anaerobic degradation process are hydrogen and acetate. When these become available in the course of fermentation processes, there are several possibilities for their further metabolism. Methanogens, sulfate-reducing bacteria, and homoacetogens all compete for hydrogen. Acetate is used both by certain methanogens (*Methanosarcina*, *Methanosaeta*) that convert it to CO_2 and CH_4 (see Sect. 5.6) and by sulfate-reducing bacteria such as

Desulfobacter (see Sect. 5.5). When sulfate is present, little methanogenesis will occur, and the sulfate-reducing bacteria will oxidize the hydrogen and the acetate. This is due to the higher affinity of the sulfate-reducing bacteria for these substrates. Methanogenesis takes over when insufficient sulfate is present. In freshwater environments, such as lakes and marshes, the sulfate concentration is often low, and methanogenesis is therefore the most important terminal process of anaerobic degradation. Sulfate reduction dominates in the marine environment in which sulfate is abundantly present. There are, however, other substrates that are converted to methane and or which sulfate-reducing bacteria do not compete, such as the methylated amines and dimethylsulfide, as discussed in Sect. 5.6.

The ability of microorganisms to bring about complete mineralization of complex organic material to mixtures of CO_2 and CH_4 is exploited in anaerobic sludge digestors. The long retention times employed enable the reactions to come close to completion. Processes very similar to those occurring in anaerobic sludge digestors occur in the rumen of ruminant animals. Also, their organic materials (starch, cellulose, proteins) are fermented to organic acids, CO_2, and hydrogen. In this case, however, the anaerobic degradation is incomplete, and the main end products are acetate, propionate, and butyrate, which are taken up into the blood stream of the animal and serve as the main carbon and energy source of the ruminant. The fact that acetate is here not converted into methane and carbon dioxide by *Archaea* such as *Methanosarcina* (Eq. (33)), and propionate and butyrate are not broken down to acetate by syntrophic proton-reducing acetogens (Eqs. (39) and (40)) is due to the short retention time of the ingested food in the rumen. Those methanogens that use acetate for energy generation have very long generation times (in the order of days), and so have syntrophic bacteria such as *Syntrophobacter* and *Syntrophomonas*. As a result, such microorganisms cannot maintain stable populations in the system in which the retention time is considerably shorter than their generation time. All the methane formed in the digestive system of ruminants – the considerable amount of about 200 l per day in an adult cow – is formed by rapid-growing species (*Methanobrevibacter ruminantium*, *Methanomicrobium mobile*) that use the hydrogen evolved during the sugar fermentations as energy source by reducing CO_2 (Eq. (32)).

6. THE CHEMOAUTOTROPHIC WAY OF LIFE

The third way of energy generation, which is unique to the prokaryote world, is the use of reduced inorganic compounds as energy source to drive autotrophic CO_2 fixation. Chemoautotrophic (chemolithotrophic) microorganisms can thus grow on inorganic compounds only, without being dependent on light energy (13, 14). The general principles behind chemoautotrophic metabolism have been previously explained in Sect. 2.2 and in Fig. 5.6. Chemoautotrophic life is possible in aerobic as well as in anaerobic environments. The different modes of energy generation by chemolithotrophs and their importance in the environment are discussed below in further depth.

6.1. Reduced Nitrogen Compounds as Energy Source

Most organically bound nitrogen in nature exists in the reduced form as $-NH_2$ groups in amino acids. Reduced nitrogen also occurs in the purine and pyrimidine bases of the nucleic acids. Upon degradation of these organic nitrogen-containing compounds, the nitrogen

is released as ammonium ions. This process of ammonification occurs both under aerobic and under anaerobic conditions. Aerobically, the amino acids and other nitrogen compounds are converted to intermediates of the central metabolic pathways such as the tricarboxylic acid (see Fig. 5.2). Anaerobically, fermentation reactions release ammonium ions as well, as exemplified in Eqs. (21) and (22).

The ammonium released not only becomes available for assimilatory uptake by microorganisms and plants (see Sect. 3.2), but can also be used as energy source for chemolithotrophic bacteria that perform the process of nitrification (33). Since the pioneering studies of Sergei Winogradsky around 1890, we know that nitrification is an autotrophic process that proceeds in two steps. First, ammonium is oxidized to nitrite with the release of six electrons (Eq. (7)), and in the second step, the nitrite is further oxidized to nitrate by a different group of bacteria with the gain of two more electrons (Eq. (8)). As explained earlier, the amount of energy that can be obtained from these reactions is relatively small. As considerable amounts of energy are needed to drive the uphill transport of electrons to form NADPH, the reductant needed for autotrophic CO_2 fixation, cell yields are low. Large amounts of substrate are therefore, transformed to products by a small biomass (see the calculations in Sect.2.2). As the equations show, nitrification is associated with net production of protons, leading to an acidification of the environment in which the process takes place.

The two groups of microorganisms involved in autotrophic nitrification have phylogenetically different affiliations. The aerobic ammonium oxidizers (genera such as *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus* and others) are all Proteobacteria (β- or γ-branch) or belong to a recently discovered group of *Crenarchaeota* (*Candiatus* 'Nitrosopumilus maritimus' and relatives). Most nitrite oxidizers (*Nitrobacter*, *Nitrococcus*, *Nitrospina*) are Proteobacteria as well (α-, γ-, and δ-branch, respectively), but the genus *Nitrospira* forms a separate deep lineage within the domain *Bacteria*. The process of nitrification occurs only under aerobic conditions, as oxygen is the terminal electron acceptor both for ammonium- and for nitrite-oxidizing bacteria. Moreover, the first enzymatic step in the oxidation of ammonium with the formation of hydroxylamine (NH_2OH) as intermediate, catalyzed by ammonium monooxygenase, uses molecular oxygen as co-substrate.

Nitrification occurs everywhere in nature where ammonium ions and molecular oxygen occur together. As explained above, ammonium is the form in which nitrogen is released during the degradation of amino acids and other nitrogen-containing cellular components. In spite of this, nitrate is present in much higher concentrations than ammonium in most aerobic environments as the result of its rapid oxidation to nitrate by nitrifying bacteria. Nitrification occurs in aquatic as well as in terrestrial ecosystems. Nitrogen fertilizer applied as ammonium salts is rapidly oxidized in the soil to nitrate. Nitrification is exploited in many wastewater purification systems; the nitrogen load of the wastewater is reduced in a two-step procedure in which the ammonium is first aerobically oxidized to nitrate (nitrification), which is subsequently reduced in an anaerobic process to gaseous nitrogen (denitrification, see Sect. 5.2).

A completely different process of chemolithotrophic oxidation of ammonium, but this time under anaerobic conditions, was discovered a few years ago. The reaction involves the oxidation of ammonium using nitrite as electron acceptor with the formation of gaseous

nitrogen (see Eq. (9) and Sect. 2.2 for additional details). This anammox process, as it is generally called, was first documented to occur in an anaerobic laboratory-scale wastewater purification system. New processes of wastewater treatment are now under development to exploit the anammox reaction in an attempt to design a one-step process for nitrogen removal to replace the conventional two-step process of aerobic nitrification followed by anaerobic denitrification (34). The process of anaerobic ammonium oxidation with nitrite as electron acceptor is ecologically important in stratified water bodies such as the Black Sea, in which significant concentrations of oxidized nitrogen compounds (nitrate, nitrite) are present in the anoxic zone. It was estimated that up to 20–40% of the N_2 formed in such environments may be derived from the anammox reaction rather than from dissimilatory nitrate reduction – denitrification (35, 36), and recent estimates even indicate that up to 30–50% of the nitrogen evolved from the world ocean may originate from the anammox process rather than from denitrification, mainly in the continental slope and hemipelagic sediments where the majority of the marine nitrogen loss takes place.

6.2. Reduced Sulfur Compounds as Energy Source

Reduced sulfur compounds are excellent electron donors for chemoautotrophic growth (37). Due to the fact that sulfide, elemental sulfur, thiosulfate, and other similar potential electron donors are stronger reductants than ammonium and nitrate (see Fig. 5.3), the amount of energy gained by their oxidation to sulfate with oxygen as electron acceptor is much higher than that available to the nitrifying bacteria:

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad \Delta G^{o\prime} = -796.3\,\text{kJ} \tag{47}$$

$$S^o + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad \Delta G^{o\prime} = -587.0\,\text{kJ} \tag{48}$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad \Delta G^{o\prime} = -818.2\,\text{kJ} \tag{49}$$

These reactions are all associated with the production of sulfuric acid, a strong acid. In some environments, this may cause severe acid pollution problems. The best-known case is that of acid mine drainage, formed by the aerobic oxidation of pyrite (FeS_2) associated with coal and metal ores to form sulfuric acid and trivalent iron by bacteria such as *Acidithiobacillus ferrooxidans* (see also Sect. 6.3). The formation of acids during chemoautotrophic sulfur oxidation can be exploited when acidification is desirable, e.g., in highly alkaline soils. Addition of elemental sulfur efficiently leads to acid production according to Eq. (48). Many of the chemoautotrophic bacteria that oxidize sulfur compounds are highly acid-tolerant or even obligatory acidophilic. Some grow optimally at pH 2–3, and growth below pH 1 is not unusual.

Chemoautotrophic oxidation can also be coupled to the reduction of nitrate, instead of oxygen with the formation of gaseous nitrogen:

$$5H_2S + 8NO_3^- \rightarrow 5SO_4^{2-} + N_2 + 4H_2O + 2H^+ \quad \Delta G^{o\prime} = -3722\,\text{kJ} \tag{50}$$

The autotrophic bacteria that oxidize reduced sulfur compounds include members of the β- and γ-Proteobacteria such as *Thiobacillus*, *Acidithiobacillus*, *Geothiobacillus*, and *Thiomicrospira*. In addition to obligate autotrophs, the group also contains species that prefer a

mixotrophic way of life, in which reduced sulfur compounds provide the energy, but most of the cellular carbon is derived from organic compounds taken up from the medium rather than from CO_2. Within the γ-Proteobacteria, we also find filamentous colorless sulfur bacteria such as *Beggiatoa*, *Thiothrix*, and *Thioploca*. These organisms are among the largest prokaryotes extant; some *Beggiatoa* types have cells of 100 μm or more in diameter, and filaments as long as several millimeters being not uncommon. The filamentous sulfide-oxidizers are sediment-dwelling bacteria that generally locate themselves at the boundary between the anaerobic sulfide-rich sediment layers and the aerobic layer that supplies the oxygen. The filaments can move through the sediment by means of an unusual mechanism of gliding movement, not involving flagella, and they follow the diurnal changes in the location of the aerobic–anaerobic boundary. Sulfide is oxidized by the filamentous sulfur bacteria with elemental sulfur as intermediate, which is stored within the cells. Sergei Winogradsky defined the concept of chemoautotrophy in 1887 on the basis of his observations of the appearance of elemental sulfur in *Beggiatoa* filaments after feeding with sulfide and their disappearance following starvation. However, *Beggiatoa* filaments are notoriously difficult to grow in the laboratory in pure culture, and it was not done until 1983 when the ability for true chemoautotrophic growth in some *Beggiatoa* strains was first unambiguously demonstrated.

Large masses of the filamentous *Thioploca* occur in the sediments of the continental shelf in upwelling areas near the Pacific coast of South America. Recent studies of the biology of *Thioploca* have shown an unexpected feature: most of the cell volume is taken up by a large vacuole, which contains a very high concentration of nitrate. *Thioploca* accumulates nitrate to serve as electron acceptor during sulfide oxidation when oxygen is in short supply. Rather than producing dinitrogen as the end product of nitrate respiration, ammonium is excreted, so that the amount of biologically available nitrogen does not decrease in the process. A similar type of metabolism was documented in *Thiomargarita namibiensis*, a filamentous organism that lives in upwelling zones off the west coast of Africa, and has the largest cells yet documented in the prokaryotes. *Beggiatoa* can accumulate nitrate in intracellular vacuoles as well.

Also among the *Archaea,* we find chemoautotrophic sulfide and sulfur oxidizers. The best-known genus is *Sulfolobus*, an aerobic thermophilic sulfur oxidizer that lives at high temperatures (optimum at 75°C, maximum at 87°C) in acidic sulfur springs worldwide.

A highly interesting ecosystem in which the chemoautotrophic sulfide-oxidizing bacteria play a key role is found around the deep-sea hydrothermal vents along the spreading zones in the Pacific and the Atlantic Oceans at several kilometers depth. In these regions of intense volcanic activity, the plates that make up the earth crust separate and new crust is added. Springs that emit sulfide-rich water are abundantly found in these areas, some of them with water temperatures of up to 350°C. These areas in which anaerobic sulfide-rich hydrothermal waters mix with the cold oxygenated seawater are ideal habitats for the development of chemolithotrophic sulfur bacteria. The springs are surrounded by dense communities of giant tube worms that can reach a length of several meters and are 10–20 cm thick. Most of the body volume of these worms is occupied by the trophosome, an organ filled with chemoautotrophic bacteria, phylogenetically associated with the γ-Proteobacteria. These bacteria grow at the expense of sulfide, oxygen, and carbon dioxide transported to them by the blood stream of the worm. The organic carbon produced by the bacteria is used as carbon and energy source for

the worm that hosts the cells. Similar symbiotic associations have been documented between clams and mussels that dwell in the sulfide-rich environment of the hydrothermal vents. Sulfide is thus the primary energy source that supports the densely populated hydrothermal vent ecosystem.

6.3. Reduced Iron and Manganese as Energy Source

Reduced iron and manganese can be used as electron donors and energy sources to drive chemoautotrophic growth of specialized bacteria. The reactions involved are:

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad \Delta G^{o\prime} = -9.0\,\text{kJ (at pH 7)} \quad (51)$$

$$\Delta G^{o\prime} = -65.8\,\text{kJ (at pH 2)}$$

$$Mn^{2+} + 0.5O_2 + H_2O \rightarrow MnO_2 + 2H^+ \quad \Delta G^{o\prime} = -71.2\,\text{kJ} \quad (52)$$

Not much is known about the bacteria that oxidize iron at neutral and alkaline pH, mainly due to the fact that divalent iron is unstable in the presence of molecular oxygen, and chemical oxidation thus competes with the biological process. The best-characterized neutrophilic chemoautotrophic iron oxidizer is *Gallionella*, a bacterium that deposits the iron hydroxides produced in the form of a stalk. *Gallionella* is often observed in environments such as draining bogs and iron-rich springs.

In acidic environments, however, divalent iron is stable, and there chemoautotrophic iron-oxidizing bacteria often have a dramatic impact on the ecosystem. In mining areas where metal ores or coal containing pyrite (FeS_2) are brought to the surface, oxidation of both the iron and the sulfur atoms of the pyrite by *Acidithiobacillus ferrooxidans* results in highly acidic waters and streams, colored orange-brown by the iron hydroxides formed (see also Sect. 6.2). Acid mine drainage is a very severe environmental problem in many parts of the world.

The existence of bacteria that oxidize divalent manganese to the tetravalent form in the presence of oxygen has been documented long ago. However, relatively little is known about the process, and it is not always clear whether the oxidation of the metal ions is coupled with CO_2 fixation. The finding of genes for RuBisCo, the key enzyme of the Calvin cycle (see Fig. 3.1) suggests that in some manganese-oxidizing bacteria the process may indeed enable autotrophic growth.

6.4. Hydrogen as Energy Source

Thanks to the very negative standard reduction potential of the couple H^+/H_2 (Fig. 5.3), oxidation of hydrogen can be coupled with the reduction of many potential electron acceptors with the gain of energy and the possibility to drive chemoautotrophic growth. The aerobic oxidation of hydrogen is energetically highly favorable:

$$2H_2 + O_2 \rightarrow 2H_2O \quad \Delta G^{o\prime} = -474.4\,\text{kJ} \quad (53)$$

A number of aquatic and soil bacteria are known to perform this reaction (the "Knallgas reaction"), including members of the genera *Pseudomonas*, *Paracoccus*, *Ralstonia* (Proteobacteria) and *Bacillus* (Gram-positive bacteria). These can all grow as chemoorganotrophs as well. It is not clear what the importance of the reaction is in soils and in aquatic habitats

of moderate temperature. As documented in Sect. 5.3, hydrogen is one of the major products of bacterial fermentations, but it is used at a very high efficiency in the same environment in which it is produced by sulfate reducing, methanogenic and homoacetogenic bacteria, which keep the hydrogen concentrations at a sufficiently low value so that syntrophic associations between proton-reducing acetogens and methanogenic *Archaea* can exist (see Sect. 5.7). The amounts of molecular hydrogen that will escape from such environments to reach oxygen-rich niches are probably small. There are also thermophilic species that perform the Knallgas reaction. These include species of the genera *Aquifex* and *Hydrogenobacter*, isolated from hydrothermal vent environments where molecular hydrogen is among the substrates present in the hot waters that emerge from the vents. Phylogenetically, these genera belong to deep lineages branching off at the basis of the phylogenetic tree of the *Bacteria*.

Anaerobically, the oxidation of hydrogen can be coupled with a range of electron acceptors. These include nitrate (in facultative anaerobes such as *Paracocccus denitrificans*), sulfate (in some species of *Desulfovibrio*, and in *Desulfonema* and *Desulfosarcina*, Eq. (23)), elemental sulfur (especially in a wide range of extremely thermophilic *Archaea*), or carbon dioxide to form methane by methanogenic *Archaea* such as *Methanobacterium* and *Methanococcus* (Eq. (32)) or to form acetate by homoacetogens such as *Acetobacterium* (Eq. (34)). Some of the organisms mentioned use the Calvin cycle for autotrophic CO_2 fixation, others use alternative pathways such as the reduction of CO_2 to CO, which is then coupled with methyl groups to form acetyl-CoA (see Sect. 3.1).

6.5. Other Substrates as Energy Sources for Chemoautotrophic Growth

In addition to the well-known electron donors such as ammonium, nitrite, sulfide, sulfur, reduced iron, and hydrogen, there are a few additional compounds whose oxidation can drive chemoautotropic growth of certain bacteria. Some can grow autotrophically on carbon monoxide under aerobic conditions:

$$CO + 0.5O_2 \rightarrow CO_2 \quad \Delta G^{o\prime} = -248.8\,\text{kJ} \tag{54}$$

Bacteria that can use this reaction generally can perform the Knallgas reaction (Eq. (53)) as well. Anaerobic growth on CO is also possible according to:

$$CO + H_2O \rightarrow CO_2 + H_2 \quad \Delta G^{o\prime} = -11.6\,\text{kJ} \tag{55}$$

A few thermophilic anaerobes (*Carboxydothermus*, *Caldanaerobacter*) use this reaction. The thermophilic methanogen *Methanothermobacter thermautotrophicus* can grow anaerobically on CO with the production of methane.

There are also indications that the oxidation of arsenite (AsO_3^{3-}) to arsenate (AsO_4^{3-}) can be coupled with autotrophic growth. Little is known yet about the organisms that perform these processes and about their ecological importance. The waters of the highly saline and alkaline Mono Lake (California) contain about 0.2 mM arsenic compounds, and a microbiological arsenic cycle is operative there, including anaerobic respiration that reduces arsenate to arsenite, and aerobic chemoautotrophic oxidation of arsenite to arsenate, as well as anaerobic arsenate oxidation with nitrate as electron acceptor (38).

7. THE BIOGEOCHEMICAL CYCLES OF THE MAJOR ELEMENTS

The preceding sections provide the basis for an understanding of the biogeochemical cycles. A short discussion will suffice to show how the different processes interact to obtain functional cycles of carbon, nitrogen, sulfur, and other elements.

7.1. The Carbon Cycle

We may consider the carbon cycle (Fig. 5.10) as a series of conversions of inorganic carbon (CO_2 from the air or CO_2, bicarbonate, or carbonate dissolved in the water) into organic carbon and vice versa. Autotrophic, energy-requiring processes enable the fixation of inorganic carbon into cell material. Oxygenic phototrophs that use light as energy source (green plants, algae, cyanobacteria) are responsible for most CO_2 fixation on Earth, but in certain ecosystems anoxygenic phototrophs and chemoautotrophs may contribute significantly to the fixation of inorganic carbon.

The pathways that lead to mineralization of organic carbon to CO_2 in any single ecosystem primarily depend on the availability of potential electron acceptors to receive the electrons released when reduced carbon is oxidized. Organisms that obtain the most energy will generally have an advantage, and for respiratory processes the amount of energy involved primarily depends on the standard reduction potential of the electron acceptor; the more oxidized, the more energy can be gained. Thus, the order of the processes is generally: aerobic oxidation > denitrification > dissimilatory sulfate reduction > methanogenesis, all in accordance with the availability of the respective electron acceptors.

Mechanistic constraints complicate the picture to some extent, as processes such as dissimilatory sulfate reduction and even more so methanogenesis function with a limited range of electron donors only. Therefore, additional stages in the anaerobic degradation of organic material are essential, notably fermentation processes that degrade complex organic compounds into a range of smaller molecules that are amenable to further mineralization by the terminal anaerobic degradation processes of sulfate reduction or (in the absence of any other electron acceptors) methanogenesis. An interesting observation is that the small amount of energy available in the anaerobic degradation is often shared between a number of partners, and that the processes performed by each partner have to be carefully coordinated so that each of the organisms can make a living. Although indications now exist that some methane can be oxidized also under anaerobic conditions, it is the aerobic oxidation of the methane formed under anoxic conditions that closes the cycle. The nature of the microorganisms involved and the relative importance of the different processes will vary for each ecosystem, but the principles are universally valid.

The above analysis of the microbial carbon cycle shows that it is closely linked and interrelated with the cycles of nitrogen and sulfur; oxidized forms of nitrogen and sulfur can serve as electron acceptors during the mineralization of organic carbon by anaerobic respiration. Similarly, oxidation of reduced nitrogen and sulfur compounds drives autotrophic fixation of CO_2, both by chemoautotrophic and by anoxygenic photoautotrophic microorganisms (in the latter case based on oxidation of sulfur compounds only; the hypothetic ammonia-oxidizing anoxygenic photoautotrophs envisaged by Broda (16) are yet to be discovered).

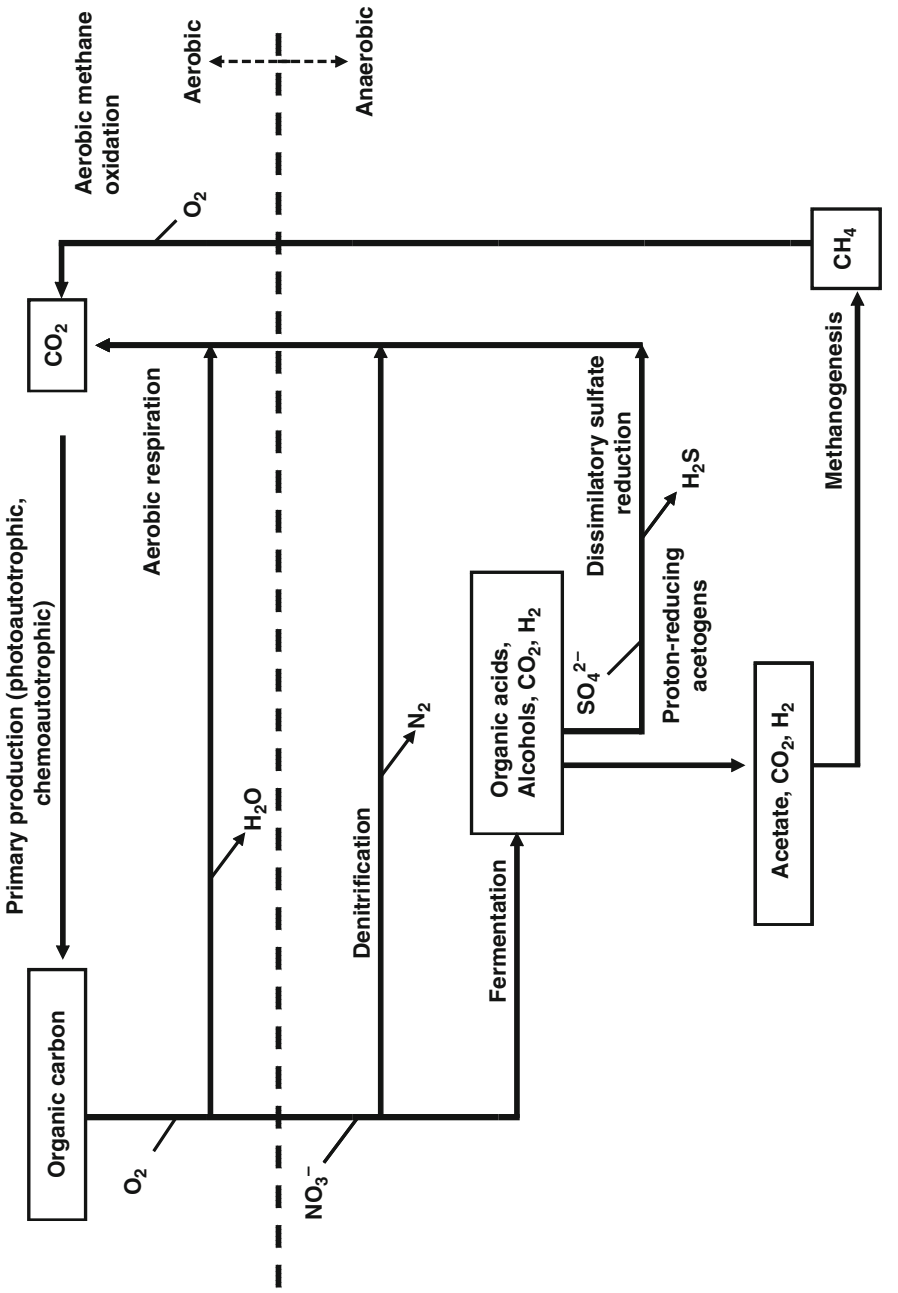

Fig. 5.10. The most important processes in the carbon cycle.

7.2. The Nitrogen Cycle

The nitrogen cycle is particularly rich in transformations of inorganic forms of nitrogen, and almost all possible oxidation states, from ammonium (N^{3-}) to nitrate (N^{5+}), are encountered. An overview of the principal processes in the nitrogen cycle is given in Fig. 5.11.

The same principles shown for the carbon cycle are operative here, such as energy-expensive assimilatory processes – which are extremely energy-costly in the case of fixation of molecular nitrogen, as well as energy sharing between partners that together perform processes that either one alone cannot accomplish – in this case the autotrophic oxidation of ammonium to nitrate, which proceeds with nitrite as obligate intermediate. Also on the assimilatory level, both inorganic and organic forms of nitrogen can be used, the latter mainly bound in the form of amino acids. The recent discovery of the anammox process, including the demonstration that at least in certain environments this reaction is also quantitatively significant, nicely demonstrates that we still may not known all processes in the biogeochemical cycles, and that new insights are continuously being obtained.

7.3. The Sulfur Cycle

Also in the sulfur cycle (Fig. 5.12), alternations in oxidation state abound. Both oxidized and reduced sulfur can be used for assimilatory purposes, reduced sulfur can serve as electron donor for photoautotrophic growth or as electron donor and energy source for chemoautotrophic growth, and oxidized sulfur compounds can be used as electron acceptors in anaerobic respiration.

One aspect of the sulfur cycle that was not discussed in-depth in the sections above but which is of considerable importance, also on a global level, is the formation and transformations of methylated sulfur compounds. DMSP, an osmotic stabilizer produced by marine algae, is degraded among other products to dimethylsulfide (DMS). Other ways DMS may be formed are by anaerobic respiration with dimethylsulfoxide as electron acceptor, a process whose ecological significance is not yet clear, as well as by anaerobic degradation of aromatic methoxylated compounds in the presence of sulfide (39). Part of the DMS dissolved in seawater escapes as a gas to the atmosphere. DMS is thus the main chemical form in which sulfur can be transported from the marine to the terrestrial environment. Oxidation of DMS in the atmosphere leads to the formation of tiny droplets of sulfuric acid, and they act as condensation nuclei for the formation of water droplets and clouds. The net flux of DMS from the oceans to the atmosphere thus directly influences cloud cover and rainfall on a global scale. DMS can also be oxidized aerobically in a process in which both the sulfur and the methyl groups are oxidized with the gain of energy, and can be anaerobically converted to sulfide and methane (Eq. (37)).

7.4. Biogeochemical Cycles of Other Elements

Many other elements are subject to microbial transformations, often associated with changes in oxidation state. We have discussed how divalent iron can be oxidized to drive chemoautotrophic and even anoxygenic phototrophic CO_2 fixation. Trivalent iron can be used as electron acceptor in anaerobic respiration. Similar phenomena have been described for

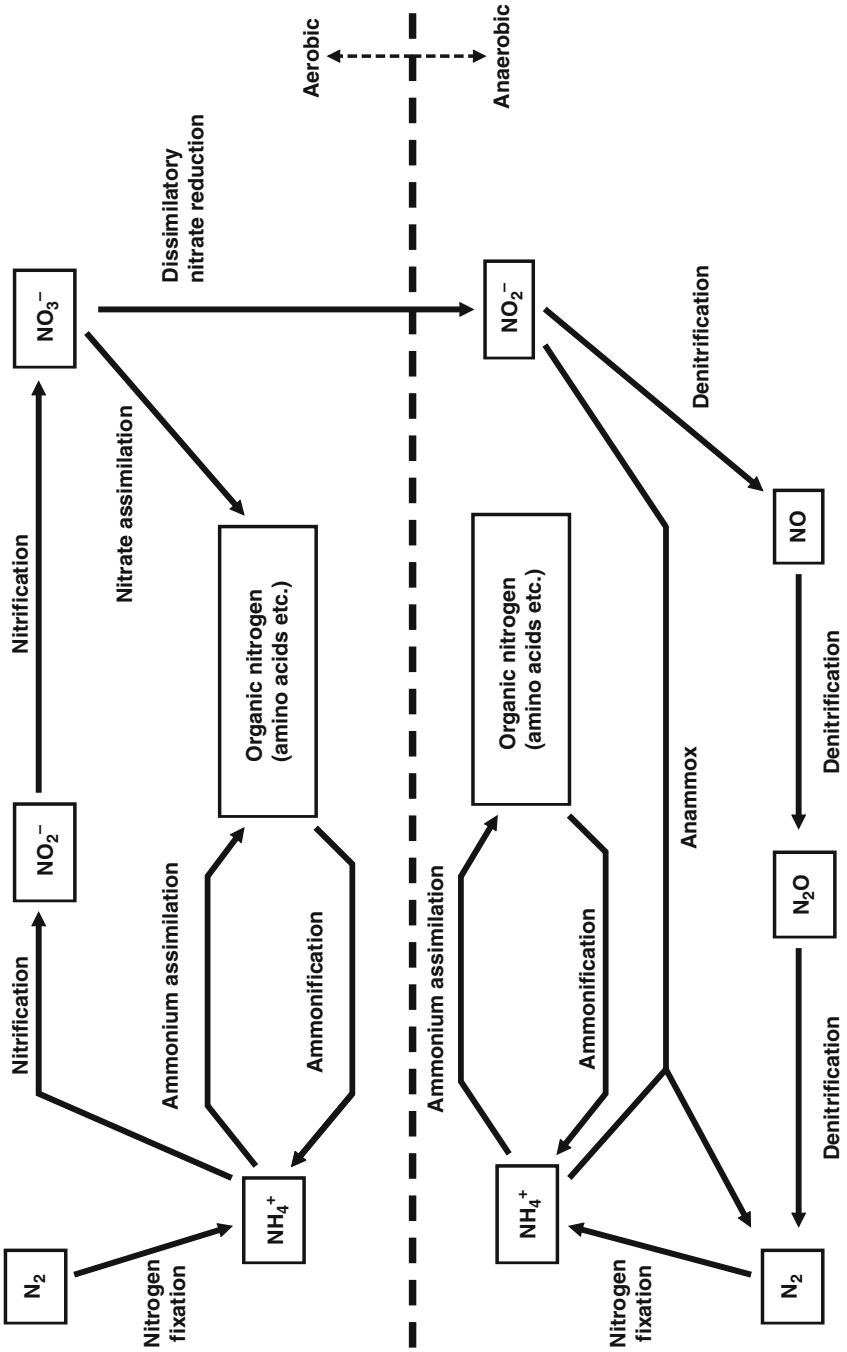

Fig. 5.11. The most important processes in the nitrogen cycle.

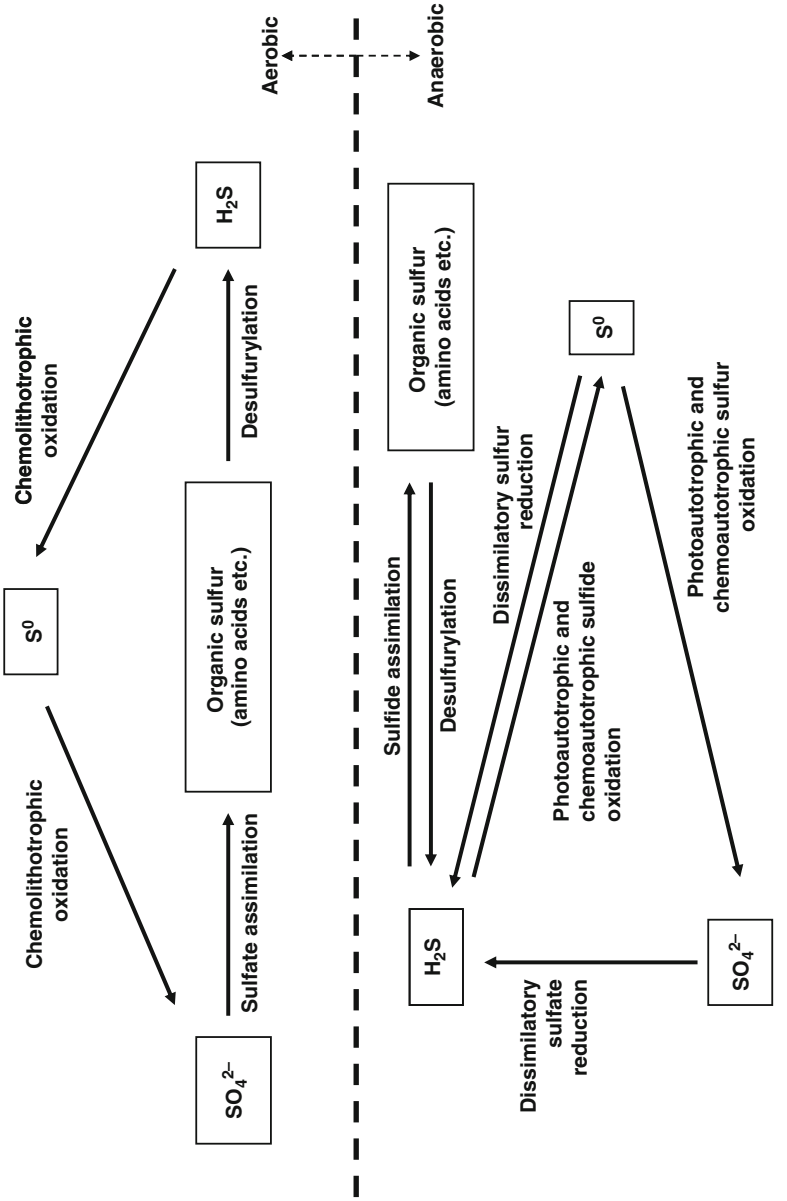

Fig. 5.12. The most important processes in the sulfur cycle.

manganese, and for ions of selenium, chromium, copper, arsenic, and additional elements. The general principle is similar in all cases, when such compounds can participate in an energy-yielding reaction, either as electron donor or as electron acceptor, some bacterium will be found that can exploit the reaction.

8. EPILOGUE

The preceding sections have provided an overview of the tremendous diversity in metabolic types among microorganisms, and especially among the prokaryotes. Hardly any process that is thermodynamically feasible remains unexploited by the microbial world. This metabolic diversity drives the biogeochemical cycles of carbon, nitrogen, sulfur, and other elements. Combination of the different processes – phototrophic and chemotrophic, autotrophic and heterotrophic, aerobic and anaerobic – enables the cycling of the elements, thus sustaining life on Earth. The processes performed by the microbes can also often be manipulated to assist man in exploiting the environment or to solve environmental problems. Understanding the metabolic potential and diversity of the microorganisms is the basis for their successful exploitation to the benefit of mankind.

NOMENCLATURE

ADP = adenosine diphosphate
APS = adenosine-5′-phosphosulfate
ATP = adenosine triphosphate
CoA = coenzyme A
E'_o = standard reduction potential of a redox couple, V
F = Faraday constant, 96.5 kJ/V
FAD = flavin adenine dinucleotide, oxidized form
FADH = flavin adenine dinucleotide, reduced form
G^o_f = free energy of formation, kJ/mol
ΔG = change in free energy during a chemical reaction, in kJ/mol
$\Delta G^{o\prime}$ = change in free energy during a chemical reaction, in kJ/mol at pH 7 under standard conditions
$\Delta\mu^+_H$ = proton electrochemical gradient over a biological membrane, mV
NAD^+ = nicotinamide adenine dinucleotide, oxidized form
NADH = nicotinamide adenine dinucleotide, reduced form
$NADP^+$ = nicotinamide adenine dinucleotide phosphate, oxidized form
NADPH = nicotinamide adenine dinucleotide phosphate, reduced form

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APPENDIX: COMPOUNDS OF ENVIRONMENTAL SIGNIFICANCE AND THE MICROBIAL PROCESSES RESPONSIBLE FOR THEIR FORMATION AND DEGRADATION

Below follows a nonexhaustive list of compounds formed during the dissimilatory metabolism of prokaryotic organisms (*Bacteria* as well as *Archaea*), with special emphasis on those compounds of importance in environmental engineering. Information is also provided on those microbial processes (assimilatory as well as dissimilatory) responsible for the disappearance of these compounds. Reference is made to the appropriate sections in the text above in which the nature of the respective processes was discussed in further depth.

Compounds of Carbon, Hydrogen, and Oxygen

Hydrogen (H_2*)*

Hydrogen is a characteristic end product of fermentation by anaerobic bacteria (representatives of the genus *Clostridium* and many others). It can be formed in ferredoxin-mediated reactions such as the oxidative decarboxylation of pyruvate to acetyl-CoA and/or by action of hydrogenase, using reducing equivalents from NADH (see Sect. 5.3). Hydrogen is also excreted by syntrophic bacteria, such as *Syntrophomonas* and *Syntrophobacter,* in the course of the oxidation of organic acids and other compounds (see Sect. 5.7). Minor amounts of hydrogen are formed also as a byproduct of nitrogenase activity in all nitrogen-fixing prokaryotes.

Hydrogen seldom accumulates at large concentrations in nature as it is effectively used by a variety of sulfate-reducing bacteria (Sect. 5.5), methanogenic bacteria (Sect. 5.6), and homoacetogenic bacteria (Sect. 5.6) (all under anaerobic conditions), and by aerobic chemolithotrophic hydrogen oxidizers ("Knallgas bacteria") (Sect. 6.4).

Oxygen (O_2*)*

Molecular oxygen is formed as a byproduct of photosynthesis by oxygenic prokaryotes (Cyanobacteria) (see Sect. 4.1), eukaryotic microalgae, macroalgae, and terrestrial plants.

Oxygen is the terminal electron acceptor in aerobic respiration, enabling degradation of about every biodegradable organic compound, as well as the chemoautotrophic oxidation of reduced nitrogen and sulfur compounds to nitrate and sulfate, respectively.

Carbon Dioxide (CO_2*)*

CO_2 is the end product of oxidation of organic material by organisms that perform aerobic respiration (animals, fungi, many bacteria) or anaerobic respiration with nitrate or sulfate as electron acceptor (see Sects. 5.1, 5.2, and 5.5). CO_2 is also released in the course of many fermentative processes together with organic fermentation products (Sect. 5.3), and in disproportionation reactions mediated by methanogenic *Archaea,* such as methanogenesis, from formate (Sect. 5.6).

Most assimilation of carbon dioxide occurs through the Calvin cycle, with ribulose bisphosphate carboxylase (RuBisCO) as the key enzyme (Sect. 3.1). This is true both for photoautotrophs and for chemoautotrophs. Alternative modes of autotrophic fixation exist in certain groups of microorganisms such as the green sulfur bacteria, the methanogenic *Archaea*,

and others. Carboxylation reactions, such as the carboxylation of phosphoenolpyruvate to oxalacetate, incorporate carbon dioxide into cellular carbon also in heterotrophic organisms.

Methane (CH_4*)*

Methane is formed only by a specialized group of *Archaea* as the end product of their energy-yielding reactions. The major precursors for methane are acetate, which is split into methane and carbon dioxide, and the reduction of carbon dioxide by molecular hydrogen (see Sect. 5.6). Methane can also be formed from formate, from methanol, from methylated amines, and from dimethylsulfide.

Methane is oxidized aerobically by methanotrophic bacteria. Anaerobic methane oxidation is possible as well in a yet incompletely understood process performed by a consortium of *Archaea* and *Bacteria* in which methane oxidation is coupled with the reduction of sulfate to sulfide (see Sect. 5.7).

Carbon Monoxide (CO)

No microorganisms are known that release CO into the environment. Carbon monoxide is an intermediate in the autotrophic fixation of CO_2 in certain autotrophs that do not use the reactions of the Calvin cycle (some sulfate-reducing bacteria, some methanogenic *Archaea*), and as such remains intracellular (see Sect. 3.1).

Carbon monoxide can be metabolized by a variety of microorganisms, aerobic as well as anaerobic. Some aerobic chemoautotrophs can obtain their energy by the oxidation of CO to CO_2. Anaerobically, CO can be converted to methane. Another anaerobic energy-yielding pathway, performed by a number of thermophilic representatives of the *Bacteria*, is its oxidation to CO_2 with concomitant formation of hydrogen (see Sect. 6.4).

Short-Chain Organic Acids

FORMIC ACID (HCOOH)

Formate is produced by pyruvate:formate lyase in a variety of fermentative processes, including, e.g., the anaerobic degradation of sugars by *Escherichia coli* under anaerobic conditions (see Sect. 5.3).

Formate can be oxidized aerobically by a variety of bacteria. Anaerobically, it can be converted to a mixture of methane and carbon dioxide in a disproportionation reaction performed by methanogenic *Archaea* (see Sect. 5.6). Alternatively, it may serve as electron donor for denitrifying bacteria or for certain sulfate-reducing bacteria.

ACETIC ACID (CH_3–COOH)

Acetate is formed as a major fermentation product by many carbohydrate- and amino acid-fermenting bacteria (see Sect. 5.3) and by proton-reducing acetogens in syntrophic partnerships (see Sect. 5.7). In addition, homoacetogenic bacteria form acetate under anaerobic conditions by reducing carbon dioxide with hydrogen as electron donor (see Sect. 5.6). Moreover, acetate can be formed in incomplete oxidation processes, aerobically as well as anaerobically. Aerobic acetic acid bacteria, such as *Acetobacter,* oxidize ethanol to acetate with molecular oxygen as electron acceptor (Sect. 5.1). Anaerobically, incomplete oxidation

of lactate, propionate, and other organic acids by dissimilatory sulfate reducing bacteria using sulfate as electron acceptor leads to acetate formation (see Sect. 5.5).

Acetate can be oxidized to carbon dioxide using oxygen, nitrate, sulfate, or trivalent iron as electron acceptors. Certain methanogenic *Archaea* split acetate into methane and carbon dioxide (see Sect. 5.6). At high temperatures, acetate can be oxidized anaerobically to carbon dioxide with the release of molecular hydrogen in a process that has to be coupled with hydrogen consumption to be energetically feasible (see also Sect. 5.7). Acetate is also used as an assimilatory carbon source by many aerobic bacteria, by facultative or obligatory photoheterotrophs, and by mixotrophic oxidizers of reduced sulfur compounds.

Propionic Acid (CH_3–CH_2–COOH)

Propionate is a characteristic fermentation product, made from sugars or from lactate by a specialized group of propionic acid bacteria (*Propionibacterium*, *Selenomonas*, *Megasphaera*) (see Sect. 5.3).

Propionate can be degraded aerobically by aerobic respiration, and anaerobically by denitrifying bacteria or sulfate-reducing bacteria such as *Desulfobulbus,* which oxidizes propionate incompletely to acetate + carbon dioxide (see Sect. 5.5). The proton-reducing acetogen, *Syntrophobacter,* converts propionate to acetate + carbon dioxide as well as hydrogen, which has to be efficiently removed for the process to be energetically favorable (see Sect. 5.7).

Butyric Acid (CH_3–CH_2–CH_2–COOH)

Butyrate is a fermentation product excreted by many fermentative prokaryotes growing on sugar or amino acids (see Sect. 5.3).

Butyrate can be oxidized to carbon dioxide by many aerobic bacteria. Under anaerobic conditions, butyrate can be converted to carbon dioxide by denitrification (Sect. 5.2) or by certain sulfate-reducing bacteria (Sect. 5.5). When no electron acceptors are available, *Syntrophomonas* converts butyrate to acetate and hydrogen in process that is thermodynamically favorable only if the hydrogen formed is efficiently removed by a syntrophic partner (see Sect. 5.7).

Lactic Acid (CH_3–CHOH–COOH)

Lactate is a product of fermentation by specialized lactic acid bacteria: homolactic organisms such as *Streptococcus* and many *Lactobacillus* species, and heterolactic species such as *Leuconostoc*, which produce a mixture of lactate, ethanol, and carbon dioxide. It is formed during other fermentations as well, such as the mixed acid fermentation of *Escherichia coli* and relatives under anaerobic conditions (see Sect. 5.3).

Lactate can be degraded by aerobic respiration, by anaerobic respiration with nitrate as electron acceptor, as well as by sulfate-reducing bacteria, such as *Desulfovibrio,* that degrade lactate incompletely to acetate + carbon dioxide (see Sect. 5.5). The sulfate-reducing thermophile *Archaeoglobus* performs complete oxidation of lactate to carbon dioxide using sulfate as electron acceptor. Lactate can also be fermented further under anaerobic conditions to a mixture of propionate, acetate, and carbon dioxide (Sect. 5.3).

SUCCINIC ACID ($COOH–CH_2–CH_2–COOH$)

Succinate is formed as a minor fermentation product in the mixed acid fermentation of enteric bacteria such as *Escherichia coli,* and is also formed by anaerobic bacteria such as *Bacteroides*, *Ruminobacter*, and *Succinomonas* that live in the digestive system of animals (see Sect. 5.3). Succinate can also be formed anaerobically as the product of anaerobic reduction of fumarate used as electron acceptor in respiration.

Succinate can be oxidized aerobically and anaerobically (by denitrification) to carbon dioxide. Moreover, it can be fermented to propionate + carbon dioxide by certain propionic acid bacteria (*Propionigenium*, *Schwartzia*) (Sect. 5.3).

Ethanol ($CH_3–CH_2OH$*)*

Ethanol is formed in many fermentation processes, both in eukaryotes (the alcohol fermentation of yeasts) and prokaryotes (*Zymomonas*, heterolactic fermenters such as *Leuconostoc*, and also as a minor product in the fermentation of enteric bacteria and some clostridia; see Sect. 5.3).

Ethanol can be oxidized aerobically (complete oxidation to CO_2 or incomplete oxidation to acetate by acetic acid bacteria; see Sect. 5.1). Under anaerobic conditions, ethanol can be oxidized to CO_2 while using nitrate as electron acceptor, to acetate by sulfate-reducing bacteria such as *Desulfovibrio* (see Sect. 5.5), or by proton-reducing acetogens under the excretion of molecular hydrogen (see Sect. 5.7).

Isopropanol ($CH_3–CHOH–CH_3$*)*

Isopropanol is formed as a minor product during carbohydrate fermentation by certain species of *Clostridium* and related organisms (see Sect. 5.3).

Isopropanol can be oxidized aerobically to CO_2. In the absence of oxygen, it can serve as electron donor for the reduction of carbon dioxide to methane in certain methanogenic *Archaea*.

n-Butanol ($CH_3–CH_2–CH_2–CH_2OH$*)*

n-Butanol is often formed during fermentation of carbohydrates by *Clostridium* species (see Sect. 5.3).

Butanol can be oxidized aerobically and anaerobically to CO_2 with oxygen, nitrate, or sulfate as electron acceptors.

Acetone ($CH_3–CO–CH_3$*)*

Acetone is a minor product of some fermentation processes, e.g., the fermentation of carbohydrates by *Clostridium acetobutylicum* (see Sect. 5.3). Furthermore, it can be formed by certain methanogenic bacteria from isopropanol that may serve as electron donor for methanogenesis.

Acetone can be oxidized aerobically by oxidation to hydroxyacetone by means of a monooxygenase, followed by oxidation to pyruvate. Anaerobic degradation by certain denitirying bacteria is possible in a pathway initiated by carboxylation to acetoacetate.

Nitrogen-Containing Compounds

Ammonium (NH_4^+)

Ammonium ions are generated as the result of aerobic as well as anaerobic degradation of amino acids and other organic compounds containing reduced nitrogen such as the purine and pyrimidine bases present in nucleic acids (ammonification, see Sect. 6.1). Ammonium ions can also be formed in the dissimilatory process of nitrate reduction, but nitrate ammonification is less common than denitrification with the formation of dinitrogen and nitrous oxide.

Ammonium can be used both aerobically and anaerobically for assimilatory purposes as nitrogen source, and can also serve as energy source in dissimilatory processes: nitrification (under aerobic conditions, where it is oxidized to nitrite), or anaerobic ammonium oxidation (the "anammox" process) in which nitrite serves as electron acceptor (see Sect. 6.1).

Nitrite (NO_2^-)

Nitrite can be formed aerobically as the product of the oxidation of ammonium ions in the first step of autotrophic nitrification by *Nitrosomonas* and related organisms as well as by ammonium-oxidizing *Archaea* (see Sect. 6.1). Anaerobically, nitrite can accumulate as an intermediate in denitrification processes during the reduction of nitrate. Certain bacteria, such as *Escherichia coli,* anaerobically reduce nitrate to nitrite as end product. Minor amounts of nitrite may also originate from $–NO_2$ residues during the aerobic breakdown of organic nitro compounds.

Nitrite can be used as nitrogen source for assimilatory purposes by a variety of photosynthetic and nonphotosynthetic microorganisms. Furthermore, it serves as energy source for autotrophic nitrifiers such as *Nitrobacter* (see Sect. 6.1). Anaerobically, it is reduced via nitric oxide and nitrous oxide to dinitrogen in the process of denitrification (see Sect. 5.2), or it may be used as the electron acceptor in anaerobic oxidation of ammonium ions (the "anammox" reaction, see Sect. 6.1).

Nitrate (NO_3^-)

Nitrate is the end product of autotrophic nitrification in which ammonium ions are aerobically oxidized via nitrite to nitrate. Minor amounts of nitrate may be formed anaerobically by the "anammox" bacteria, which use nitrite as electron donor to provide electrons for autotrophic fixation of carbon dioxide (see Sect. 6.1).

Nitrate can be consumed both in assimilatory processes when it serves as nitrogen source to plants, microalgae, and many aerobic bacteria (Sect. 3.2), as well as in dissimilatory processes: nitrate respiration – denitrification with the formation of more reduced products: nitrite, nitric oxide, nitrous oxide, dinitrogen, or ammonium ions (Sect. 5.2).

Dinitrogen (N_2)

Nitrogen is the major end product of denitrification – the dissimilatory reduction of nitrate and nitrite under anaerobic conditions (see Sect. 5.2) – as well as the product of anaerobic oxidation of ammonium ions with nitrite as electron acceptor in the "anammox" process (see Sect. 6.1).

Nitrogen can be used as a nitrogen source for assimilatory purposes by a limited number of prokaryotes, many of them living in symbiotic associations with higher organisms, in an energy-expensive process catalyzed by the enzyme nitrogenase (see Sect. 3.2).

Nitrous Oxide (N_2O*)*

Nitrous oxide is a product of dissimilatory nitrate respiration – denitrification (see Sect. 5.2), and is generally found as a minor end product besides dinitrogen. There are also indications that activity of nitrifying bacteria may be responsible for the formation of part of the nitrous oxide present in the marine environment.

Nitrous oxide can be further reduced to dinitrogen during denitrification.

Trimethylamine [$(CH_3)_3N$*] and Other Methylated Amines*

Trimethylamine and other methylated amines can be formed during degradation of choline (a component of the lipid phosphatidylcholine) or glycine betaine, a compound found as an intracellular osmotic stabilizer in many halophilic and halotolerant microorganisms inhabiting hypersaline environments.

Methylated amines can be oxidized aerobically by a variety of methylotrophic bacteria. Anaerobically, they can be used as energy source by many methanogenic *Archaea* with the production of methane, carbon dioxide, and ammonium ions.

Putrescine ($NH_2–CH_2–CH_2–CH_2–CH_2–NH_2$*), Cadaverine (*$NH_2–CH_2–CH_2–CH_2–CH_2–CH_2–NH_2$*), Agmatine (*$NH_2–CH_2–CH_2–CH_2–CH_2–NH–C(NH_2) = NH$*), and Related Organic Amines*

Putrescine, cadaverine, agmatine, and related bad-smelling compounds can be formed by decarboxylation of amino acids (ornithine, lysine, and arginine, respectively) by a variety of anaerobic fermentative bacteria.

Little is known about the further metabolism of these compounds in the absence of molecular oxygen. Putrescine can be fermented to acetate, butyrate, hydrogen, and ammonium ions. The amines can all be oxidized to carbon dioxide and ammonium ions under aerobic conditions.

Sulfur-Containing Compounds

Hydrogen Sulfide (H_2S*)*

Hydrogen sulfide may be formed by desulfurylation during anaerobic degradation of amino acids (cysteine, methionine) and other organic sulfur compounds. In addition, major amounts of sulfide are produced as the end product of dissimilatory reduction of sulfate, elemental sulfur, and other oxidized inorganic sulfur compounds under anaerobic conditions (see Sect. 5.5).

Sulfide is unstable under aerobic conditions and is oxidized abiotically in the presence of molecular oxygen. Moreover, it serves as the energy source for chemolithotrophic aerobic sulfide oxidizers such as *Thiobacillus* and *Beggiatoa* (see Sect. 6.2). Anaerobically, sulfide can be oxidized by green and purple phototrophic sulfur bacteria, in which it serves as electron donor for autotrophic fixation of carbon dioxide (Sect. 4.2), or by certain denitrifying sulfide oxidizers, in which it acts both as energy source and as electron donor for autotrophic growth (Sect. 6.2).

Sulfate (SO_4^{2-})

Sulfate is formed as the end product of both photosynthetic (Sect. 4.2) and chemosynthetic (Sect. 6.2) oxidation of sulfide and other reduced sulfur compounds. Photosynthetic sulfide oxidation occurs in anaerobic environments in which sufficient light is available to serve as energy source. Chemoautotrophic sulfur oxidation occurs aerobically, but can also proceed anaerobically in the presence of nitrate as electron acceptor.

Sulfate can be used as source of sulfur for assimilatory purposes by plants, algae, and many bacteria (see Sect. 3.4). Sulfate is also used as terminal electron acceptor for anaerobic respiration by sulfate-reducing bacteria (Sect. 5.5).

Elemental Sulfur (S^0)

Sulfur can be formed both by the abiotic oxidization of hydrogen sulfide and as an intermediate during the oxidation of sulfide to sulfate by green and purple photosynthetic bacteria (see Sect. 4.2).

Under aerobic conditions, elemental sulfur is used as electron donor and energy source by chemolithotrophic bacteria (*Bacteria* of the *Thiobacillus* group; at high temperatures *Archaea* such as *Sulfolobus*), causing acidification of the medium (see Sect. 6.2). Under anaerobic conditions, elemental sulfur can be an electron donor to photosynthetic green and purple bacteria, which oxidize it to sulfate (Sect. 4.2), or as an electron acceptor in anaerobic respiration by *Bacteria* such as *Desulfuromonas* or a variety of thermophilic *Archaea* (Sect. 5.5).

Dimethylsulfide (CH_3–S–CH_3) and Methylmercaptan (CH_3–SH)

Dimethylsulfide and methylmercaptan (methylsulfide) can be produced during the anaerobic degradation of the amino acid methionine and other organic compounds that contain reduced sulfur. A major source of dimethylsulfide in the marine environment is the degradation of DMSP, an intracellular osmotic stabilizer of many marine algae. Dimethylsulfide can also be formed as the product of anaerobic respiration processes with dimethylsulfoxide as electron acceptor. Finally, anaerobic degradation of methoxylated aromatic compounds in the presence of hydrogen sulfide can lead to the formation of dimethylsulfide.

Under aerobic conditions, dimethylsulfide can be oxidized by chemolithotrophic sulfur oxidizers and by methylotrophs, leading to the formation of carbon dioxide and sulfate. In the absence of molecular oxygen, dimethylsulfide can be used as energy source by certain methanogenic *Archaea*.

Other Elements

IRON OXIDES

Oxidized forms of iron (Fe^{3+}) are formed as the result of the chemolithotrophic oxidation of divalent iron by bacteria such as *Acidithiobacillus ferrooxidans* (see Sect. 6.3). Massive accumulations of iron hydroxides [$Fe(OH)_3$ and other forms] are often found in mine drainage waters, accompanied by low pH caused by autotrophic oxidation of reduced sulfur compounds (pyrite and others) present in many ores. Another organism that deposits trivalent iron is the autotrophic *Gallionella*, which produces iron oxide stalks. An intermediate state of oxidation as magnetite (Fe_3O_4) is found intracellularly in magnetotactic bacteria (see Sect. 3.5).

Under anaerobic conditions, trivalent iron can be reduced to divalent iron by iron-reducing bacteria such as *Geobacter* and *Shewanella* (see Sect. 5.4).

MANGANESE (Mn^{2+}, Mn^{4+})

Oxidized forms of manganese (Mn^{4+}) are formed as the result of the chemolithotrophic oxidation of divalent manganese (see Sect. 6.3).

Under anaerobic conditions, tetravalent manganese can be reduced to the divalent form in anaerobic respiration processes.

SELENATE (SeO_4^{2-}), SELENITE (SeO_3^{2-}), AND ELEMENTAL SELENIUM (Se^o)

Selenate can be used as an electron acceptor for anaerobic respiration, and is respired to selenite (SeO_3^{2-}) or to a mixture of selenite and elemental selenium. Furthermore, it can be taken up for assimilatory use by many microorganisms and used in the biosynthesis of selenocysteine, an unusual amino acid that is incorporated into some proteins.

ARSENATE (AsO_4^{3-}) AND ARSENITE (AsO_3^{3-})

Arsenate can be used as an electron acceptor for anaerobic respiration by a number of bacteria, who reduce it to arsenite (AsO_3^{3-}).

Arsenite can be used as an electron donor for chemoautotrophic arsenite oxidizers, both under aerobic conditions and anaerobically, using nitrate as electron acceptor, causing its oxidation to arsenate (AsO_4^{3-}).