Chapter 3

Thermodynamics and Microbial Metabolism

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1. INTRODUCTION

Life, like all chemical processes, obeys the laws of thermodynamics. Indeed, life has evolved to exploit them. For example, all life on Earth has associated reaction pathways, such as the utilization of adenosine triphosphate (ATP) (see Section 8.4), where seemingly thermodynamically unfavorable reactions can be promoted. Furthermore, life has evolved to survive by using countless energetically favorable oxidation-reduction reactions provided by the natural environment. Also, the competition between microbial populations in nature, as well as their mutualistic cohabitation, is often governed by thermodynamic considerations. Thermodynamic considerations explain the mechanisms of chemical transport of materials into and out of microbial cells, and the cell membrane itself typically houses a symphony of chemical processes engaged in energy conservation. An appreciation of chemical thermodynamics, therefore, provides us with a window into basic aspects of microbial metabolism. Furthermore, many fundamental principles of microbial ecology are explained by thermodynamics.

In this Chapter we consider basic aspects of chemical thermodynamics as relevant for understanding microbial metabolisms in nature and for defining the chemical environments of the microbial world. In addition, we consider cellular architecture and its relationship to how organisms gain energy for their growth and metabolism. Finally, we discuss some of the basic aspects of cellular metabolism and explain how different metabolisms are named.

2. STATE FUNCTIONS

2.1. Enthalpy

The First Law of Thermodynamics states that energy cannot be created or destroyed. As a natural outcome of this law (see Stumm and Morgan, 1996, for formal derivations), a function called enthalpy, ΔH, is defined. Enthalpy is equivalent to the heat added to, or subtracted from, a system as a result of a chemical process at constant pressure; it is also known as the heat of reaction. By convention, when a system evolves heat, ΔH is negative, and the reaction is exothermic. By contrast, when heat is absorbed, ΔH is positive, and the reaction is endothermic. Enthalpy is one of several functions defining the state of a system regardless of the system's prior history. Therefore, enthalpy is called a state function. We define ΔH^{o} relative to a standard state (STP), taken usually as one atmosphere (1 ATM) total pressure and 25 °C (298.15 K). Other standard states may be defined, but

this one is of particular relevance for many (but not all) biological systems at the Earth's surface, and it is the standard state for which most thermodynamic data are available. Enthalpies for individual compounds or elements are given, by convention, as the heat of reaction necessary to form, isothermally, one mole of a substance from its elementary components at standard state. Enthalpies of formation at standard state are designated as ΔH_f^o, and the ΔH_f^o of the most stable phase of the element is taken as 0 kJ/mole. For example, the ΔH_f^o for elemental sulfur (S^o), oxygen gas (O_2), and graphite ($C_{graphite}$) are all 0 $kJ\,mol^{-1}$, whereas the ΔH_f^o for diamond ($C_{diamond}$) is not, at 1.88 $kJ\,mol^{-1}$. Diamond is the high-pressure phase of elemental carbon.

If we write a general reaction (Equation 3.1) in which n_1 is the number of moles of compound of element X_1,

$$n_1X_1 + n_2X_2 \rightarrow n_3X_3 + n_4X_4 \tag{3.1}$$

the enthalpy, ΔH^o, for this reaction is calculated from Hess's Law of Summation as

$$(n_3\Delta H^o_{f,X_3} + n_4\Delta H^o_{f,X_4}) - (n_1\Delta H^o_{f,X_1} + n_2\Delta H^o_{f,X_2}) \tag{3.2}$$

or, more generally,

$$\Delta H^o = \Sigma(n_i\Delta H^o_{fi})_{products} - \Sigma(n_i\Delta H^o_{fi})_{reactants} \tag{3.3}$$

As a specific example we consider the dissolution of NaCl, written as $NaCl_{(s)} \rightarrow Na^+_{(aq)} + Cl^-_{(aq)}$. The ΔH^o for this reaction is $\Delta H^o = (1 * \Delta H^o_{f,Na^+} + 1 * \Delta H^o_{f,Cl^-}) - (1 * \Delta H^o_{f,NaCl}) = 3.63\ kJ\,mol^{-1}$. This is an endothermic reaction. Values of ΔH_f^o for compounds and elements of biological and geochemical interest are compiled in Appendix 2 and Appendix 3.

2.2. Entropy and Gibbs free energy

The endothermic dissolution of NaCl demonstrates that chemical reactions need not be exothermic to be spontaneous. This is because the spontaneity of a reaction is governed also by another attribute of the reacting system; this is an outcome of the Second Law of Thermodynamics. The second law may be stated in various ways, but for our purposes a good definition is "for any spontaneous process there is an increase in the entropy of the universe." We thus define entropy, S, as a new state function. "The universe" consists of the reacting system and its surroundings; therefore, the change in the entropy of the universe, or ΔS_{total}, may be broken into its component parts as follows:

$$\Delta S_{\text{total}} = \Delta S_{\text{surroundings}} + \Delta S_{\text{system}} \tag{3.4}$$

At constant temperature and pressure, the entropy change in the surroundings is equivalent to the heat added to the surroundings by the system divided by the temperature (K) at which the heat is added:

$$\Delta S_{\text{surroundings}} = \Delta H_{\text{system}}/T \tag{3.5}$$

Equations 3.4 and 3.5 are combined and rearranged to yield

$$T\Delta S_{\text{total}} = -(\Delta H_{\text{system}} - T\Delta S_{\text{system}}) \tag{3.6}$$

Since ΔS_{total} must be positive for a spontaneous change, so must $T\Delta S_{\text{total}}$. In turn, the term $(\Delta H_{\text{system}} - T\Delta S_{\text{system}})$ must be negative for spontaneous change. From here we define a new state function, called Gibbs free energy, G, such that

$$\Delta G = \Delta H - T\Delta S, \text{or at standard state, } \Delta G^{\text{o}} = \Delta H^{\text{o}} - T\Delta S^{\text{o}} \tag{3.7}$$

If ΔG for a reaction is negative, then the reaction is spontaneous, and it is known as an exergonic reaction. If ΔG is positive, then the reaction is spontaneous written in the other direction, or requires energy to proceed in the direction written, and the reaction is known as endergonic. If ΔG is equal to 0, then the system is at equilibrium. The driving force for a reaction depends on its ΔG, so the more negative ΔG, the more favorable the reaction. The Gibbs free energy for reaction at standard state, ΔG^{o}, is calculated in a way comparable to how enthalpy, ΔH^{o} is calculated (Equation 3.3):

$$\Delta G^{\text{o}} = \Sigma(n_i \Delta G^{\text{o}}_{\text{f}i})_{\text{products}} - \Sigma(n_i \Delta G^{\text{o}}_{\text{f}i})_{\text{reactants}} \tag{3.8}$$

and through a calculation of Gibbs free energy, we can evaluate whether a chemical reaction should be thermodynamically favorable. We therefore obtain a powerful tool for understanding the chemical circumstances favoring specific microbial metabolisms. We can also predict the possibility of new microbial metabolisms not previously described. However, standard state (STP) Gibbs free energy, ΔG^{o}, does not represent the normal chemical circumstances experienced by microorganisms in nature. Most significantly, concentrations of chemical species are generally much less than the unit molar concentrations used to calculate ΔG^{o}.

Thus, for any component of a system whose concentration deviates from standard conditions, the Gibbs free energy of that component can be determined from the following:

$$\Delta G_i = \Delta G^{\text{o}}_i + RT\ln a_i \tag{3.9}$$

where R is the gas constant (=8.314 J mol^{-1} K^{-1}), T is temperature in Kelvin (K), and a_i is a quantity known as the activity. Activity is related to concentration, c (molar), through the activity coefficient (dimensionless), γ.

$$a_i = \gamma_i c_i \tag{3.10}$$

For a gas,

$$f_i = \gamma_{fi} P_i \tag{3.11}$$

where f_i is fugacity, γ_{fi} is the fugacity coefficient and P_i is pressure. Activity coefficients and fugacity coefficients represent the deviation of the component of interest from ideal behavior in solutions and gases. For ions in solution, for example, the electrical interactions with other ions and the crowding of ions in concentrated solutions cause the ions to interact at effective concentrations different from the actual concentrations in the solution. These issues will be considered in more detail in Section 3.5.

2.2.1. Gibbs free energy under environmental conditions

Equation 3.9 considers how the Gibbs free energy of individual components of a system is influenced by deviations from unit molar concentration, but what about the reacting system as a whole? Consider the following general reaction, which could represent any biologically important chemical process:

$$a\mathrm{A} + b\mathrm{B} \leftrightarrow c\mathrm{C} + d\mathrm{D} \tag{3.12}$$

The components are indicated by the capital letters, and the stoichiometric amounts of each component are indicated by the lowercase letters. The Gibbs free energy of this system, ΔG, is related to the Gibbs free energy at standard state, ΔG^{o} by the following expression:

$$\Delta G = \Delta G^{\mathrm{o}} + RT\ln(a_{\mathrm{C}}^{\mathrm{c}} a_{\mathrm{D}}^{\mathrm{d}})/(a_{\mathrm{A}}^{\mathrm{a}} a_{\mathrm{B}}^{b}) \tag{3.13}$$

Let us consider the thermodynamics of the rather newly discovered microbial disproportionation of elemental sulfur (see Chapter 9). Sulfur-disproportionating organisms are anaerobic and have been found in abundance in anoxic surface sediments (Thamdrup *et al.*, 1993). The general reaction is written as

$$4\mathrm{H_2O_{(l)}} + 4\mathrm{S^o_{(s)}} \rightarrow 3\mathrm{H_2S_{(aq)}} + \mathrm{SO^{2-}_{4(aq)}} + 2\mathrm{H^+_{(aq)}}$$

The ΔG^o for this reaction is, from Equation 3.8,

$$\Delta G^o = 2[0] + [-744.6] + 3[-27.87] - [4(0) + 4(-237.18)] = 120.5 \text{ kJ mol}^{-1}$$
$$\quad H^+_{(aq)} \quad SO^{2-}_{4(aq)} \quad H_2S_{(aq)} \quad S^o_{(s)} \quad H_2O_{(l)}$$

This reaction is not thermodynamically favorable under standard conditions, yet we know the organisms promoting S^o disproportionation thrive in nature. Let us explore the free energy change actually experienced by S^o disproportionating organisms in nature. We assume a temperature of 25 °C. Furthermore, the concentration of $H_2S_{(aq)}$ is taken as 100 μM, a value not atypical in near-surface marine sediments, and a seawater $SO^{2-}_{4(aq)}$ concentration of 28 mM is used. We assume a sediment pH value of 7.5, and furthermore, we assume that activity equals concentration. The activities of liquid water and solid phases are set as 1 by definition, and from Equation 3.13 we calculate the ΔG for S^o disproportionation under environmental conditions as

$$\Delta G = 120.5 + (8.314 \times 10^{-3})\ (298)\ \ln(a^3_{H_2S} a_{SO_4} a^2_{H^+}) = -42.4 \text{ kJ mol}^{-1}$$

This is a thermodynamically favorable reaction and explains how S^o disproportionation can occur in sediments where the concentrations of reacting species differ markedly from standard state.

3. EQUILIBRIUM

All thermodynamically favorable chemical reactions will proceed, barring kinetic barriers, until the distribution of reacting components in the system reaches equilibrium. The distribution of chemical species at equilibrium is defined by the equilibrium constant, K_{eq}, which, for the general reaction presented in Equation 3.12, is given by

$$K_{eq} = (a^c_C a^d_D)/(a^a_A a^b_B) \tag{3.14}$$

Furthermore, at equilibrium, the ΔG for a reaction is equal to 0. Thus, after setting ΔG to 0 and substituting Equation 3.14 into Equation 3.13, we obtain the following:

$$\Delta G^o = -RT \ln K_{eq} \tag{3.15}$$

This expression may be rearranged and used to calculate equilibrium constants from ΔG^o.

$$\ln K_{eq} = -\Delta G^o / RT$$

or,

$$K_{eq} = e^{-\Delta G^o/RT} \tag{3.16}$$

4. INFLUENCE OF TEMPERATURE ON THERMODYNAMIC PROPERTIES

Microorganisms, of course, generally live at temperatures different from the standard state temperature (25 °C) at which thermodynamic properties are typically defined. Therefore, to fully appreciate the thermodynamics surrounding microbial metabolism in a given environment, thermodynamic calculations should be corrected for temperature deviations from the standard state. As long as deviations are within approximately 20 °C of the standard state temperature, the influence of temperature on ΔG^o can be evaluated directly from Equation 3.7, whereas the influence of temperature on K_{eq} can be evaluated from Equation 3.16. For temperature differences greater than approximately 20° from the standard state, ΔH and ΔS must be recalculated from heat capacity data, and these new values then can be used to recalculate ΔG and K_{eq}. This topic falls beyond the subjects we wish to emphasize in the present Chapter.

5. ACTIVITY COEFFICIENT CALCULATIONS

Ionic species interact with each other in solution relative to their respective activities, not concentrations (Equation 3.10). Therefore, in order to evaluate the state of chemical equilibrium of ionic components of a biological system, we must calculate the activities of the components, which requires a calculation of activity coefficients. Derived from first principles, the Debye-Hückel equation is frequently used to calculate activity coefficients. The activity coefficient for any component "i" is calculated as follows:

$$-\log\gamma_i = AZ_i^2(I)^{\frac{1}{2}} \tag{3.17}$$

where A is a constant depending only on temperature and pressure (Table 3.1), Z_i is the charge of the ion, and I is the ionic strength of the solution. This is calculated as

$$I = \frac{1}{2}\Sigma m_i Z_i^2 \tag{3.18}$$

Table 3.1 Parameters used for the Debye-Hückel equation at 1 atm

Temp (°C)	*A*
0	0.4883
5	0.4921
10	0.4960
15	0.5000
20	0.5042
25	0.5085
30	0.5130
35	0.5175
40	0.5221
45	0.5271
50	0.5319
55	0.5371
60	0.5425

From Garrels and Christ (1965).

where m_i is the molar concentration of the species of interest. The Debye-Hückel equation is, however, only valid for dilute solutions with ionic strengths (I) of $<5 \times 10^{-3}$ M. Empirically based extensions of the Debye-Hückel expression have been proposed, and a common alternative, used for ionic strengths up to 0.5 M, is a simplified version of the Davies equation:

$$-\log\gamma_i = [AZ_i^2(I)^{\frac{1}{2}}/(1 + (I)^{\frac{1}{2}})] + 0.2I \tag{3.19}$$

As an example, let us calculate the activity coefficient for Ca^{2+} in lake water with the following major ion composition:

Ionic species	mM
Na^+	5
K^+	1
Ca^{2+}	1
Mg^{2+}	1
HCO_3^-	1
Cl^-	5
SO_4^{2-}	2

The ionic strength for the solution is $I = \frac{1}{2}(5*1^2 + 1*1^2 + 1*2^2 + 1*2^2 + 1*1^2 + 5*1^2 + 2*2^2) \times 10^{-3} = 14 \times 10^{-3}$ M, and using the Davies equation, the activity coefficient for Ca^{2+} at 20 °C is 0.58. This is quite a deviation from ideal behavior.

6. GAS SOLUBILITY AND HENRY'S LAW

We know from common experience that gases dissolve into water. We also know that the amount of gas held by water is highly sensitive to salt and temperature. Thus, concentrated brine holds very little gas, and we know that as we bring a kettle of water to boil massive bubble formation occurs, representing solution degassing, well before the boiling actually begins. The amount of gas dissolved into water is expressed by equilibrium thermodynamics similar to what we have already seen. Thus, at equilibrium, and for dilute solutions and low gas pressures, the relationship between the partial pressure of gas in the gaseous phase and the amount of gas dissolved in water is given by Henry's Law and is expressed as

$$C_{(\mathrm{aq})i} = P_i K_{Hi} \tag{3.20}$$

where $C_{(\mathrm{aq})i}$ (M) is the concentration of the gas, in aqueous solution, P_i (atm) is the partial pressure of the gas and K_{Hi} is the Henry's Law constant (M atm^{-1}). A list of the Henry's Law constants for gases of biological interest at 25 °C is presented in Table 3.2. In concentrated solutions, and at high pressures, the concentration, $C_{(\mathrm{aq})i}$, should be replaced with activity, a_i (Equation 3.10), and partial pressure, P_i, should be replaced with fugacity, f_i (Equation 3.11). We will assume that fugacity equals partial pressure for atmospheric constituents.

Table 3.2 Henry's law constants (K_H, M atm^{-1}), salting out coefficient (k_i) and heats of solution (ΔH_{sol}) for some gases of biological interest at 25 °C

Gas	K_H	k_i	(kJ mol^{-1}) ΔH_{sol}
CO_2[a]	3.4×10^{-2}	0.095	19.6
NH_3	5.7×10^{1}		
H_2S	1.0×10^{-1}	0.02	
N_2	6.5×10^{-4}	0.131	8.8
O_2	1.3×10^{-3}	0.122	12.5
CO	9.6×10^{-4}	0.134	15.9
CH_4	1.3×10^{-3}	0.092	13.4
NO_2	1.0×10^{-2}		
NO	1.9×10^{-3}		11.3
N_2O	2.5×10^{-2}		20.1
H_2O_2	1.0×10^{5}		
O_3	9.4×10^{-3}		
H_2	7.8×10^{-4}		1.3

[a] $CO_{2(g)} + H_2O_{(g)} \leftrightarrow H_2CO_{3(aq)}$.

Data from Stumm and Morgen (1996) and Millero (1996).

6.1. Influence of salt on gas solubility

The "salting out" of gases occurs in solution as the ionic strength, I, of the solution increases. The activity coefficient, γ_{gl}, for a gas in solution is approximated as

$$\gamma_{gl} = 10^{k_i I} \tag{3.21}$$

and the concentration of a gas in solution $C_{(aq)i}$ is related to the concentration in an infinitely dilute solution, $C^{o}_{(aq)i}$, through the activity coefficient as follows:

$$C_{(aq)i} = C^{o}_{(aq)i}/\gamma_{gl} \tag{3.22}$$

Values for k_i vary from gas to gas, but 0.1 is typical (Table 3.2). So, let us compare the solubility of O_2 in both dilute solution and sea water. The concentration of $O_{2(aq)}$ in dilute solution in equilibrium with atmospheric oxygen at 25 °C ($p_{O_2} = 0.21$ atm) is, from Equation 3.20 (and the K_H value from Table 3.2), 2.64×10^{-4} (M), or 264 μM. The k_i (Equation 3.21) value for O_2 is 0.122 (Table 3.2), and the ionic strength of sea water, I, is 0.7. Thus, an activity coefficient, γ_{gl}, for $O_{2(aq)}$ in seawater of 1.22 is calculated, yielding an air equilibrium concentration for $O_{2(aq)}$ of 217 μM, a significant reduction in solubility over the dilute solution.

6.2. Influence of temperature on gas solubility

All gases decrease their solubility as water temperature increases, by amounts varying from gas to gas. The influence of temperature on gas solubility may be approximated from the Clausius-Clapeyron equation in the form

$$\ln(C_0/C_1) = \Delta H_{sol}/R[1/T_1 - 1/T_0] \tag{3.23}$$

which rearranges to the following:

$$\ln(C_1) = \ln(C_0) - \Delta H_{sol}/R[1/T_1 - 1/T_0] \tag{3.24}$$

where ΔH_{sol} is the heat of solution for the gas in water at 25 °C (Table 3.2), R is the gas constant, C_0 is the saturation concentration of the gas at 25 °C, C_1 is the concentration at the desired temperature, T_1 is the desired temperature (K), and T_0 is the standard state temperature of 298.15 K. This equation is useful for relatively small deviations around the standard state temperature.

7. OXIDATION-REDUCTION

The life process is intimately coupled to oxidation-reduction reactions, including electron transfers associated with carbon fixation, fermentation, proton potential generation leading to ATP formation, and countless biochemical processes within the cell. Also, many important microbial respiration reactions are coupled to the oxidation of organic carbon with electron acceptors such as oxygen, nitrate, and sulfate. The localization of organisms promoting these different respiration reactions in the environment depends on the availability of the electron acceptor and also very much on the thermodynamics of the respiration reactions. Therefore, understanding oxidation-reduction allows us to appreciate aspects of cellular metabolism, as well as the ecology of microbes in nature.

7.1. Half reactions and electrode potential

Oxidation-reduction reactions, also known as redox reactions, involve electron transfer. If a chemical species gains electrons it is said to be reduced, whereas if it loses electrons it is said to be oxidized. Electrons do not accumulate in solution, so all oxidation reactions must be coupled to reduction reactions, and vice versa. It is easiest to consider oxidation and reduction reactions separately. For example, the photosynthetic production of oxygen gas can be broken into its component oxidation and reduction reactions. Each of these is known as a half reaction and represents a redox pair or redox couple:

$$4H^+ + CO_2 + 4e^- \rightarrow CH_2O + H_2O \ (\text{reduction, gain of } e^-)$$

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \ (\text{oxidation, loss of } e^-)$$

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \ (\text{overall, reduction + oxidation})$$

The ease with which chemical species gain or lose electrons varies greatly, and the ability of a chemical species to gain or liberate electrons is referred to as the electrode potential, E, often called the redox potential.

By convention, electrode potentials are compared for reactions written as reduction reactions. Furthermore, electrode potentials are generally reported relative to a standard redox reaction known as the standard hydrogen electrode, SHE. The SHE represents the following half reaction, written as a reduction reaction:

$$2H^+_{(aq)} + 2e^- \rightarrow H_{2(g)}(\text{SHE}, a_{H^+} = 1, p_{H_2} = 1\,\text{atm})$$

under the standard conditions of 1 atm $H_{2(g)}$ and 1 M $H^+_{(aq)}$ activity. The SHE is given a standard electrode potential, E^o, of 0.000 volts. The standard electrode potential of other redox pairs at unit activity may be determined directly by coupling to a SHE, or alternatively, relative to other reference electrodes such as the calomel electrode ($Hg_2Cl_{2(s)} + 2e^- \rightarrow 2Hg_{(l)} + 2Cl^-_{(aq)}, E^o = 0.241$ V), with appropriate corrections back to the SHE scale. When written relative to the SHE, electrode potential is designated as E^o_H.

The oxidized form of the redox pair with the most positive E^o_H value is the strongest oxidant. By contrast, the reduced form of the redox pair with the most negative E^o_H value is the strongest reductant. A list of E^o_H values for important half reactions of biological interest is shown in Table 3.3, arranged in order of decreasing electrode potential. An ordered arrangement like this is known as an electrochemical series. Of particular importance is the fact that the oxidized form of a redox pair will oxidize the reduced form of a redox pair lower in the electrochemical series. Therefore, sulfate can act as an oxidant for methane, but not for Fe^{2+}. Of course, the conditions expressed in Table 3.3 are standard conditions that are not normally met in the environment. We will see below how to make calculations under natural conditions.

Table 3.3 E^o_H values for some redox pairs of biological interest at 25 °C, calculated from Equation 3.25 and at standard state

Note	Oxidized form		Reduced form	E^o_H (v)
	$NO_2^- + 4H^+ + 4e^-$	$\rightarrow$	$1/2N_{2(g)} + 2H_2O$	1.51
	$O_{2(aq)} + 4H^+ + 4e^-$	$\rightarrow$	$2H_2O$	1.27
	$NO_3^- + 6H^+ + 5e^-$	$\rightarrow$	$1/2N_{2(g)} + 3H_2O$	1.24
Pyrolusite	$MnO_2 + 4H^+ + 2e^-$	$\rightarrow$	$Mn^{2+} + 2H_2O$	1.23
	$NO_3^- + 10H^+ + 8e^-$	$\rightarrow$	$NH_4^+ + 3H_2O$	0.88
Amorphous oxide	$Fe(OH)_3 + 3H^+ + e^-$	$\rightarrow$	$Fe^{2+} + 3H_2O$	0.88
	$NO_3^- + 2H^+ + 2e^-$	$\rightarrow$	$NO_2^- + H_2O$	0.85
	$Fe^{3+} + e^-$	$\rightarrow$	Fe^{2+}	0.77
	α-$FeOOH + 3H^+ + e^-$	$\rightarrow$	$Fe^{2+} + 2H_2O$	0.67
	$SO_4^{2-} + 8H^+ + 6e^-$	$\rightarrow$	$S^0_{(s)} + 4H_2O$	0.35
	$SO_3^{2-} + 2H^+ + 2e^-$	$\rightarrow$	$H_2S_{(aq)} + 3H_2O$	0.34
	$S^0_{(s)_2} + 2H^+ + 2e^-$	$\rightarrow$	$H_2S_{(aq)}$	0.29
	$SO_4^{2-} + 10H^+ + 8e^-$	$\rightarrow$	$H_2S_{(aq)} + 4H_2O$	0.23
	$HCO_3^- + 9H^+ + 8e^-$	$\rightarrow$	$CH_{4(aq)} + 3H_2O$	0.21
Acetate	$HCO_3^- + 9/2H^+ + 4e^-$	$\rightarrow$	$1/2C_2H_3OO^- + 2H_2O$	0.19
Ethanol	$HCO_3^- + 7H^+ + 6e^-$	$\rightarrow$	$1/2C_2H_5OH + 5/2H_2O$	0.17
Lactate	$HCO_3^- + 14/3H^+ + 4e^-$	$\rightarrow$	$1/3C_3H_5O_3^- + 2H_2O$	0.16
Glucose	$HCO_3^- + 5H^+ + 4e^-$	$\rightarrow$	$1/6C_6H_{12}O_6 + 2H_2O$	0.10
	$2H^+ + 2e^-$	$\rightarrow$	$H_{2(g)}$	0.00

Table 3.4 Thermodynamics of glucose oxidation with oxygen

Reaction	E_H^o(v)	ΔG^o (kJ)	$\ln k_{eq}$
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	1.27	−490.68	198
$1/6C_6H_{12}O_6 + 2H_2O \rightarrow 5H^+ + HCO_3^- + 4e^-$	−0.10	40.4	−15.6
$1/6C_6H_{12}O_6 + O_2 \rightarrow HCO_3^- + H^+$	1.17	−450.28	182.4

Values for the electrode potential are independent of the number of electrons transferred in the balanced equation. They also are additive. As an example, we can consider the electrode potential associated with the oxidation of glucose with oxygen (Table 3.4). We compile first the electrode potentials for the individual half reactions and then sum these to obtain the electrode potential for the overall reaction. Note that the oxidation of glucose to carbon dioxide is an oxidation reaction (Table 3.4), and the sign of the electrode potential associated with this reaction is reversed from the value presented in Table 3.3.

7.2. Gibbs free energy and electrode potential

Electrode potentials are related to the Gibbs free energy, ΔG, through the following relationship:

$$\Delta G = -nFE, \text{or relative to SHE, } \Delta G^o = -nFE_H^o \qquad (3.25)$$

where n is the number of electrons transferred in the reaction, either half reaction or coupled oxidation-reduction reaction, and F is Faraday's constant, with a value of 96.53 kJ volt^{-1}. Thus, as opposed to the electrode potential, the free energy change of a reaction depends on reaction stoichiometry and the number of electrons transferred. As discussed earlier, thermodynamically favorable reactions are given by negative values for ΔG. Therefore, positive values for electrode potential, E or E_H^o, represent favorable reactions, and negative values for E or E_H^o indicate that the reaction is favorable in the opposite direction.

An example is given in Table 3.4, where the standard state free energy change, ΔG^o, associated with the oxidation of glucose with oxygen is calculated from the electrode potential, E_H^o, for the individual redox couples using Equation 3.25. The ΔG^o is −450.3 kJ mol^{-1} per mole of O_2, and the reaction is clearly favorable. The ΔG^o of a coupled oxidation-reduction reaction may also be calculated from the ΔG^o of the individual reactants and products as shown in Equation 3.8, or from entropy and enthalpy data as shown in Equation 3.7.

7.3. Equilibrium constant and electrode potential

The electrode potential is also related to the equilibrium constant, K_{eq}, for the reaction. Thus, we can combine Equation 3.15 with Equation 3.25 to yield the following relationship:

$$\ln K_{eq} = nFE^{o}_{H}/RT \tag{3.26}$$

Using this equation, the equilibrium constants for the individual half reactions, as well as for the overall reaction expressing the oxidation of glucose with oxygen, are shown in Table 3.4. For balanced oxidation-reduction reactions, K_{eq} may also be computed from free energy data, as shown in Equation 3.15.

7.4. Electrode potential in non-standard conditions

The standard conditions represented by E^{o}_{H} values are rarely found in nature, except perhaps in some extreme examples of acid production in abandoned metal sulfide mines. In order to represent realistic natural conditions, and to accommodate the variability of chemical environments found in nature, we must calculate electrode potentials for situations far removed from the standard state. Consider a reduction half reaction of the following general form:

$$a\mathrm{A}_{oxid} + b\mathrm{e}^{-} + c\mathrm{H}^{+} \rightarrow d\mathrm{A}_{red} - g\mathrm{G} \tag{3.27}$$

Here, A refers to a redox-active species undergoing reduction, and G is a possible non-redox active reaction product, while a, c, d, and g are stoichiometric coefficients. The electrode potential for this reaction under non-standard conditions is determined from the Nernst equation:

$$E = E^{o}_{H} + (RT/nF)\ln(a^{a}_{A_{oxid}} a^{c}_{H^{+}})/(a^{d}_{A_{red}} a^{g}_{G}) \tag{3.28}$$

Note that the oxidized form of the redox pair is in the numerator while the reduced form is in the denominator, and the electron does not enter into the equation. To see how this equation is used, consider the reduction of nitrate to nitrogen gas at 25 °C, a pH of 7, a nitrate concentration of 30 μM and a partial pressure of nitrogen gas of 0.78 atm. We assume concentration equals activity and partial pressure equals fugacity, and with E^{o}_{H} values from Table 3.3:

$$\mathrm{NO}_3^{-} + 6\mathrm{H}^{+} + 5\mathrm{e}^{-} \rightarrow \frac{1}{2}\mathrm{N}_{2(g)} + 3\mathrm{H}_2\mathrm{O} \tag{3.29}$$

Thus,

$$E = 1.24 + [(8.314 * 10^{-3} * 298.15)/(5 * 96.53)] \ln[(30 * 10^{-6})(10^{-7})^6/(0.78)^{\frac{1}{2}}] = 0.69 \text{ V} \quad (3.30)$$

The electrode potential associated with nitrate reduction to nitrogen gas under typical environmental conditions is very different from the electrode potential relative to the SHE, as shown in Table 3.3.

Commonly, electrode potentials for biological and environmental systems are calculated relative to pH = 7. This is done to more faithfully represent the chemistry of the environment or of a cell, as opposed to the 1 M H^+ activity used for the SHE. Electrode potentials calculated in such a fashion are designated variably as $E^o(w)$, E_{m7} or E'_0, and the calculation is easily accomplished with Equation 3.28. Frequently, E'_0 values are arranged in an "electron tower" such as the one shown in Figure 3.1, and as for the electrochemical series presented in a tabular form (Table 3.3), the oxidized form of a redox pair can oxidize the reduced form of a redox pair lower on the tower. Still, electrode potentials calculated relative to a neutral pH are

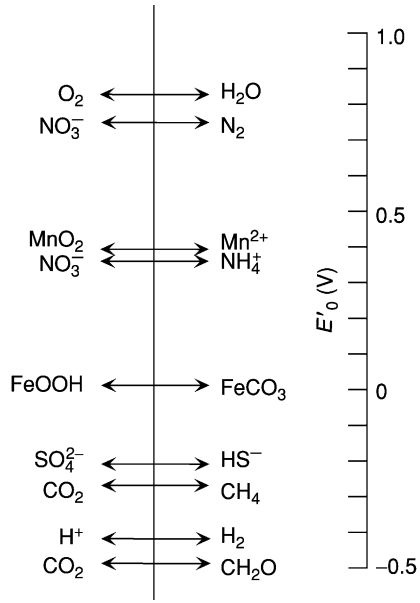

Figure 3.1 Electron tower showing the electrode potential of various oxidation-reduction pairs of environmental interest at a pH of 7, but otherwise at standard state. Concept after Fenchel *et al.* (1998).

approximations of the natural environment. Significantly, unit activity is assumed for reactants and products other than H^+, and furthermore, excursions from neutral pH are normal. Electrode potentials should be calculated for the chemistry of the specific environment of interest.

7.5. *pε*

Geochemists traditionally express redox intensity relative to the dimensionless parameter $p\varepsilon$, which gives the potential activity of electrons in solution and is defined as

$$p\varepsilon = -\log(a_{e^-}) \tag{3.31}$$

The activity of electrons, a_{e^-}, is only hypothetical, as already discussed; electrons do not accumulate free into solution. Rather, $p\varepsilon$ expresses the tendency of a redox pair to either liberate or accept electrons. The derivation of $p\varepsilon$ and its practical use is beyond the scope of the current Chapter; however, a straightforward relationship exists between $p\varepsilon$ and electrode potential:

$$p\varepsilon = [F/(2.303RT)]E, \text{ and } p\varepsilon^o = [F/(2.303RT)]E^o_H \tag{3.32}$$

8. BASIC ASPECTS OF CELL BIOCHEMISTRY

8.1. Energy gain, catabolism, and anabolism

Prokaryotes are clever little chemists. They exploit, with complex biochemical machinery, numerous energy-yielding chemical interfaces met within the environment. Indeed, microbial enzymes such as nitrogenase, promoting nitrogen fixation, and Rubisco, promoting carbon fixation in the Calvin cycle, easily perform chemical reactions that frustrate the bench chemist. Ultimately, usable energy within a cell is derived from electrons transferred in oxidation-reduction reactions. Light drives energy-gaining oxidation-reduction reactions in photosynthesis (see Chapter 4), while in the absence of light, energy may be gained from electron transfer between primary electron donors such as organic carbon and primary electron acceptors such as oxygen. This is known as respiration. Energy can also be gained from the fermentation of organic compounds, where the same organic molecule acts as both the electron donor and the electron acceptor. The breakdown of

organic and inorganic compounds by an organism, whether by respiration or by fermentation, is known as catabolism. Much of the energy gained during cellular catabolism, or from light (photosynthesis), is used for the biosynthesis of cell constituents from simple molecules. The process of biosynthesis, therefore, needs energy, and it is known as anabolism.

8.2. Mobile electron carriers

Regardless of the process from which the energy is derived or how it is used, the transfer of electrons in a cell relies on numerous different electron carriers. Mobile, freely diffusible electron carriers, of which coenzymes NAD^+/NADH and $NADP^+$/NADPH are the most common, are involved in oxidation-reduction reactions within the cell necessitating the transfer of hydride ($H^- = H^+ + 2e^-$). These co-enzymes are similar, with a low electrode potential, E'_0, of −0.32 V (Figure 3.2); however, NAD^+/NADH is used principally in catabolic pathways while $NADP^+$/NADPH is used in anabolic pathways. The oxidized forms of these electron carriers gain electrons, and become reduced, from redox pairs with lower electrode potential.

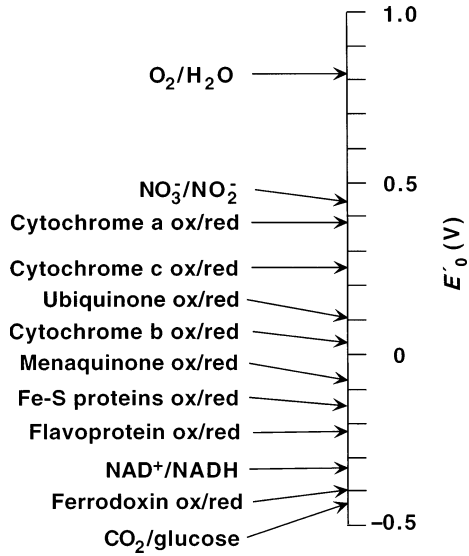

Figure 3.2 Electron tower showing the electrode potentials of various redox couples involved in electron transport chains leading to ATP formation by oxidative phosphorylation. Electrons may be transferred up the tower from redox couples with lower electrode potential to those with progressively higher electrode potentials. Electrode potentials are calculated at a pH of 7, but otherwise at standard state. Data from Thauer *et al.* (1977) and Madigan *et al.* (2003).

Once reduced, they can donate electrons to redox couples with a higher electrode potential. For example,

Substrate oxidation:

$$\begin{array}{c} NAD^+ + H^+ + 2e^- \rightarrow NADH \\ \hline Sub_{(red)} \rightarrow Sub_{(ox)} + 2e^- \\ \hline Sub_{(red)} + NAD^+ + H^+ \rightarrow Sub_{(ox)} + NADH \end{array}$$

Substrate reduction:

$$\begin{array}{c} NADH \rightarrow NAD^+ + H^+ + 2e^- \\ \hline Sub_{(ox)} + 2e^- \rightarrow Sub_{(red)} \\ \hline Sub_{(ox)} + NADH \rightarrow Sub_{(red)} + NAD^+ + H^+ \end{array}$$

A real-world example is the reduction of pyruvate to lactate, coupled to the oxidation of NADH to NAD^+:

$$Pyruvate + NADH + H^+ \rightarrow lactate + NAD^+$$

8.3. Membrane-bound electron carriers and oxidative phosphorylation

Electron carriers are also bound in the cell membrane, which is a semi-permeable barrier separating the inside of the cell from the environment (Figure 3.3). In most prokaryotic cells, a rigid protective layer, the cell wall, is found just outside of the cell membrane. Membrane-bound electron carriers are arranged in a series, comprising an electron transport system, and they promote the transfer of electrons between an electron donor and an electron acceptor. The transfer of electrons, however, is not direct, and numerous small steps are used to ensure that energy is conserved in a form that can be used by the cell. Several of the enzymes in an electron transport chain direct positively charged protons to the outer surface of the cell membrane (Figure 3.4), generating a voltage gradient across the membrane. A pH gradient is also established, and the combined electrical and proton gradients are known as a proton motive force. The relaxation of these gradients is carefully controlled through an enzyme known as ATPase, which couples the energy gained from the import of protons across the cell membrane, to the synthesis of ATP. The import of three to four protons is coupled to the production of one ATP. This process of ATP generation is known as oxidative phosphorylation and is the principal means of ATP formation during respiration and photosynthesis (see Chapter 4).

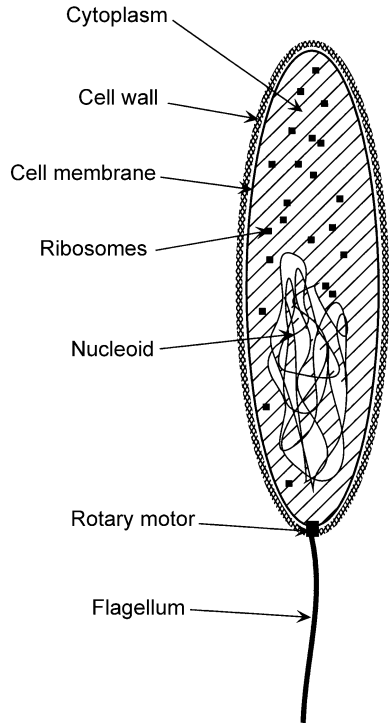

Figure 3.3 Key components of a gram-negative prokaryotic cell. Figure inspired by Margulis and Schwartz (1998).

8.4. ATP

As mentioned above, the energy gained from cellular catabolism and photosynthesis is derived ultimately from coupled oxidation-reduction reactions. These reactions are carefully controlled within the cell to maximize the transfer of chemical energy to the formation of ATP. ATP is constructed from the nucleoside adenosine (a ribose sugar combined with the nitrogen base adenine; see Chapter 1) connected to a triphosphate group through a phosphate ester linkage (Figure 3.5). The hydrolysis of the terminal phosphate on ATP, forming ADP (Figure 3.5), has a high-energy yield with a ΔG^{o} of approximately -32 kJ mol^{-1} of ATP (Thauer *et al.*, 1977). ATP drives to completion, in cooperation with the appropriate enzymes, otherwise thermodynamically unfavorable reactions. Consider the following generic example of an unfavorable biosynthetic reaction:

$$A + B \rightarrow C + D \text{ (unfavorable)} \tag{3.33}$$

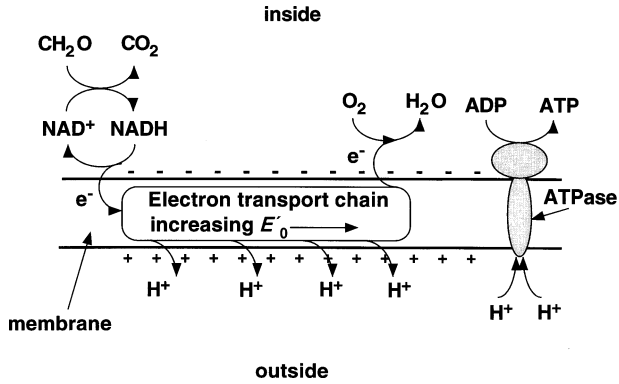

Figure 3.4 The principal features of ATP generation by oxidative phosphorylation. Electrons are derived at a low redox potential from the oxidation of a reduced electron donor. These electrons are passed through a series of membrane-bound redox couples, known as an electron transport chain, in which energy is conserved by translocating protons to the outer surface of the membrane. ATP is generated through the energy produced by the controlled mobilization of protons back into the cell through the enzyme ATPase. Electrons are finally consumed through the reduction of an electron acceptor, in this case oxygen, at a high redox potential.

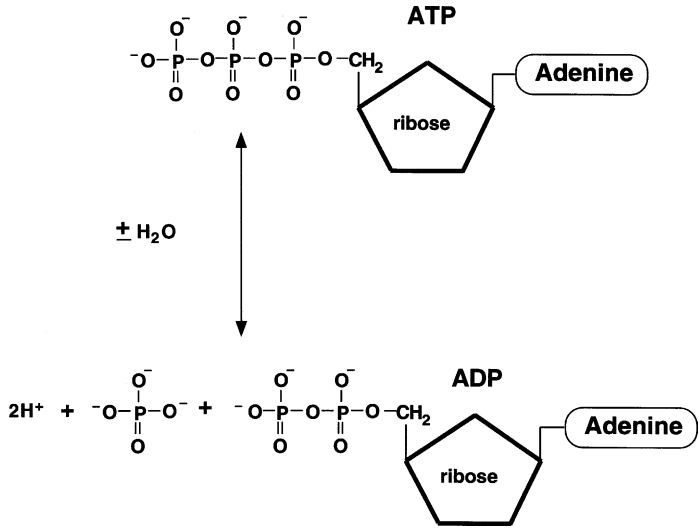

Figure 3.5 Schematic drawings of ATP and ADP and the relationship between the two.

This reaction sequence can be broken down into two favorable reactions with the release of energy during the hydrolysis of ATP and the formation of the high-energy intermediate compound, A-P. This is illustrated in Equations 3.34 and 3.35, which, upon addition, give the reaction in Equation 3.36, made favorable due to the hydrolysis of ATP to ADP.

$$\mathrm{A} + \mathrm{ATP} \rightarrow \mathrm{A\text{-}P} + \mathrm{ADP}\ (\text{favorable}) \tag{3.34}$$

$$\mathrm{A\text{-}P} + \mathrm{B} \rightarrow \mathrm{C} + \mathrm{D} + \mathrm{P}\ (\text{favorable}) \tag{3.35}$$

$$\mathrm{A} + \mathrm{ATP} + \mathrm{B} \rightarrow \mathrm{C} + \mathrm{D} + \mathrm{ADP} + \mathrm{P}\ (\text{favorable}) \tag{3.36}$$

An example is the reaction of glucose plus fructose to yield sucrose and water with an unfavorable ΔG^{o} of 23 kJ mol^{-1}:

$$\text{glucose} + \text{fructose} \rightarrow \text{sucrose} + \mathrm{H_2O},\ \Delta \mathrm{G^o} = 23\ \mathrm{kJ\,mol^{-1}}$$

However, when this reaction is coupled to the energy released during the hydrolysis of 2ATP to 2ADP, the formation of sucrose becomes favorable:

$$\text{glucose} + \text{fructose} + 2\mathrm{ATP} \rightarrow \text{sucrose} + 2\mathrm{ADP} + \mathrm{H_2O} + 2\mathrm{P}, \Delta \mathrm{G^o} = -41\ \mathrm{kJ\,mol^{-1}}$$

8.5. Fermentation and ATP generation

As mentioned previously, during fermentation organic compounds undergo coupled oxidation and reduction reactions, with no utilization of external electron acceptors such as oxygen or nitrate. Numerous different types of fermentation reactions are accomplished by microorganisms, and a few common fermentation pathways are presented below, including the fermentation of ethanol to acetate and H_2 gas (Equation 3.37), the fermentation of glucose to ethanol and CO_2 (Equation 3.38), the fermentation of glucose to lactate (Equation 3.39), and the fermentation of acetate to CO_2 and methane (Equation 3.40):

$$\mathrm{CH_3CH_2OH} + \mathrm{H_2O} \rightarrow \mathrm{CH_3COO^-} + 2\mathrm{H_2} + \mathrm{H^+} \tag{3.37}$$

$$\mathrm{C_6H_{12}O_6} \rightarrow 2\mathrm{C_2H_6O} + 2\mathrm{CO_2} \tag{3.38}$$

$$\mathrm{C_6H_{12}O_6} \rightarrow 2\mathrm{C_3H_4O_3^-} + 2\mathrm{H^+} \tag{3.39}$$

$$H^+ + CH_3COO^- \rightarrow CO_2 + CH_4 \quad (3.40)$$

The oxidation-reduction reactions involved in these fermentation reactions are obvious, except perhaps for the fermentation of glucose to lactate (Equation 3.39), in which the oxidation and reduction occurs between the carbon atoms in glucose and in lactate. Thus, if glucose is written as $HCO(HCOH)_4H_2COH$ and lactate as $CH_3(HCOH)COO^-$, we see that the methyl carbon in lactate is more reduced (charge of -3), and the carboxyl carbon is more oxidized (charge of $+3$), than any of the carbon atoms in glucose (range of -1 to $+1$).

Generally, oxidative phosphorylation is not used to generate ATP during fermentation. A notable exception is the fermentation of acetate to methane and CO_2 (acetoclastic methanogenesis), in which a unique biochemistry generates a proton potential that is used to form ATP through ATPase (see Chapter 10). In most cases, however, ATP is formed during fermentation through the formation of phosphorylated intermediates in a process known as substrate level phosphorylation. The ATP yield during fermentation is not high. For example, the fermentation of glucose generally yields 2–4 ATPs per molecule of glucose fermented, whereas the oxidation of glucose with oxygen produces 32 ATPs (Fenchel *et al.*, 1998). However, the main advantage to fermentation is that no external electron acceptor is required. As we shall see in Chapter 5, fermentation plays a critical role in the anaerobic degradation of organic material.

When H_2 is produced during fermentation, the energetics of the process depend critically on the ambient concentration of H_2. Thus, under standard conditions, the fermentation of ethanol to acetate and H_2, as shown in Equation 3.37, has a positive Gibbs free energy change, ΔG°, of 49.52 kJ mol^{-1} ethanol. This reaction is clearly not favorable, and even at a pH of 7, with equal concentrations of ethanol and acetate, ΔG is still unfavorable (from Equation 3.13) at 9.55 kJ mol^{-1} ethanol. With an H_2 partial pressure of 0.1 atm, the reaction becomes barely favorable with a ΔG of -1.87 kJ mol^{-1} ethanol, and it becomes increasingly more favorable as pH_2 decreases (Figure 3.6).

Due to the low solubility of H_2 gas in water (Table 3.2), an $H_{2(g)}$ partial pressure of 0.1 atm is equivalent to only 80 μM $H_{2(aq)}$ at 25 °C. It is obvious that to maintain active fermentation in natural environments, some mechanism must be in place to limit the accumulation of H_2 in solution. Therefore, active H_2 production also requires active H_2 removal, and this is accomplished with microbial metabolisms coupling H_2 as an electron donor with a variety of different electron acceptors.

Indeed, H_2 provides an excellent electron donor to numerous types of microbial metabolisms, including methanogenesis by CO_2 reduction,

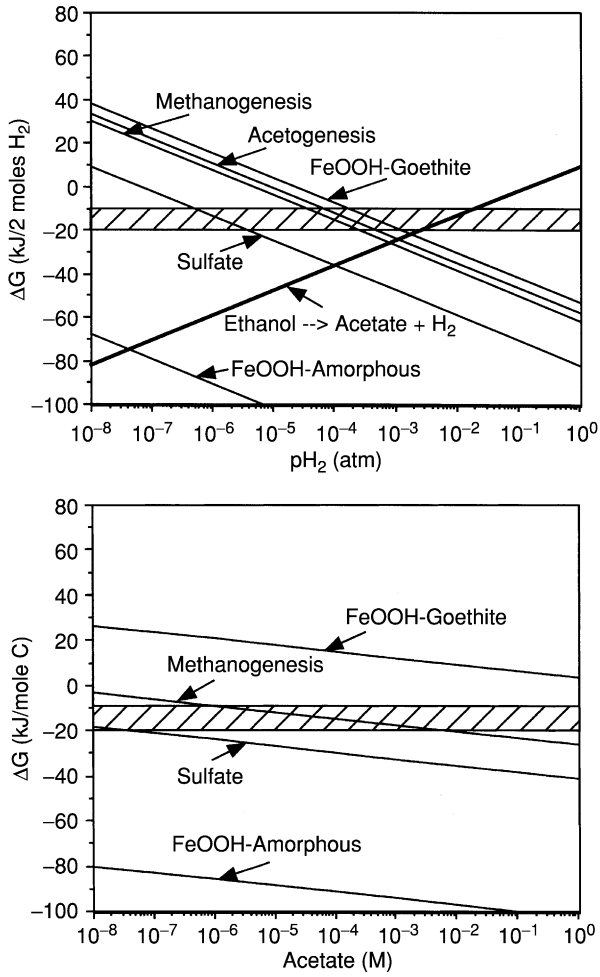

Figure 3.6 The Gibbs free energy change for mineralization reactions with various electron acceptors using H_2 and acetate as electron donors. Free energy has been calculated for reactions yielding four electrons transferred, at a pH of 7, and for reasonable environmental concentrations of reactants and products. Also shown is the free energy change associated with the fermentation of ethanol to acetate and H_2.

acetogenesis, sulfate reduction, iron reduction, manganese reduction, and others (Table 3.5). The transfer of H_2 between fermenting organisms and organisms utilizing H_2 is known as interspecies H_2 transfer. This is a syntrophic relationship (see Chapter 2) and is just one of many types of mutually beneficial metabolic associations found in nature. Similar to H_2, the

Table 3.5 Examples of H_2 consuming respiratory reactions in nature

Reaction	Organisms
$O_2 + 2H_2 \rightarrow 2H_2O$	Hydrogen bacteria
$2\ Fe(OH)_3 + H_2 + 4H^+ \rightarrow 2Fe^{2+} + 6H_2O$	Fe reducers
$MnO_2 + H_2 + 2H^+ \rightarrow Mn^{2+} + 2H_2O$	Mn reducers
$CO_2 + 4H_2O \rightarrow CH_4 + 2H_2O$	Methanogens
$2CO_2 + 4H_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	Acetogens
$H_2 + S^o \rightarrow H_2S$	Sulfur reducers
$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$	Sulfate reducers

accumulation of other fermentation products, such as acetate, may also affect the thermodynamics of the fermentation process. Thus, active fermentation also requires active removal of fermentation products other than H_2 (Lovley and Phillips, 1987).

8.6. Minimum energy for growth

To sustain growth, organisms need to utilize a reaction with a ΔG considerably lower than zero (Thauer *et al.*, 1977). The threshold for microbial growth is usually considered as the energy needed to produce ATP, and as mentioned previously, under standard state conditions the production of ATP from ADP has a free energy of $-32\ kJ\ mol^{-1}$. However, a larger ΔG of about $-50\ kJ\ mol^{-1}$ is required to produce ATP under the chemical conditions of a growing cell, where the concentrations of ATP, ADP, and phosphate deviate considerably from standard state (Schink, 1997). In addition, accounting for the energy lost as heat, ATP formation requires a ΔG of approximately $-70\ kJ\ mol^{-1}$ of ATP synthesized (Schink, 1997). This, however, is not the minimal energy needed for microbial growth. Recall that the formation of one ATP during oxidative phosphorylation is coupled to the mobilization of three to four protons across a semipermeable membrane. Therefore, the minimal metabolically convertible energy is considered to be the energy needed to translocate one proton, or to form 1/3 to 1/4 ATP. This is therefore around $-20\ kJ$ per 1/3 to 1/4 mole of ATP.

Anaerobic systems in nature are often poised at what appears to be a threshold near the minimal energy necessary to sustain microbial growth (Conrad *et al.*, 1986; Conrad, 1999). For example, when respiration reactions are written as four electron transfers (equivalent to the oxidation of one organic carbon; see below), the free energy gain associated with anaerobic metabolism during sulfate reduction, methanogenesis, and acetogenesis is consistently around $-20\ kJ\ mol^{-1}$ of organic carbon oxidized (Table 3.6).

Table 3.6 ΔG values for anaerobic mineralization processes *in situ* and in laboratory experiments with sediment slurries. Values are calculated from the chemistry of the environment or the slurry experiments

Process	ΔG (kJ per mole org C)[a]		Reference
Sulfate reduction	−23	±1.2	Hoehler *et al.* (1998)
Methanogenesis	−20	±0.6	Hoehler *et al.* (1998)
Methanogenesis	−15	±4	Lovley and Goodwin (1998)
Acetogenesis	−18	±1.1	Hoehler *et al.* (1998)

[a]Or equivalent, two moles of H_2 are equivalent to one mole of organic carbon.

As we shall see, this has implications for the competition between different anaerobic microbial populations for electron donors in the environment. Note that nitrate reduction and Mn reduction (and possibly also Fe reduction in some cases) conduct their metabolisms at energy yields considerably more negative than the minimal threshold discussed here (see below and also Hoehler *et al.*, 1998).

9. ENERGETICS OF ORGANIC MATTER MINERALIZATION DURING RESPIRATION

9.1. Free energy gain

The free energy gain associated with the oxidation of electron donors such as H_2, acetate or other organic compounds varies with the different electron acceptors. A careful consideration of these differences helps us to understand the stratification of microbial communities in environments such as sediments and anoxic water columns. To illustrate this point, we calculate the free energy gain associated with the oxidation of H_2 and acetate under standard state conditions (Table 3.7). The order of the sequence varies somewhat depending on the electron donor used. Also, the specific electron donors used in the calculation, H_2 and acetate, are more appropriate for anaerobic metabolisms (without O_2) than for aerobic metabolisms (utilizing O_2) (see Chapter 5). Nevertheless, we see that, consistent with numerous previous discussions (e.g., Berner, 1980), the greatest free energy gain is associated with oxic respiration, whereas the lowest free energy gain is associated with methanogenesis. Therefore, based strictly on energetic considerations, oxic respiration is the most favorable process of organic carbon mineralization, whereas methanogenesis is the least favorable. This sequence in free energy gain is roughly coincident with the depth distribution

Table 3.7 Standard Gibbs free energy calculated for the principal respiratory pathways of organic matter mineralization in nature, with H_2 and acetate as electron donors

Reaction	kJ per reaction	
	ΔG^0 (H_2)	ΔG^0 (acetate)[a]
Oxic respiration		
$O_2 + 2H_2 \rightarrow 2H_2O$	−456	–
$O_2 + 1/2C_2H_3O_2^- \rightarrow HCO_3^- + 1/2H^+$	–	−402
Denitrification		
$4/5H^+ + 4/5NO_3^- + 2H_2 \rightarrow 2/5N_2 + 12/5H_2O$	−460	–
$4/5NO_3^- + 3/5H^+ + 1/2C_2H_3O_2^- \rightarrow 2/5N_2 + HCO_3^- + 1/5H_2O$	–	−359
Mn reduction (pyrolusite)		
$4H^+ + 2MnO_2 + 2H_2 \rightarrow 2Mn^{2+} + 4H_2O$	−440	–
$7/2H^+ + 2MnO_2 + 1/2C_2H_3O_2^- \rightarrow 2Mn^{2+} + HCO_3^- + 2H_2O$	–	−385
Fe reduction (freshly precipitated amorphous FeOOH)		
$8H^+ + 4FeOOH + 2H_2 \rightarrow 4Fe^{2+} + 8H_2O$	−296	–
$15/2H^+ + 4FeOOH + 1/2C_2H_3O_2^- \rightarrow HCO_3^- + 4Fe^{2+} + 6H_2O$	–	−241
Sulfate reduction		
$H^+ + 1/2SO_4^{2-} + 2H_2 \rightarrow 2H_2O + 1/2H_2S$	−98.8	
$1/2H^+ + 1/2SO_4^{2-} + 1/2C_2H_3O_2^- \rightarrow 1/2H_2S + HCO_3^-$	–	−43.8
Methanogenesis		
$1/2H^+ + 1/2HCO_3^- + 2H_2 \rightarrow H_4 + 3/2H_2O$	−74.8	–
$1/2H_2O + 1/2C_2H_3O_2^- \rightarrow CH_4 + 1/2HCO_3^-$	–	−19.9

[a]Values are standardized to a four e^- transfer equivalent to the oxidation of one mole of organic carbon with a charge of 0, as in carbohydrates. Calculation conditions: 25 °C and unit activity for all reactants and products.

of electron acceptor utilization in sedimentary environments (Froelich *et al.*, 1979; Canfield *et al.*, 1993a,b). Thus, oxic respiration occurs highest in the sediment column, followed generally by denitrification, and so on, until finally methanogenesis occurs after the other electron acceptors are depleted.

9.2. Competition for electron donors

We can explore the underlying ecological reasons for this tendency of microbial communities to stratify in nature by considering the competition between microbial communities for substrate. This competition is dictated

by the energetics of the respiratory processes and, therefore, has an underlying thermodynamic rationale. We begin by calculating the free energy gain associated with the various significant respiratory processes, with both H_2 and acetate as electron donors. These calculations are performed at a pH of 7, and with realistic concentrations for all of the dissolved and gaseous species used or produced during bacterial metabolism (Figure 3.6).

The energetics of oxic respiration, denitrification, and Mn reduction are all highly favorable for environmental concentrations of H_2 and acetate, and they are not included in Figure 3.6. Considerations other than competition for an electron donor probably determine the stratification of these microbial populations. By contrast, the thermodynamic favorableness of Fe reduction, sulfate reduction, methanogenesis, and acetogenesis is highly dependent on electron donor concentration (Figure 3.6). This dependency, and the relative differences in the energetics of the processes, forms the basis for the competitive exclusion of one respiratory process over another. Thus, with H_2 as an electron donor, an H_2 partial pressure of 10^{-7} atm (equivalent to around 0.08 nM $H_{2(aq)}$, a typical value for a sediment supporting active Fe reduction; see below) allows a highly favorable free energy gain for Fe reduction of approximately -75 kJ per 2 moles of H_2 oxidized (for reactions see Table 3.7). However, whereas Fe reduction may proceed at 10^{-7} atm H_2, the other processes are not energetically favorable. Therefore, if Fe reducers can metabolize at H_2 partial pressures below those at which the other processes are thermodynamically favorable, then Fe reduction will dominate, and the other anaerobic respiration pathways will be inhibited. The energetics of Fe reduction, however, depend critically on the nature of the solid iron phase being reduced. Thus, while the reduction of amorphous FeOOH is favorable at a pH_2 of 10^{-7} atm, the reduction of crystalline goethite is highly unfavorable.

After amorphous iron oxides become utilized, pH_2 should rise until the energetics of the next respiration process becomes favorable. Thus, sulfate reduction becomes favorable at a pH_2 of around 10^{-5}. This level of H_2 will produce a free energy gain of -20 kJ mol^{-1} per 2 moles of H_2 oxidized, which should be sufficient to fuel microbial growth. Importantly, the other respiration processes, methanogenesis and acetogenesis, are not thermodynamically possible. Thus, if sulfate reducers can maintain a pH_2 near their threshold for growth, then methanogens and acetogens are inhibited. These processes become favorable after sulfate is depleted and pH_2 rises further. In a similar way, the maintenance of low acetate concentrations by Fe reducers can inhibit sulfate reducers (again, depending on the nature of the iron oxide), and sulfate reducers can inhibit methanogens at somewhat higher acetate concentrations (Figure 3.6).

In nature, it appears that Fe reducers, sulfate reducers, methanogens, and acetogens metabolize at near the minimum energy needed for ATP

Table 3.8 Concentrations of H_2 and acetate in sediments supporting different respiratory processes

Process	H_2 (nM)	Acetate (μM)
NO_3^- respiration	0.05–0.04	
Fe reduction	0.05	±0.1
Sulfate reduction	0.5–3.0	±0.2
Methanogenesis	2–12	±0.8
Acetogenesis	150 ± 50	

Data from Hoehler *et al.* (1998), Lovley and Phillips (1987a,b), and Lovley and Goodwin (1988).

generation and growth (Lovley and Goodwin, 1988; Hoehler *et al.*, 1998; Conrad, 1999). By doing so, the concentrations of electron donors are maintained too low for other processes with a lower energy yield. Supporting this scenario is a strong relationship between H_2 concentration and the type of microbial metabolism occurring in sediments (Table 3.8).

This, however, is only part of the story. The calculations presented in Figure 3.6 have been made at a constant pH of 7. In the environment, pH may range greatly, but values between 6 and 9 are common. Furthermore, the thermodynamics of some of the respiratory processes are highly pH dependent, and of the processes considered here, Fe reduction is the most highly affected. Thus, Fe reduction with goethite is favorable compared to sulfate reduction only at pHs below around 6.3 (Figure 3.7), and Fe reduction with amorphous FeOOH is favorable at pHs below 9. These calculations assume an acetate concentration of 10^{-6} M and a pH_2 of 10^{-4} atm (realistic average values for sediments; see above). Environmental pH is therefore an important controlling factor on the significance of Fe reduction in nature. We underscore the necessity of carefully considering the thermodynamics of the microbial processes of interest in any given environment.

10. NAMING ENERGY METABOLISMS

We have already discussed how catabolic (also called dissimilatory) processes and light provide the energy for the anabolic (also called assimilatory) synthesis of cellular material. A vast array of different energy-providing metabolisms exist in nature, and a common nomenclature has been adopted whereby these metabolisms are named based on their (1) energy source, (2) electron sourcer, and (3) carbon source (Figure 3.8). Thus, energy may be provided either by light, whereby the organism is known as a phototroph, or from chemical energy in the absence of light, whereby the organism is known as

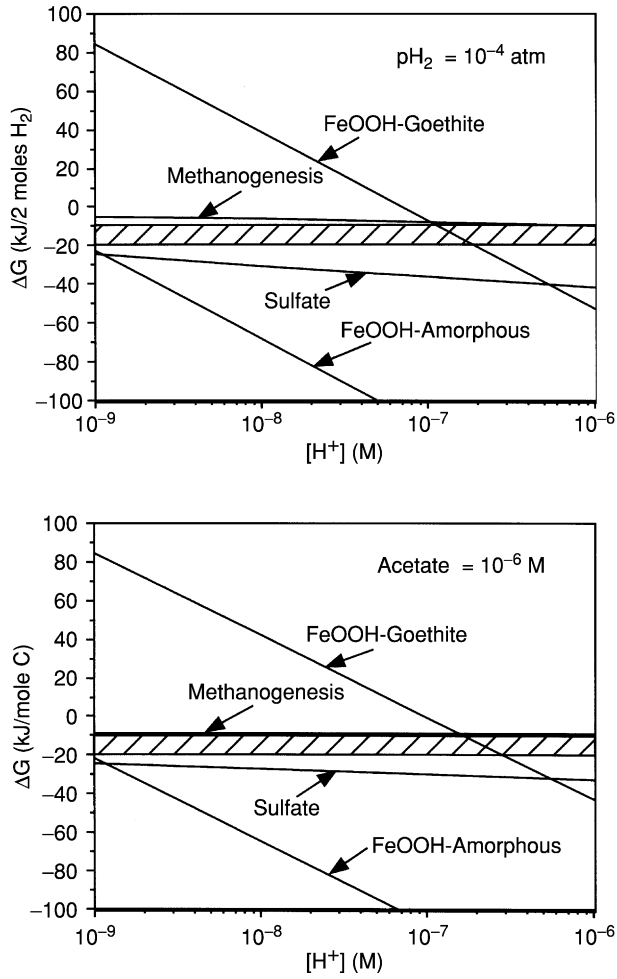

Figure 3.7 Relationship between the free energies associated with Fe reduction, sulfate reduction, and methanogenesis and pH, with both H_2 and acetate as electron donors.

a chemotroph. The electron source may be an inorganic compound, whereby the organism is a lithotroph, or an organic compound, whereby the organism is an organotroph. If the carbon source is CO_2, the organism is an autotroph, and if it uses organic compounds, it is a heterotroph.

In principle, all three descriptors should be used to name an organism's metabolism in the following order: energy source → electron source → carbon source. For example, an organism using chemical energy, an

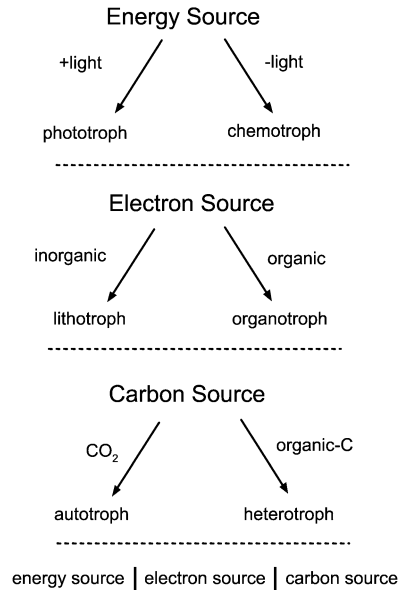

Figure 3.8 Naming energy metabolisms.

inorganic electron donor, and CO_2 for carbon is a chemolithoautotroph. This is a common type of metabolism at interfaces of electron donor and electron acceptor, such as, for example, the O_2-H_2S interface in sediments or the water column. An organism using light energy, an inorganic electron donor, and an organic source of carbon is known as a photolithoheterotroph. Many anoxygenic photosynthetic purple bacteria can be classified this way (see Chapter 9).