Bacteria and Mineral Cycling

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Preface

This book is a general text, describing the influences of bacterial metabolism on the chemical environment of the biosphere. Being a general review, it should be of value to those working in various aspects of this field. General microbiologists should find it interesting to read about the ecological significance of their favorite organisms, and ecologists lacking a background in microbiology should find this book useful for understanding the role of bacteria in natural ecosystems. This book will perhaps also prove useful as a textbook for graduate courses in microbial ecology. Finally, those working in more applied fields involving microbial ecosystems (e.g., soil science, environmental engineering) hopefully will find that our book may add a new and more general perspective to their fields of study.

Although our book presumes some elementary knowledge of general microbiology, biological energetics, and ecological principles, we include two appendices, as aids to the reader. The first appendix shows how free energy yields of biological processes are calculated. Appendix II is a simplified systematic index of the prokaryotes, with notes on their physiology and natural history. We have included a large number of references to recent literature, and we have reviewed work which has not previously found its way into textbooks.

Many recent books on microbiology and microbial ecology use rather fuzzy definitions of "microorganisms", often including, in addition to the bacteria, some part of the unicellular eukaryotes, up to the fungi, including mushrooms. Our book deals exclusively with prokaryotes, including the blue-green algae (referred to as blue-green bacteria or cyanobacteria). Research carried out in the last decade has shown that from structural, physiological, and evolutionary viewpoints, the distinction between prokaryotes and eukaryotes is the most fundamental classification of living organisms. This is, to a large extent, also true from an ecological point of view (discussed in detail in Chapters 1 and 3). We have, therefore, confined our discussion to prokaryote metabolism and ecology, although the importance of bacteria relative to eukaryotes with respect to carrying out different processes in natural ecosystems is discussed in several chapters.

Three aspects of our subject are stressed. The energetics of bacterial processes is treated in some detail and is used as a general framework for most of the topics of the book. Energetic considerations may yield much insight into the understanding of which processes are actually important in natural ecosystems. Secondly, evolutionary aspects are emphasized, viz., the chemical evolution of the biosphere which largely parallels prokaryote evolution. Finally, we have attempted to show how the different element cycles interconnect in natural ecosystems.

The first chapter is devoted to bacterial transformations of matter, as described in terms of energetics of the processes. In particular, we discuss in detail the dissimilatory reduction–oxidation processes involving carbon, nitrogen and sulfur, and the assimilatory reductions of these elements. This chapter gives a general background for the remaining portion of the book.

Chapter 2 discusses theories about the evolution of prokaryote metabolic pathways, in context with palaeontological and geochemical evidence and with current ideas on the evolution of the atmosphere. It is concluded that the chemical composition of the biosphere and its important biologically mediated element cycles are largely the product of prokaryote evolution.

The remainder of the book concentrates on the extant biosphere. In Chapter 3, it is shown that the microbial mineral cycling is mainly driven by the chemical energy of detritus, viz., dead organic material, which is mostly derived from plants. The dominating role of bacteria in the mineralization of this material, which in many ecosystems represents the dominant part of the primary productivity, is explored. These considerations are followed by a treatment of the mineralization of dissolved and particulate organic material in different types of habitats, the hydrolysis of structural compounds, the breakdown of hydrocarbons, and the microbial role in the fossilization of organic matter.

The following three chapters (4–6) are central to the book; they treat the microbial transformations of carbon, nitrogen, and sulfur compounds, respectively. Examples from several different types of habitats (sediments, soils, sewage treatment plants, etc.) are given, but first of all, the general principles of these element cycles and their interconnections are stressed.

Chapter 7 discusses bacterial transformations of other elements. Some attention is given to the controversial dissimilatory oxidation of iron and manganese compounds. This is followed by a short account of some bacterial transformations of trace metals and of the biological phosphorus cycle.

In Chapter 8, examples of mineral cycling connected with microbial symbiosis are discussed. Most emphasis is given to the symbiotic degradation of structural carbohydrates, especially in ruminants. This system is probably still the best understood natural microbial community, and it gives a good illustration of many of the principles discussed in previous chapters, par-

ticularly with respect to the ecology of fermentation. Symbiotic nitrogen fixation is also discussed at some length. Finally, the chapter offers a general discussion of mutualistic relationships between microorganisms and the theory of "serial endosymbiosis" for explaining the origin of the eukaryotic cell from the viewpoint of mineral cycling in symbiotic relationships.

We conclude with a chapter on the global cycling of carbon, nitrogen and sulfur, with special emphasis on the quantitative and qualitative role of prokaryotes. There are still great gaps in our knowledge with respect to causal relationships between processes, and in defining and quantifying transfer rates; this has often not been sufficiently emphasized by textbook authors or by prophets predicting the effects of human activity on the future of the biosphere. The chapter also serves as a summary of the most important principles of mineral cycling discussed in previous chapters.

The book covers a large field relative to the combined knowledge and research experiences of the authors. Readers may therefore, quite justifiably find that it is biased in some places, and that some topics and examples are over-emphasized whereas others are treated superficially, or even contain misunderstandings. Still, we hope that the general principles of the subject will be understood.

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CHAPTER 1

The Requirements of the Bacterial Cell

1.1. ENERGY YIELDING PROCESSES

Bacteria require a nutrient for only one of two processes (1) to utilize in energy yielding processes or (2) to assimilate for cell synthesis. These processes are closely related since a considerable portion of a cell's energy budget is expended on biosynthetic reactions (Table I). Protein biosynthesis accounts for about 60% and nutrient transport for about 18%. If the nutrients require to be reduced before assimilation into cell constituents, energy must be expended on the reductive processes. This is considered in detail in the second half of this chapter.

It is well established that adenosine triphosphate (ATP) is the main energy-coupling agent in all cells (Lehninger, 1971). It is generated in the reaction:

$$\underset{\text{adenosine diphosphate}}{\text{ADP}} + \underset{\text{phosphate}}{\text{Pi}} + \text{energy} \rightleftharpoons \underset{\text{adenosine triphosphate}}{\text{ATP}} + H_2O.$$

When a chemical reaction results in the production of heat it is said to be *exothermic* and to have a negative change in *enthalpy* (ΔH is negative); the reaction proceeds in the forward direction, e.g.:

$$\text{glucose} + 6O_2 \rightleftharpoons 6CO_2 + 6H_2O; \qquad \Delta H = -673 \text{ kcal mol}^{-1}.$$

TABLE I. Bacterial Energy Budget.

Process	% Energy (ATP) expended on each process[a]
Synthesis:	
Polysaccharide	6·5
Protein	61·1
Lipid	0·4
Nucleic acid	13·5
Transport into cells	18·3

[a] Based on Stouthamer (1973) for a cell grown on glucose.

The maximum amount of energy that can be utilized in such a reaction is known as the free energy and is related to enthalpy change as follows:

$$\Delta G = \Delta H - T\Delta S,$$

when ΔG is the change in the free energy in the system, ΔH the heat transferred between the system and its surroundings, T the absolute temperature, and ΔS the entropy change in the system. The free energy can be calculated from the equilibrium constant of a reversible reaction:

$$\Delta G^\circ = -RT \ln K_{eq},$$

where R is the gas constant and K_{eq} is the equilibrium constant. The symbol ΔG° represents the change in *standard free energy*, when one mol of a reactant is converted to one mol of product at 25°C, 1 atm. The standard free energy at pH 7 is represented by $\Delta G'_\circ$. The standard free energy change, $\Delta G'_\circ$, for the oxidation of glucose is -686 kcal mol^{-1} and represents the maximum amount of energy that may be gained from the reaction. It is common practice to calculate ΔG° from the *energy of formation* of compounds in a reaction, as illustrated in Appendix I.

Another useful relationship in considering energy transformations in oxidation/reduction reactions is:

$$\Delta G^\circ = -nF\,\Delta\varepsilon,$$

where n is the number of electrons, F is the Faraday (23 kcal mol^{-1}) and $\Delta\varepsilon$ is the difference in standard oxidation/reduction potentials of the reactants.

The $\Delta G'_\circ$ for the hydrolysis of ATP is -7 kcal mol^{-1}:

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+.$$

It therefore follows that at least 7 kcal mol^{-1} reactant must be available to generate one mol ATP. Reactions with a $-\Delta G'_\circ$ of less than 7 kcal mol^{-1} cannot be coupled directly to ATP generation although there are possibilities for indirect coupling which will be discussed later.

It was noted that $\Delta G'_\circ$ is defined using *molar* concentrations of reactants but since these concentrations are definitely non-biological, the calculated free energy changes are not always very precise. Table II shows a variety of energy yielding reactions which sustain bacterial growth, presumably because they are capable of generating ATP. Two principal methods of ATP synthesis are used by bacteria in these reactions: *substrate level phosphorylation* and *electron transport* (*oxidative*) *phosphorylation* of ADP. These are briefly described before the chemotrophic energy yielding reactions (Fig. 1) themselves are considered.

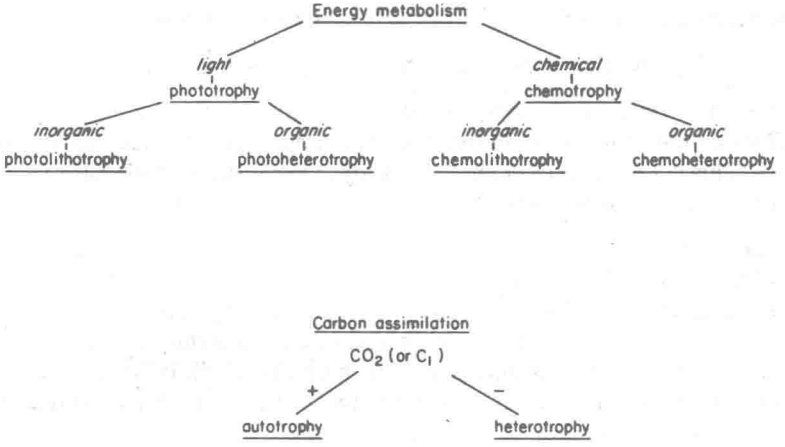

Fig. 1. Bacterial classification based on nutritional requirements. Above in terms of energy requirements and below in terms of carbon assimilation. An additional term, mixotroph, describes a facultative autotroph growing under conditions where it simultaneously uses autotrophic and heterotrophic pathways.

TABLE II. Chemotrophic Reactions Sustaining Bacterial Growth[a].

Oxidant (e^- acceptor)	Reductant (e^- donor)							
	H_2	CHO	CH_4	HS^-	NH_4^+	N_2	NO_2^-	Fe^{2+}
H^+/H_2O	−	+	−	−	−	−	−	−
CHO	+	+	−	−	−	−	−	−
CO_2	+	+	−	−	−	−	−	−
SO_4^{2-}	+	+	?	−	−	−	−	−
NO_3^-	+	+	?	+	−	−	−	−
O_2	+	+	+	+	+	−	+	+

[a] The electron donors are listed in order (left to right) of decreasing capacity to donate electrons (H, C, S, N, Fe). The electron acceptors are listed in order (top to bottom) of increasing capacity to accept electrons (H, C, S, N, O). There is thus an increasing difference in E_o between the couples in going down a series and a decreasing difference in E_o in going across a series. The larger the difference in E_o values, the greater is the free energy that is available for the reaction. CHO is used to represent reduced carbon of undefined composition.

1. *Substrate level phosphorylation.* In the reactions:

$$\text{glyceraldehyde} + \text{phosphate} \xrightarrow[(\text{minus } 2e^-)]{\text{oxidation}} 2H + H_2O + \text{3-phosphoglyceric acid},$$

$$\text{3-phosphoglyceric acid} + \text{ADP} \rightarrow \text{glyceric acid} + \text{ATP},$$

the $\Delta G'_o$ of -7 kcal for the oxidation of the aldehyde to the acid is preserved in ATP and the coupled reactions occur with a much smaller decrease in free energy. This is substrate level phosphorylation because one molecule of substrate must be phosphorylated for each molecule of ATP synthesized.

2. *Electron transport phosphorylation.* In this process no direct phosphorylation of a large quantity of compound occurs in the cells. The mechanism has not been completely elucidated but it may involve a reversal of ATPase, driven by an electrochemical proton gradient across a membrane containing the electron transporting components of a chain (Mitchell, 1977). The capacity of electrons to generate enough energy to synthesize ATP may be related to the change in potential.

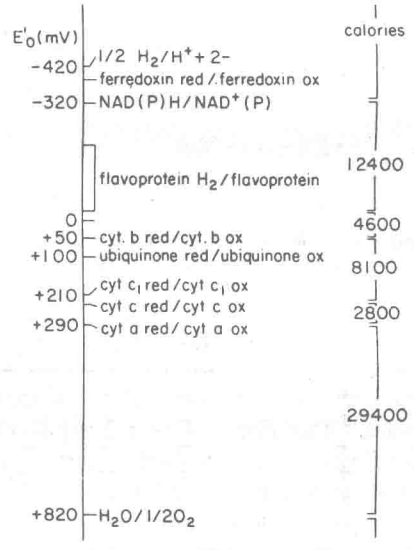

Fig. 2. An electron transport chain. On the left are shown the oxidation/reduction potentials of various couples in a representative electron transport chain. The distance between potentials reflects the energy difference between the corresponding electron carriers. This energy may be expressed in calories (right hand axis) where it is seen that sufficient energy is available between three carriers (12·4, 8·1 and 29·4 kcal) to generate ATP (7 kcal), if some form of direct coupling is necessary.

Standard oxidation/reduction potentials, E'_o, are based on the assignment of an arbitrary value of zero volts to the hydrogen electrode at pH 0 when H_2 at 1 atm is 50% ionized:

$$\tfrac{1}{2}H_2 \rightleftharpoons H^+ + e^-.$$

E'_o is the potential of the half reduced system at pH 7; it is -420 mV. At the other end of the scale we have:

$$\tfrac{1}{2}O_2 + 2e^- \rightleftharpoons O^{2-}; \qquad E'_o = +820\,\text{mV}.$$

Figure 2 shows the E'_o values for these two extremes (O_2 and H_2) with the intermediate values of the components of the mitochondrial or aerobic bacterial electron transporting chain. Many bacteria have cytochromes other than those shown in Fig. 2 (Jones, 1977). The $\Delta G'_o$ values between the components are given for comparison. A potential difference of 200 mV for a two electron transfer between donor and acceptor is necessary to give a $\Delta G'_o$ of -9 kcal mol^{-1}, or approximately the amount of energy required to ensure the generation of one ATP. This may not be necessary if a proton gradient drives phosphorylations. There are only three coupled reactions in the transport chain at which ATP is traditionally thought to be generated. The reduced state of the electron carrier NAD(P)H is generated partly during glycolysis but principally from the oxidation of acetate to carbon dioxide through the tricarboxylic acid (TCA) cycle; Lehninger (1971) should be consulted for further details. The glycolytic oxidation of glucose yields only 2 mol ATP per mol of substrate, whereas the electron transport phosphorylating system should yield a further 36 mol per mol substrate. Considerably more energy is thus obtained when the E'_o of the reactants are widely separated (CHO/O_2) than when they are close together (CHO/CHO) as in glycolysis (CHO is used to denote reduced organic carbon of undefined composition). It is more relevant to consider differences in E'_o between reacting compounds in defining energy yield than it is to define, e.g., glucose as a "high energy" nutrient. Glucose can only yield a large amount of energy when it is coupled to a powerful oxidizing agent, e.g., oxygen. It would be just as logical to consider oxygen as a "high energy" nutrient.

The ATP which is generated can be related to the quantity of cellular material synthesized. Bauchop and Elsden (1960) have shown that 10 g dry weight of cells are produced per mol ATP synthesized by substrate level phosphorylation.

1.1.1. Chemotrophic Oxidation Reduction Reactions

The significance of the term chemotrophic is seen in Fig. 1 where some other processes are also defined. In a more detailed consideration of oxidation/

reduction reactions (Table II) each reductant (electron donor) is considered against the various oxidants (electron acceptors). Only a few examples of each reaction are discussed in the following which may be considered a catalogue of the principal energy yielding chemotrophic reactions found in bacteria. All these reactions may be termed *dissimilative* since neither of the reactants are assimilated. The oxidation/reduction reactions are discussed in the order in which they are listed vertically in Table II, i.e., with increasing energy yield going downwards but decreasing energy yields in each series from left to right.

1. *H_2 oxidations*

Molecular hydrogen participation in biological reactions is mediated by hydrogenases. Hydrogen is highly reactive and rarely accumulates in any environment. It is produced in significant quantities only in anaerobic situations. It is largely used by methane producers but some bacteria may oxidize it by alternative mechanisms as shown by the following examples.

H_2/CHO — The following process has been described for *Vibrio succinogenes* (Iannotti *et al.*, 1973):

$$\underset{\text{fumarate}}{COOHCHCHCOO^-} + H_2 \rightleftharpoons \underset{\text{succinate}}{COOHCH_2CH_2COO^-}$$

$$\Delta G'_o = -20{\cdot}6 \text{ kcal.}$$

This type of reaction probably occurs infrequently, only when high concentrations of suitable CHO e^- acceptors are present with H_2, a combination which is not common.

H_2/CO_2 — The process:

$$H_2 + \tfrac{1}{4}CO_2 \rightleftharpoons \tfrac{1}{4}CH_4 + \tfrac{1}{2}H_2O; \qquad \Delta G'_o = -8{\cdot}3 \text{ kcal},$$

is carried out by all methanogenic bacteria (Wolfe, 1972) and is quantitatively the most important process involving H_2 in non-sulfate environments. In *Clostridium aceticum* the following process may take place:

$$H_2 + \tfrac{1}{2}CO_2 \rightleftharpoons \tfrac{1}{4}CH_3COO^- + \tfrac{1}{2}H_2O; \qquad \Delta G'_o = -4{\cdot}3 \text{ kcal},$$

(Wieringa, 1940; Mah *et al.*, 1976). Although this reaction is less favorable than methane production, it may occur preferentially in sewage sludge. The acetate is then converted into methane by other bacteria (Mah *et al.*, 1976).

H_2/SO_4^{2-} — The oxidation with sulfate is carried out by *Desulfovibrio* spp. (Postgate, 1969):

$$H_2 + \tfrac{1}{4}SO_4^{2-} + \tfrac{1}{4}H^+ \rightleftharpoons \tfrac{1}{4}HS^- + H_2O; \qquad \Delta G'_o = -9{\cdot}1 \text{ kcal}.$$

The oxidation by sulfate yields more energy than the oxidation by carbon dioxide and presumably occurs preferentially when both oxidants are present.

H_2/NO_3^- The process:

$$H_2 + 2/5NO_3^- + 2/5H^+ \leftrightharpoons 1/5N_2 + 6/5H_2O;$$

$$\Delta G'_o = -53{\cdot}6 \text{ kcal},$$

has been described for *Paracoccus* (*Micrococcus*) *denitrificans* and *Alcaligenes* (*Hydrogenomonas*) *eutrophus* (Schlegel, 1975). It yields considerable free energy but hydrogen and nitrate may never reach significant concentrations in the same environments.

H_2/O_2 The two above mentioned bacteria may also carry out the reaction:

$$H_2 + \tfrac{1}{2}O_2 \leftrightharpoons H_2O; \qquad \Delta G'_o = -56{\cdot}7 \text{ kcal}.$$

The probability is slight that significant hydrogen oxidation occurs by this process in nature.

2. *CHO oxidations*

These oxidations are performed by *heterotrophic bacteria*. The oxidations are important since a major portion of the dissimilatory carbon cycle flows through them. The reactions are very diverse and many different types of bacteria perform them. The following series illustrates particularly well the increasing energy yields per substrate carbon as one progresses down the series (increases in $\Delta\varepsilon$).

CHO/H_2O The following process is carried out by *Desulfovibrio* according to Bryant (1969):

$$\underset{\text{lactate}}{CH_3CHOHCOO^-} + H_2O \leftrightharpoons \underset{\text{acetate}}{CH_3COO^-} + CO_2 + 2H_2;$$

$$\Delta G'_o = +5{\cdot}7 \text{ kcal}.$$

The energy yield is negative and the bacteria can only grow when hydrogen is removed by coupling it to methane production. This also applies to the reaction:

$$\underset{\text{ethanol}}{CH_3CH_2OH} + H_2O \leftrightharpoons \underset{\text{acetate}}{CH_3COO^-} + 2H_2;$$

$$\Delta G'_o = +8{\cdot}3 \text{ kcal},$$

carried out by the "S-organism" (Bryant *et al.*, 1967). In a mixed culture Bryant *et al.* (1967), observed the reaction:

$$\underset{\text{propionate}}{CH_3CH_2COO^-} + 2H_2O \rightleftharpoons \underset{\text{acetate}}{CH_3COO^-} + CO_2 + 3H_2;$$

$$\Delta G'_o = +25{\cdot}1 \text{ kcal.}$$

This is again an example of a process where an apparently unfavorable thermodynamic oxidation can occur when one of the products, in this case hydrogen, is removed from the system. Wolin (1974) illustrates this in the reaction:

$$NADH + H^+ \rightleftharpoons NAD^+ + \tfrac{1}{2}H_2.$$

When the hydrogen partial pressure is 1 atm the K is $6{\cdot}7 \times 10^{-4}$ and $\Delta G'_o$ is $+4{\cdot}33$ kcal; when the partial pressure of hydrogen is reduced to 10^{-6} atm, the equilibrium constant is $6{\cdot}7 \times 10^2$ and $\Delta G'_o$ is $-3{\cdot}83$ kcal (at the specified pressure). Thus, at very low hydrogen pressures the production of hydrogen from NADH is favored. Even when hydrogen production is not coupled to phosphorylations, this type of reaction where NADH is oxidized by H^+ may be very important in providing a mechanism for NADH oxidation, without the necessity of utilizing a portion of the substrate, as must occur in typical fermentations, in order that the supply of the electron acceptor NAD^+ be renewed.

CHO/CHO

This type of oxidation, where part of the substrate is oxidized at the expense of a part of the substrate being reduced, ATP being generated by substrate level phosphorylation, is termed *fermentation*. The extent to which substrate level phosphorylation is involved in all the following reactions is not clear. The most common type of fermentation is that of sugars. This is carried out by a variety of bacteria, among them species of *Bacteroides*, *Escherichia*, *Ruminococcus*, *Bacillus*, *Clostridium*, and *Lactobacillus*. They produce a variety of end-products: carbon dioxide, formate, acetate, propionate, butyrate, succinate, lactate, hydrogen and alcohols, with a range of ATP yields. In mixed cultures (e.g., rumen, sewage sludge) the products are the simple volatile acids plus methane and carbon dioxide. This is partly due to the other products being re-metabolized but it is also due to the fermentation products of individual bacteria being altered by the mechanisms suggested above. In this way, through