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SEDIMENT BACTERIA: Who's There, What Are They Doing, and What's New?

Kenneth H. Nealson
Center for Great Lakes Studies, University of Wisconsin, Milwaukee, Wisconsin 53204; e-mail: knealson@alpha1.csd.uwm.edu

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ABSTRACT

The prokaryotes (bacteria) comprise the bulk of the biomass and chemical activity in sediments. They are well suited to their role as sediment chemists, as they are the right size and have the required metabolic versatility to oxidize the organic carbon in a variety of different ways. The characteristic vertical nutrient (electron donor and electron acceptor) profiles seen in sediments are produced as a result of microbial activities, with each nutrient a product or reactant of one or more metabolic groups. Thus, understanding the mechanisms by which the chemical environment of a sediment is generated and stabilized requires a knowledge of resident populations, something that has been very difficult to obtain, given the techniques available to microbiologists. However, the new approaches of molecular biology, which have added insights into the phylogenetic relationships of the prokaryotes, have also provided tools whereby sedimentary populations can be examined without the need for culturing the organisms. These techniques, in concert with new methods of microscopy, isolation of new metabolic groups, and the study of new ecosystems, suggest that there is much that will be learned about the microbiology of sedimentary environments in the coming years.

INTRODUCTION

When I accepted the invitation to write this review, I was challenged to present an overview of microbial structure, metabolism, and ecology that could be related to the processes that occur in stratified microbial communities like those found in sediments—all this while making the material palatable for the nonexpert. In response, I present below a discussion of some of the properties of prokaryotes, dealing with basic issues like size, structure, and metabolic diversity. This is

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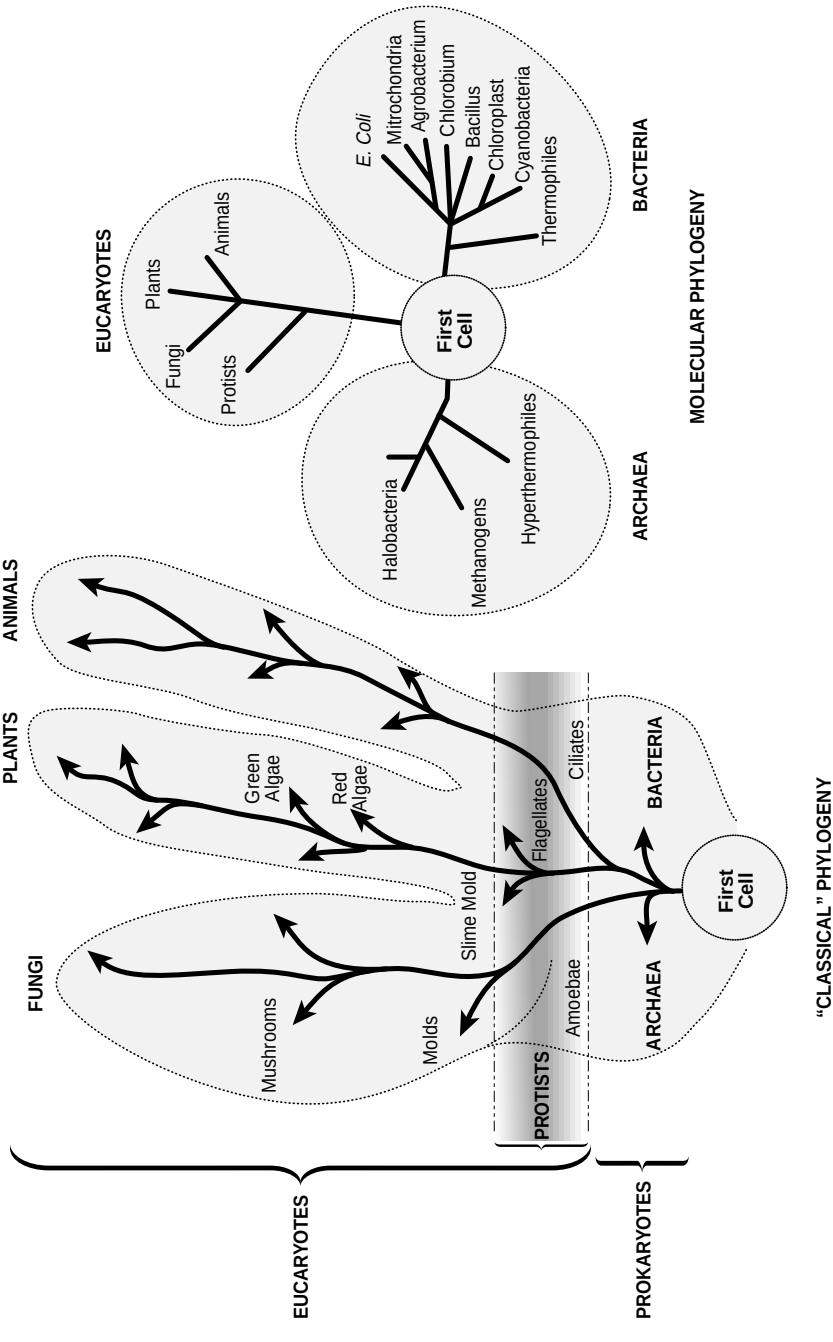
followed by a discussion of some emerging techniques and some new findings in microbial ecology that may have relevance to sediments and the role(s) that the prokaryotes play in these environments. Only the two prokaryotic domains of life [Archaea and (eu)Bacteria] are dealt with here, because eukaryotes are usually minor components of sediments.

In Figure 1, the three domains of life, as defined by sequences of 16S ribosomal RNA (rRNA) sequences (Olsen et al 1986, 1994, Pace et al 1986, 1993, Stahl & Amann 1991, Woese 1987), are contrasted to the more classical version of the five kingdoms of life. Based on such molecular phylogenies, which are established by rRNA sequence comparisons, the major genetic diversity on the planet resides in the two prokaryotic domains, whereas the genetic diversity of the eukaryotes is actually quite limited. For example, in terms of evolutionary distance, humans are only slightly removed from the fungi or the plants, and the distance between seemingly similar bacteria is quite impressively large. This view is consistent with what we know of evolution of the biota, given that the planet is believed to have been inhabited by prokaryotes for more than 3.5 billion years, whereas eukaryotes (those organisms with chromosomes, nuclei, nuclear membranes, and many visible exterior structures) are in comparison relatively recent inhabitants. The prokaryotes have remained small and simple throughout evolution; their diversity is expressed in terms of physiology and metabolism, whereas that of the larger eukaryotes is expressed in terms of structures and behavior.

Sediments share some properties with soils and yet are distinct from soil environments for a variety of reasons, many of which are of great importance to the populations of microbes that reside there. Sediments are, in general, overlain by a permanent water body, be it an ocean, fjord, lake, river, or reservoir. Thus, although the chemistry of the water may vary substantially, as may the level of primary productivity (autochthonous input) and contribution from runoff or rivers (allochthonous input), sediments share the property of being continuously wet. Oxygen solubility in water limits the level of oxygen to

Figure 1 The domains of life, as defined by 16S rRNA sequence analysis and compared to the classical five kingdoms of life defined by morphology and behavior. On the left, the five-kingdom phylogeny is shown, which presents the tree of life and diversity with the eukaryotic kingdoms as the dominant and diverse types. On the right is the view obtained from sequence analysis of the 16S rRNA genes. Most of the functional groups that are discussed here are in the Bacteria domain, while some of the hydrogen oxidizers, sulfur oxidizers, and the methanogens are included in the domain Archaea. This illustration is not meant to show details of group relationships, just the general concept of the groups. To learn about detailed relationships between bacterial groups, the reader is referred to Woese (1994). [CR Woese. 1994. There must be a prokaryote somewhere: microbiology's search for itself. *Microbiol. Rev.* 58:1–9]


EUCARYOTES
PROKARYOTES
FUNGI
PLANTS
ANIMALS
PROTISTS
ARCHAEA
BACTERIA
First Cell
Mushrooms
Molds
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Red Algae
Flagellates
Amoebae
Ciliates
"CLASSICAL" PHYLOGENY
EUCARYOTES
Plants
Animals
Fungi
Protists
Halobacteria
Methanogens
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Bacillus
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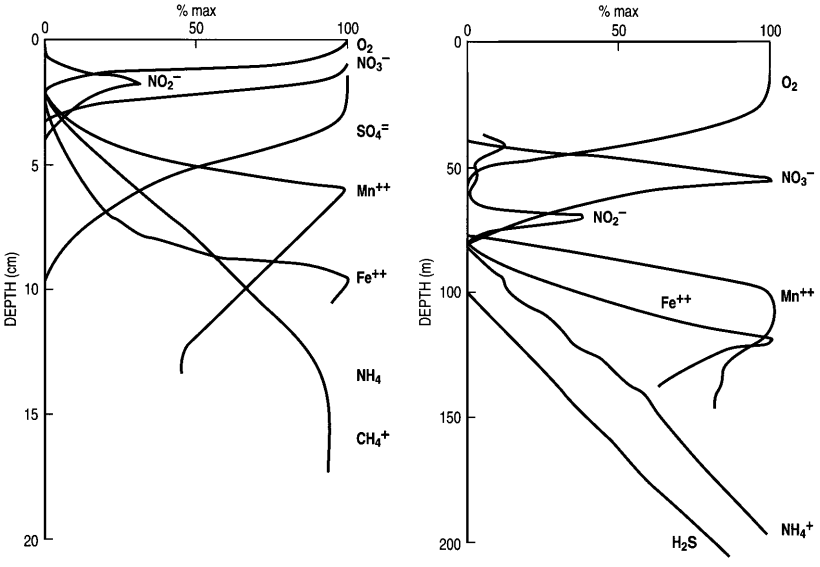


Figure 2 Vertical profiles of nutrients in typical environments: (*left*) freshwater (Lake Michigan) and (*right*) marine (Black Sea). These profiles are meant to act as general guidelines of what might be expected upon analysis of porewater components from marine or freshwater sediments (Froelich et al 1979, Reeburgh 1983). The upper regions are oxic and thus compatible with eukaryotic life, whereas lower portions are anoxic and primarily the domain of prokaryotes. The depth of oxygen depletion is a function of the amount of organic carbon that reaches the sediment. The primary difference between the freshwater and marine sediments relates to the amount of sulfate in the latter and the resulting dominance of the sulfur cycle, whereas in the freshwater sediments, methane formation is the terminal step, which dominates carbon metabolism at depth.

The numbers presented here are percentages of maximum values that may be encountered in these environments: freshwater/marine: O_2, 300–400 μM for both; NO_3^- and NO_2^-, a few μM for both; $SO_4^=$, 100–200 μM in freshwater; 25 mM in marine systems (for this reason sulfate depletion is often not seen until deep in profiles, and methane production often is minor in marine systems); Mn^{++}, 100 μM/10 μM; Fe^{++}, 10 μM/25 nM; NH_4^+, few micromoles in both; H_2S, usually not seen in freshwater, and rarely exceeds a few micromoles in marine systems—in this system, the H_2S is in the micromolar range and will not reach micrometer values until very deep (hundreds of meters). Thus, no significant sulfate depletion (sulfate profile not shown) will occur over this range. CH_4, This will range from a few nanomoles to saturation, forming bubbles that are exported out of the system; because of the high sulfate in marine systems, methane is not usually a major component.

the order of 300–400 μM (depending on water temperature); so, if organic matter is present that can be aerobically respired, then undisturbed sediments almost universally become anoxic with depth. After oxygen is depleted, a series of rather stable horizontal gradients is set up within the sediments, in which various electron acceptors are then consumed, usually in the order of decreasing redox potentials (Figure 2). The gradients are a function of organic

input, microbial metabolic abilities (Table 1), and the geochemistry of the environment (marine vs freshwater, mineral content, etc). Assuming that mixing is minimal, gradients will be formed whenever the production or consumption of any product or nutrient exceeds the diffusion rate of that product or reactant.

Although similar conditions may exist in waterlogged soils, the situation in soils can change dramatically as drying occurs, oxygen gas is introduced to the soils, and microgradients form around soil particles. In addition, the situation is temporarily unstable and is susceptible to perturbations such as water introduction from rain or irrigation, introduction of nutrients and/or dissolved gases from plant roots, and physical disruption from aerobic macroorganisms. Clearly, soils have the potential for being much more complex, both with regard to microscale chemistry and microbiology and in terms of the properties of the microbes, which may need to tolerate large changes in levels of hydration, temperature, and nutrient stress.

The typically stratified sediments described above are repositories of their respective overlying water columns and, in the absence of microbial activity, would present us with a geochemical and biological record of the past—a chronological record of what had occurred above them. To some degree, such records exist, but only after severe alteration and diagenesis due to the activities of a wide range of microbes, which competed for the rich sources of energy deposited there and used that energy to make more biomass.

A final important characteristic of sediments that they share with soils is the abundance of minerals (clays, carbonates, silicates, metal oxides, etc). Minerals can be both reactants with and/or products of microbial metabolism, and they undoubtedly impact the microbial ecology and metabolism of the surrounding environments, both structurally and functionally.

The primary inhabitants of sediments are the simpler, smaller prokaryotes. As opposed to eukaryotes, the prokaryotes have few modes of behavior other than growth and division: Their small size and rigid cell walls preclude their being predators in the classical sense. Diversity is expressed in terms of metabolism rather than structure, and prokaryotes have optimized their biochemistry for the uptake and utilization of a wide variety of nutrients. In sedimentary environments, prokaryotes so efficiently exploit the energy sources present that they outcompete the larger, metabolically less-efficient eukaryotes. Many aspects of prokaryotic metabolic diversity are dealt with briefly here; for more detailed treatments of metabolism and organisms, readers are referred to Balows et al (1991).

Metabolic optimization includes versatility, and here, when compared to their eukaryotic counterparts, the prokaryotes are notable for their utilization of both energy sources (fuels) and electron acceptors (oxidants) (Tables 1, 2; Figure 3).

Table 1 Commonly measured pore water components—biological sources, sinks, processes, and organisms involved[a]

Source (process)/organism(s)	Component	Sink (process)/organism(s)
Oxygenic photosynthesis/ cyanobacteria and algae	Oxygen	Organic carbon oxidation/aerobic heterotrophs Inorganic (H_2, H_2S, etc) oxidation/aerobic chemolithotrophs
Ammonia oxidation/nitrifying bacteria	Nitrate (NO_3^-)	Organic carbon oxidation/denitrifiers Inorganic (H_2, H_2S, Fe^{++}) oxidation/anaerobic chemolithotrophs
Ammonification/protein degrading bacteria	Ammonia (NH_4^+)	Aerobic oxidation/nitrifiers
Manganese reduction/manganese reducers	Manganese (Mn^{++})	Manganese oxidation/manganese oxidizers
Iron reduction/Fe reducers	Iron (Fe^{++})	Iron oxidation/aerobic Fe and anaerobic (NO_3^+) Fe oxidizers; photosynthetic Fe oxidizers
Sulfur (H_2S, S°, $S_2O_3^=$) oxidation/aerobic S oxiders	Sulfate ($SO_4^=$)	Heterotrophic sulfate reduction/sulfate reducing bacteria Sulfate assimilation/most bacteria
Sulfur (S°, $S_2O_3^=$, SO_4) reduction/sulfate, sulfur, and thiosulfate reducers	Hydrogen sulfide (H_2S)	Aerobic sulfide oxidation/aerobic sulfide oxidizers Anaerobic sulfide oxidation/anaerobic sulfide oxidizers Photosynthetic sulfide oxidation/sulfur photosynthetic bacteria
Methanogenesis/methanogens	Methane (CH_4)	Aerobic methane oxidation/methanotrophs Anaerobic methane oxidation/unknown consortium
Fermentation/fermentative bacteria Proton reduction/syntrophic bacteria	Hydrogen (H_2)	Aerobic H oxidation/hydrogen chemolithotrophs Anaerobic H oxidation/many bacteria Methanogenesis/methanogens Acetogenesis/acetogens
Fermentation/fermentative bacteria Respiration/many bacteria	Carbon dioxide (CO_2)	Autotrophy/chemoautotrophs or photoautotrophs Methanogenesis/methanogens Acetogenesis/acetogens

[a]This table is designed to complement Figure 1, presenting in the center column the components that are commonly measured when pore waters are analyzed. In the left column are some of the processes and organisms that supply these components, while on the right are those processes and organisms that act as sinks for the components. Clearly, many organisms can be involved, and one expects that profiles like those shown in Figure 1 must be the result of many competing processes by many organisms.

Table 2 Metabolic types of prokaryotes

General type	Carbon source	Energy source	Electron donor	Electron acceptor
Heterotroph	Organic C	Organic C		
Aerobes			Organic C	O_2
Denitrifiers			Organic C	NO_3^-
Mn reducers			Organic C	Mn (IV)
Fe reducers			Organic C	Fe (III)
SRBs			Organic C	$SO_4^=$
Sulfur reducers			Organic C	S°
Methanogens			Organic C/H_2	CO_2
Syntrophs			Organic C	Organic C
Acetogens			Organic C/H_2	CO_2
Fermentors			Organic C	Organic C
Phototroph	CO_2	Light		
Cyanobacteria			H_2O	
Photosynthetic bacteria			S compounds, H_2, Organic C	
Lithotroph	CO_2/organic C	Inorganics		
H_2 oxidizers			H_2	O_2, NO_3^-, Mn (IV), Fe(III), $SO_4^=$, CO_2
Fe oxidizers			Fe (II)	O_2, NO_3^-
S oxidizers			H_2S, S°, $S_2O_3^=$	O_2, NO_3^-
N oxidizers			NH_3, NO_2^-	O_2
CH_4 oxidizers			CH_4	O_2

Eukaryotes are limited to energy sources that can be converted into glucose or breakdown products of glucose, like pyruvate, and to oxygen as the only electron acceptor used to burn their metabolic fuel. In contrast, prokaryotes utilize a wide array of electron donors, both organic and inorganic, as well as many different alternative electron acceptors or "oxygen substitutes" for respiration in the absence of molecular oxygen, which makes them extremely versatile with regard to energy. Some microbes are quite versatile themselves, whereas others specialize with remarkable efficiency in their own niches, and it is common to find these microbial specialists in intricate metabolic symbioses with other specialists (Schink 1991). This contrasts with the eukaryotic domain, where metabolic versatility is rare, but morphological (and resulting behavioral) versatility is the rule, resulting in complex food chains, predator-prey relationships, behavioral symbioses, and many other phenomena.

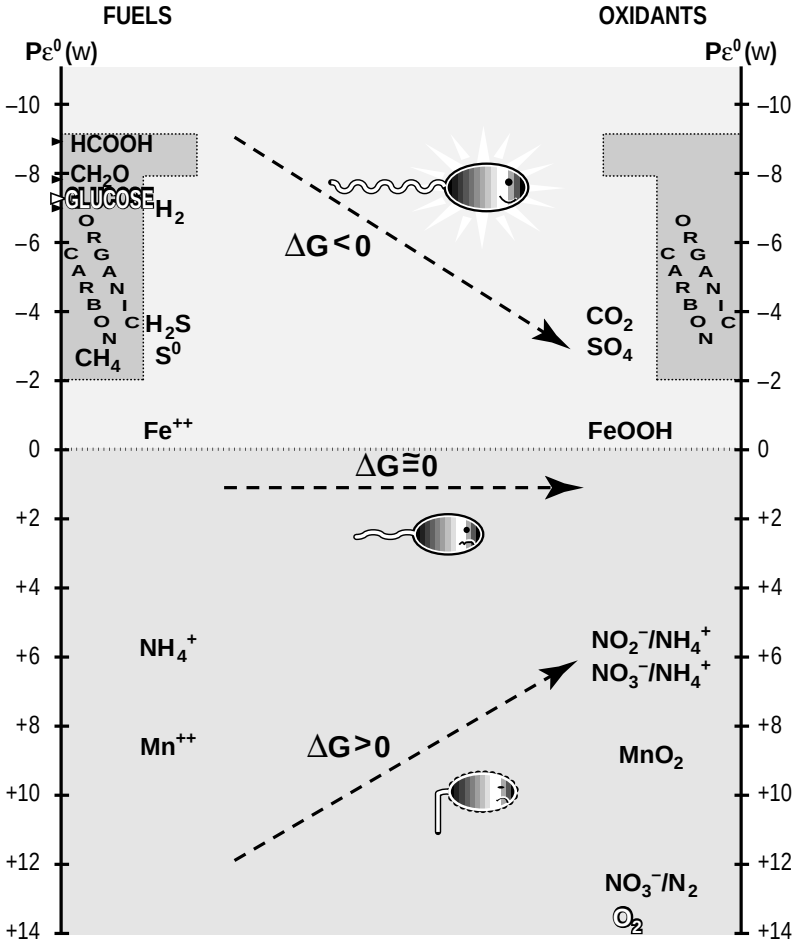


Figure 3 Electron donors and acceptors of life. This cartoon is designed to show the various redox couples known to be utilized by living organisms. On the left are the fuels or energy sources (organic and inorganic), and on the right are oxidants used to burn these fuels. If the arrow between energy source and oxidant has a negative slope, then (if kinetic properties allow) an organism should be able to harvest this energy. Prokaryotes have exploited most of these energetic niches, whereas eukaryotes are confined to just a few organic compounds as fuels and to molecular oxygen as oxidant.

PROPERTIES OF MICROBES

Size

Although everyone knows that bacteria are small, the import of this simple fact is not always appreciated. Given that the major role of these organisms is metabolism, it is probably no accident that they have, for the most part, remained small over evolutionary time. Chemical reactions are strongly impacted by the surface to volume ratio (S/V) of the reactants, and living cells often try to maximize this parameter. Thus small bacterial cells, with their cell size of 0.5 to a few micrometers in diameter, have S/V values 100–1000 times higher than typical eukaryotic cells, which may range from 20 μm to millimeters in diameter (Nealson 1982), making it difficult for eukaryotes to energetically compete. This must also be kept in mind in consideration of terms like "biomass." Clearly, if the S/V value of bacterial biomass is 100 times that of its eukaryotic neighbors, then even when bacteria represent only a few weight percent of the total biomass, they have a potential reactivity and environmental impact equal to that of the total. In many sediments, the bacteria probably account for 90–99% of the biomass, thus making the prokaryotes by far the dominant group in terms of metabolic potential. Clearly, it is an advantage for prokaryotes to remain small—given 3.5 billion years of evolution, if it were an advantage to get larger, they would have done it! In marked contrast, the eukaryotes tend to get larger in response to behavioral advantages of size, such as in predator-prey relationships.

However, there must also be lower size limits to metabolically effective life; the small size of bacteria may put limits on the chemistry that is possible. For example, the intracellular volume of a bacteria that is 0.5 μm is sufficiently small that at a pH value of 7.5, there are only a few free protons per cell. As bacteria get even smaller, problems are encountered in terms of liquid phase chemistry. As shown in Table 3a, the number of molecules of solutes in cells smaller than 100 nm may in fact be prohibitively small. Given that concentrations of various compounds (substrates, solutes, etc) range from micromolar to several millimolar, it would seem that when cells reach the size of 20 nm or less, life as we know it is impossible, and even for 50-nm size cells, it is extremely difficult. In a 20-nm sphere, for example, even at concentrations of 1 mM, there is not sufficient volume for even one molecule of a given solvent! Typical K_m values for enzymatic reactions are in the range of 1 to 10 μM. At these concentrations, for cell sizes less than 0.1 μm, the number of molecules per cell approaches only one. Thus, the trend to become small for biological advantage must be limited by chemical reality as a function of available volume. In fact, the situation is probably much worse than illustrated in Table 1, which assumes that no volume will be taken by other components and that no space is required for a rigid cell wall. Given the need for enzyme catalysts, DNA, and ribosomes, the volume

Table 3a Size considerations for bacterial life and metabolism

Cell size (μm)[a]	Radius (μm)[b]	Volume (μm^3)	mol/cell[c]				
			1 M	10 mM	1 mM	10 μM	1 μM
1	0.495	0.12	3.06 E8	3.06 E6	3.06 E5	3058	305.8
0.5	0.245	0.015	3.71 E7	3.71 E5	3.71 E4	371	37.1
0.2	0.095	8.57 E−4	2.16 E6	2.16 E4	2.16 E3	21.6	2.16
0.1	0.045	9.11 E−5	2.29 E5	2.29 E3	229	2.29	0.229
0.05	0.02	8.0 E−6	2.02 E4	202	20.2	0.202	0.0202
0.02	0.005	1.25 E−7	315	3.15	0.315	3.15 E−3	3.15 E−4

[a]For the purposes of this discussion, the cell is assumed to be spherical. Smaller bacteria are on the order of 500 nm, while those that pass through 0.2-μm filters are referred to as ultramicrobacteria. Bacteria-like particles in the 20–50 nm range are referred to as nanobacteria.
[b]Radius is assumed to be half the diameter, and then 5 nm are subtracted for the width of the double membrane. No space is assumed for the rigid cell wall, so these estimates are conservative in the direction of high volumes.
[c]These calculations are meant to show the number of molecules of any given compound (substrate, solute, etc) within the spherical volume specified. As shown, when bacteria get to a size of 50 nm, there is space for only about 20 molecules when concentrations reach 1 mM. At 10-μM concentration, a 100-nm–sized sphere has only 2 molecules per cell! Clearly, there must be some lower limits that chemistry sets for a metabolizing cell.

Table 3b Size considerations for bacterial life and metabolism

Sizes of some bacterial components	
Cell membrane thickness	5–10 nm
Ribosome diameter[a]	20–25 nm
Genome diameter[b]	500 nm
Flagella diameter	25 nm

[a]This is an average number obtained from studies of ribosomes of several different bacteria (Lake, 1985).
[b]This is the size of the genome of *Escherichia coli* when the DNA is supercoiled. Other genomes are substantially smaller, with 1/5 to 1/10 the number of genes

available to substrates and solvents may be in fact substantially less, especially in the smaller cells, where these components will occupy a larger percentage of the small volume.

Structure

Two items are noted in terms of bacterial structure: simple cellular architecture and rigid cell walls. Although prokaryotic cells are very sophisticated and highly regulated metabolic machines, they are structurally simple in comparison to eukaryotes. They have a single piece of double-stranded DNA, with no nucleus or nuclear membranes. They have almost no intracellular organelles, and no tissues or organs characteristic of eukaryotes, i.e. few complex parts that can fail. For these reasons they are often found in extreme environments, in conditions that preclude the growth or survival of the structurally complex (fragile) eukaryotes.

The rigid structure of the bacterial cell wall imposes certain restrictions on metabolism of the cell and the way in which bacteria interact with their environment. A common mode of nutrient uptake available to eukaryotic cells is that of phagocytosis and food engulfment; it is one property that lends advantage to being large and developing predatory behavior. Bacteria do not have this luxury—their rigid cell wall precludes the phagocytotic mode, and probably in response, they have advanced two modes of nutrient modification and uptake to a state of near perfection: (*a*) the use of extracellular enzymes to convert polymeric large molecules into smaller oligomers and monomers and (*b*) the use of specific transport systems to move nutrients against concentration gradients into the cytoplasm. There are many variations on these themes among the different groups of bacteria, but the generalization that extracellular enzymes and specific transport systems predominate in bacteria must always be remembered when considering their potential ecological role(s).

Metabolic Versatility

Although eukaryotic versatility is expressed in terms of structures (and behaviors that are possible because of these structures), prokaryotic versatility is expressed in metabolic terms. While the prokaryotes are unified with the rest of life through the mechanism of energy conservation, namely the generation of a chemoosmotic gradient called the proton motive force (pmf), which consists of an electrochemical potential and a pH gradient, and the use of this pmf for the generation of biologically useful energy in the form of adenosine triphosphate (ATP), they are decidedly different from the eukaryotes with regard to the variety and types of fuels and oxidants utilized to generate pmf. The eukaryotes can utilize very few oxidants (only oxygen) and reductants (only a few organic molecules such as glucose and pyruvate) shown in Figure 3; the other fuels and oxidants constitute the metabolic realm of the prokaryotes.

The diversity of prokaryotic metabolism requires a separate vocabulary for the description of the metabolic groups (Table 2). The vocabulary is based on the energy source used by the bacteria, so that they are called either phototrophs or chemotrophs; among the chemotrophs are included those that use organic carbon (chemoorganotrophs) or inorganic energy sources (chemolithotrophs). If an organism uses organic carbon as its source of carbon, it is referred to as a heterotroph, whereas if it fixes its carbon from CO_2, it is an autotroph. Combinations of these terms thus surface as the versatility of a given organism is revealed, and the situation can be semantically complex if an organism is capable of many types of metabolism; for example, some organisms, called mixotrophs, can grow both autotrophically and heterotrophically. However, Table 2 does serve to point out that bacteria groups are known that utilize many of the environmental redox pairs. Such bacteria reside in a remarkable array of environments where energy would normally flow, and by using

enzyme catalysts to speed up otherwise slow reactions, they make use of a large number of energy sources and oxidants that are not available to eukaryotes. It seems likely that if chemical kinetics are sufficiently slow to allow bacteria to compete, then almost any redox couple that yields energy will be exploited.

Mechanisms of Energy Conservation and Patterns of Metabolism

One of the revelations of the past 25 years in biology was the elucidation of a central mechanism of metabolism that allowed microbial energy conservation to be understood as a unified feature. The so-called chemiosmotic theory (see Harold & Maloney 1966, Gottschalk 1994) proposed that it should be possible to transform chemical energy of a variety of forms into an electrochemical potential across a membrane: a so-called proton motive force (pmf). This pmf is then used to drive the synthesis of ATP via membrane-bound enzymes called ATPases, which utilize the energy in the electrochemical gradient to drive the synthesis of high-energy phosphate bonds that could then be used by cells for many purposes. The basic requirements of the chemiosmotic theory are presented in Figure 4 and discussed below:

1. A cellular membrane that is impermeable to charged molecules, so that a charge separation can be achieved across the membrane.

2. Electron carriers (e-carriers) and hydrogen carriers (H-carriers) that can be oriented in the membrane in order to achieve a net flux of protons across the membrane. The energy needed to drive this process is derived from the flow of electrons from reduced compounds (substrates) to more oxidized ones (oxidants).

3. An enzyme that can convert the membrane (electrochemical) gradient into cellular-useful chemical energy in the form of ATP.

4. Other enzymes and systems that can directly use the membrane pmf for specific functions, such as transport or motility.

The chemiosmotic model is unifying in the sense that all living organisms utilize it, or some variation of it, to synthesize ATP and drive cellular functions. It allows, in principle, any electron potential between electron donor and acceptor to be used to pump protons to the exterior of the cell, thus establishing the pmf that can be utilized to synthesize ATP. This scheme allows one to think of microbial metabolism in a unifying chemical way. Reduced H-carriers are produced (by enzymatic oxidation of a substrate, by photoreduction, etc),

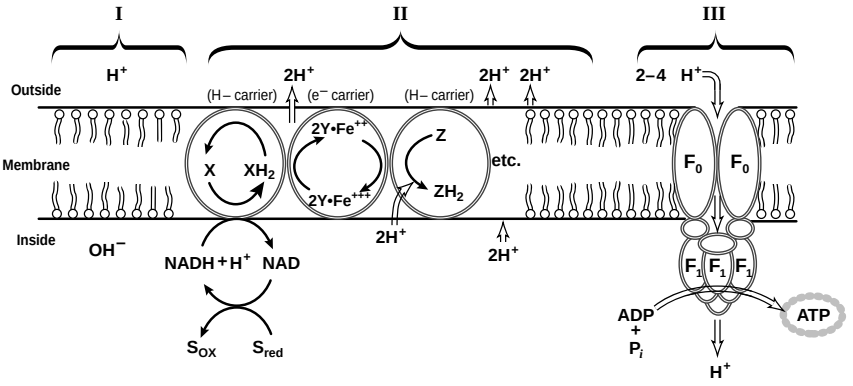


Figure 4 Energy conservation in living organisms. This cartoon is meant to show, in diagrammatic form, the basics of the chemiosmotic theory—in essence, how living organisms on this planet harvest chemical (redox) energy from the environment and conserve it as biologically useful energy (adenosine triphosphate, ATP). The three features shown are as follows: 1. A semipermeable membrane, which is impermeable to charged molecules and can thus be used to separate charges. Once a charge separation is achieved, energy can be harvested. 2. A vectorial electron transport chain, in which H-carriers and e-carriers alternate in the flow of reducing power from substrate to oxidant. As electrons flow toward the oxidant, protons are pumped to the outside of the membrane, creating the electrochemical gradient (a proton motive force or pmf, which consists of a combination of pH and charge gradient). 3. An enzyme to convert the pmf into useful cellular energy. In this case, the enzyme shown is the membrane bound ATPase, which allows protons to flow back into the cell through pores in the membrane it creates and, during this flow, uses the energy to synthesize ATP from adenosine diphosphate (ADP) + inorganic phosphate (P_i). In addition to the ATPase, other systems are present that are activated by the pmf, such as transport systems to bring in or excrete substrates, as well as the flagellar "motor" that causes the bacterial flagella to rotate, giving the organism its motility.

and these interact with a membrane-bound electron transport chain, which ultimately routes the electrons to the appropriate oxidant while pumping protons to the exterior in the process. For this model, the energy source can be organic carbon, inorganic substrates, or light—it really makes no difference as long as the cell possesses the enzymatic machinery to harvest the energy and transfer it to the membrane-bound electron transport chain. The difference between the versatile prokaryotes and the limited eukaryotes is that most eukaryotes have very few choices of reductants and can utilize only one oxidant, molecular oxygen. It may be instructive in viewing the sedimentary processes discussed in the next section to keep this mechanism in mind, remembering that almost all of the reactants and products that are discussed are either oxidants or reductants that fit nicely into the scheme proposed by chemiosmotic model.

THE PROCESSES

Pore water profiles like those shown in Figure 2 are the result of a series of redox processes and are ultimately driven by the input of organic carbon to the sediments (Froelich et al 1979, Reeburgh 1983). The commonly measured components in the study of sediments are shown in the center column of Table 2; the processes and organisms that generate them are in the left columns, and those that consume them in the right columns. It is these processes that are discussed in this section, followed by a discussion of the causative organisms. I deal here with a deep sediment outside the photic zone, where conditions are stable with regard to temperature and carbon input, and a minimal amount of physical mixing occurs. Conditions get more complex in terms of both organisms and carbon cycling if one considers shallow sediments, stratified lakes and fjords, and estuarine sediments, where episodic input of organic carbon and/or photosynthesis are included. The following discussions proceed downward into the sediment, discussing each component listed in Table 2 and shown as profiles in Figure 2. Though such profiles can be used to identify general zones of metabolic processes, some—like fermentation, for which there are no easily measured or distinctive chemical markers [oxygen, sulfide, methane, Mn(II), etc]—are much more difficult to fix spatially in the sediment environment.

Aerobic Respiration

Organic matter that reaches the sediments is aerobically respired (to CO_2 and H_2O) until it exceeds the amount of oxygen that can be delivered to the site by diffusion. In sediments overlain by deep water, much of the organic matter is respired during transit to the sediments, leaving very little carbon to be further oxidized. The result is that deep-sea surface sediments are usually oxidized, and oxygen can remain at high levels for many centimeters downward. However, in shallower, more carbon-rich sediments, it is usual to see oxygen depletion within millimeters or centimeters of the sediment surface.

Nitrification and Denitrification

As one proceeds downward across the oxygen depletion zone, it is common to see a zone in which nitrate concentration increases. This occurs at low concentrations of oxygen, where ammonia diffusing upward from below is converted into nitrate via a process called nitrification. Dissimilatory reduction of N_2 results in the decrease of nitrate with the concomitant oxidation of organic carbon CO_2. The magnitudes of nitrification and denitrification are not easy to measure because they occur in spatially adjacent samples and because for both, the product of one process is the reactant of the other. Thus, with small concentrations of nitrate, the effect on the nitrogen and carbon cycles can be substantial if the cycling rate is large.

Methane, Manganese, and Iron Oxidation

Although some anaerobic methane oxidation occurs in nature (Reeburgh 1983), by and large the major oxidant for methane is molecular oxygen. The profiles of methane observed in sediments usually show methane depletion at the intersection of the oxygen minimum.

As Mn(II) and Fe(II) diffuse upward from reduced sediments, they are deposited as metal oxide layers or crusts in the presence of low levels of oxygen. The layer of oxidized manganese, commonly in the form of MnO_2, typically overlays a layer of oxidized iron, which is more rapidly oxidized in the presence of low levels of oxygen and precipitates just below the MnO_2. Such ferromanganese layers are common in sediments. The oxidation of both Mn(II) and Fe(II) are thermodynamically favored, but the kinetics of the two processes are substantially different at neutral pH values common to most sediments. Mn(II) is kinetically stable (Stumm & Morgan 1981) and usually requires biological catalysis, whereas Fe(II) oxidation is very rapid at neutral pH and biological catalysis is assumed to be unnecessary.

Sulfur Oxidation

Reduced sulfur species such as sulfide, thiosulfate, or polysulfide are produced as a result of organic carbon oxidation in deep sediments, and as they diffuse upward, they are oxidized. These processes are chemically complex and poorly quantified in sediments. As sulfide diffuses upward, it is oxidized by Fe(III), Mn(IV), and oxygen, of which the latter two reactions are quite rapid. Each oxidant generates different sulfur intermediates that can interact with other compounds, which makes the system sufficiently complex to defy most efforts to quantify the separate parts. In most systems, the oxidizing potential of the sediment is such that sulfide is consumed within the sediment, either by oxygen itself or by other oxidants, such as nitrate or metals.

Manganese and Iron Reduction

The zones of metal reduction exist below the nitrate reduction zone, where organic carbon is oxidized by manganese and iron oxides. This results in increased levels of porewater Mn(II) and Fe(II). Fe(II) is a good reductant of MnO_2 and is reoxidized to Fe(III) during this reaction (Myers & Nealson 1988b). Mn(II) increases, followed by the appearance of Fe(II). In freshwater sediments, the profiles are often clearly defined, whereas in marine sediments, iron can be difficult to follow, in part because of the active sulfur cycle in marine systems in which upward diffusion of sulfide tends to remove Fe(II) as iron sulfide (pyrite).

Manganese and iron reduction can be either biological or abiological. Manganese is easily reduced by organic compounds (Stone et al 1994) as well as by

several inorganics, such as sulfide (Burdige & Nealson 1986) or Fe(II) (Myers & Nealson 1988b). Iron can also be reduced by sulfide (Stumm & Morgan 1981) or organics, but it is considerably more resistant to chemical reduction, and some reports maintain that all iron reduction in nature is due to biological catalysis (Lovley et al 1991).

Sulfur Reduction

Sulfate reduction is well characterized in sediments, whereas thiosulfate and sulfur reductions are much less well quantified. With the exception of reduction by very high temperatures, such as those found in hydrothermal waters, sulfate is stable unless reduced biologically—probably no chemical reduction of sulfate is known in sediment systems. Once below the zone of metal reduction, the next major reduced species to appear in porewaters is sulfide, and this is generally attributed to sulfate-reducing bacteria. The importance of other sulfur intermediates, such as thiosulfate or elemental sulfur (polysulfide), remains to be elucidated, probably because of the complexity of sulfur chemistry.

The production of sulfide (and the generation of other reduced sulfur species) as a result of sulfate reduction is one of the major biogeochemical differences between freshwater and marine systems. In freshwater systems, sulfate is in the range of 100–250 μM, whereas in marine systems, it is approximately 25 mM. Sulfate is thus the dominant electron acceptor in marine sediments, dwarfing even oxygen. In contrast, in freshwater systems, the sulfur cycle is less dominant than in marine systems. The profiles in Figure 2 show that in marine sediments, the consumption of oxidants occurs owing to the upward diffusion of reduced sulfur species and that these species are produced largely by the oxidation of organic carbon in the sediments.

Methanogenesis

As in sulfate reduction, methanogenesis is a process that occurs only as a result of biological catalysis at temperatures and conditions common to most sediments. As sulfate reduction to sulfide dominates marine sediments, so does CO_2 reduction to methane dominate freshwater ones. Methane appears in porewaters just below the oxic/anoxic interface, and it is the major indicator of organic carbon turnover in freshwater sediment systems.

THE ORGANISMS

Sediment microbes are present because of the energy to be harvested and should thus be expected to vary in type and number as the input of energy to the given sediment varies in quantity and quality. The organisms can be discussed in terms of the processes observed in the sediments (as discussed above), but this

approach can be dissatisfying because many organisms are facultative, i.e. capable of crossing the boundaries between the processes. Thus, to speak of denitrifiers as a group may be overlooking the important point that most denitrifiers also use oxygen, and many use other electron acceptors and/or are fermentative bacteria. Given the energy-conserving mechanisms outlined above, metabolic plasticity is not particularly surprising. Many bacteria enjoy a wide versatility, from sulfate reducers that are known to utilize electron acceptors up to the potential of nitrate to aerobes that can utilize sulfite and elemental sulfur. Simply identifying an organism will not give information as to what it is actually doing; it is only the beginning of defining the environment.

Aerobic Heterotrophs

A wide array of bacteria possess the ability to degrade organic matter using molecular oxygen as an electron acceptor. Only a few of these are obligately aerobic, and even for these, there may be alternative modes of survival or growth under anoxic conditions. For example, some of the genera long considered to be aerobes, such as *Pseudomonas* and *Bacillus*, have quite a number of species that do well under anoxic conditions, either through some form of fermentation or via the use of alternate electron acceptors like nitrate. It is safe to say that there will not be a shortage of aerobic heterotrophs as long as there is oxygen and organic carbon, and these efficient organisms, given a high enough flux of oxygen, will leave little organic carbon undegraded.

Chemolithotrophs

In marked contrast to the aerobic heterotrophs, the chemolithotrophs (organisms that utilize inorganic energy sources) are restricted to a few groups of organisms and tend to be specialists (Shively & Barton 1991). An exception is the group of H_2-utilizing bacteria, which are widespread, both environmentally and biologically.

HYDROGEN OXIDIZING BACTERIA The most widespread of the chemolithotrophs are those that use molecular hydrogen as an energy source, via the enzyme hydrogenase, which produces reduced NAD (NADH + H^+) by the reaction shown below. The oxidation of NADH is then coupled to the reduction of some electron acceptor, as discussed above, to yield energy in the form of a pmf for growth and metabolism.

$$H_2 + \text{NAD} \xrightarrow{\text{hydrogenase}} \text{NADH} + H^+.$$

Many bacteria are known to utilize H_2 as an energy source, ranging from aerobes like *Alcaligenes eutrophus* to facultative organisms like *Paracoccus denitrificans* and *Shewanella putrefaciens*, which can couple hydrogen oxidation to the

reduction of a variety of different electron acceptors, and including obligately anaerobic bacteria like sulfate reducing bacteria (SRB) and methanogens. Some of these organisms are autotrophs, growing with CO_2 as the sole source of carbon, while others, like *S. putrefaciens*, simply use the hydrogen as a source of energy and grow heterotrophically on organic carbon.

SULFUR-OXIDIZING BACTERIA Organisms that oxidize sulfur compounds lithotrophically are usually quite specialized. Most are autotrophic, utilizing sulfur compounds as the sole source of energy and CO_2 as the sole carbon source, and are incapable of growing heterotrophically on organic carbon sources. Once the commitment is made to this metabolism, other modes of metabolism are not common. The substrate of the sulfur oxidizers is usually thought of as hydrogen sulfide, although many sulfur oxidizers will also oxidize elemental sulfur and/or thiosulfate as well. Given the rapid kinetics of sulfide oxidation by molecular oxygen, sulfide-oxidizing organisms are in a continuous struggle with chemical oxidation. Therefore, they are often found at interfaces where anoxic waters are mixing slowly with oxic waters above them. At such boundaries, sulfur oxidizers position themselves between the two reactants and take advantage of natural gradients, thus harvesting abundant energy.

Another approach to utilizing sulfide in an oxic world is seen with the sulfide-oxidizing symbionts of hydrothermal-vent eukaryotes. In some of these systems, the eukaryotic hosts have developed sulfide-binding proteins that harvest sulfide from the vent waters and transport the sulfide (and oxygen) to the bacteria in their symbiotic organelles (trophosomes), where the bacteria can deal with each substrate separately (Jannasch 1995).

The sulfur oxidizers include those that are restricted to low pH environments (acidophiles) and the neutral pH types. The acidophiles are commonly isolated from acid-mine drainage environments, where sulfur-rich coals are exposed to oxygen. These bacteria utilize the sulfide in the coal as their energy source, converting it to sulfuric acid, thus creating an environment of pH4 or less, where they thrive and other organisms are excluded. The neutral pH sulfur oxidizers include several *Thiobacillus* species as well as an array of structurally complex sulfur bacteria in the genera *Beggiatoa*, *Thioploca*, *Thiothrix*, and others. These bacteria have been traditionally difficult to culture but are often abundant in both marine and freshwater sediments. In some cases, such as with *Beggiatoa* species, the bacteria are clearly capable of growth on sulfide. In others, involvement with sulfide is suspected because of the environment from which they are isolated and, often, from the presence of either intracellular or extracellular sulfur granules associated with the cells. Fossing et al (1995) reported that *Thiothrix* cells contain vesicles in which nitrate is stored for later utilization, so that the organisms could accumulate nitrate (up to 500 mM!) in oxic environments, then migrate downward as much as 10 cm, where

they could use the nitrate as an electron acceptor to oxidize sulfide under aerobic conditions. If such microbial "scuba divers" are common, the delicate profiles presented in Figure 2 could be missing major metabolic microenvironments.

Despite the difficulties in culturing sulfur-oxidizing bacteria, many have now been identified by using 16S rRNA probes. Their abundance and distribution, as well as some aspects of their diversity, will undoubtedly be further elucidated in the near future.

IRON-OXIDIZING BACTERIA As with the sulfur oxidizers, the iron oxidizers are in competition with chemical oxidation—at neutral pH, the oxidation of ferrous to ferric iron is extremely rapid, so it is difficult to compete except in gradient-like environments. At low pHs, as in acid mines, where Fe(II) is abundant, species like *Thiobacillus ferrooxidans* grow very well using ferrous iron as their only source of energy. In neutral pH sediments, other iron oxidizers may compete. For most neutral pH iron oxidizers, like *Gallionella ferruginea*, the proof of iron oxidation as a mode of energy conservation has been difficult. In the laboratory, it is hard to duplicate environments where reduced iron is produced and made available to bacteria without the interference from contaminating oxygen.

NITRIFYING BACTERIA AND METHANOTROPHS Nitrifying bacteria consist of two groups, those that oxidize ammonia to nitrite and those that oxidize nitrite to nitrate. The first group—the ammonia oxidizers—include many species in the genera *Nitrosobacter*, *Nitrosococcus*, and *Nitrosomonas* and are noted for internal membranes and the presence of a primary amine oxidase (PMO) that catalyzes the oxidation of ammonia with the production of $NADH + H^+$. The second group (including many species in the genera *Nitrobacter*, *Nitrococcus*, and *Nitrospira*) are slow-growing specialists dependent on the supply of nitrite from the first group. Like the nitrate oxidizers, these bacteria are replete with internal membranes, and cell shape and internal membrane structure is often used for identification.

Bacteria that grow on methane as the sole source of carbon and energy are called methanotrophs, while those that grow on methanol and more oxidized species of organic carbon are called methylotrophs. Methanotrophs (including the genera *Methylomonas*, *Methylococcus*, and *Methylosinus*) share many properties with the ammonia-oxidizing bacteria, including the presence of abundant intracellular membranes. On the basis of membrane structure, they are usually divided into major taxonomic groups. Ecologically, they also share a habitat with the ammonia oxidizers and are abundant at the oxic-anoxic interface. Methylotrophs, which include many different genera, are less specialized and are often capable of heterotrophic growth on other carbon compounds.

FERMENTATIVE BACTERIA Fermentative bacteria consist of a wide array of different metabolic types that are specialists in the disproportionation of organic carbon. The carbon is taken into the cell, usually split into smaller molecules, and part of it oxidized while other parts are reduced. This results in the excretion of both oxidized and reduced fermentation end-products (H_2, CO_2, acetate, lactate, etc), almost all of which are important in the metabolism of other organisms in the anaerobic food chain. Many fermentative bacteria, rather than being specialists in fermentation, are facultative and are able to convert to a fermentative mode when extracellular oxygen is depleted.

NITRATE-RESPIRING BACTERIA A great number of bacteria can catalyze the reduction of nitrate to N_2 gas in the denitrification process, and although nitrate is often at low concentrations in the environment, these organisms are ubiquitous and play a role in the cycling of carbon and nitrogen in sedimentary systems. When oxygen becomes limiting, nitrate is typically the next major biological electron acceptor utilized. There is a great variation among organisms that accomplish nitrate reduction, with some that reduce the nitrate all the way to ammonia and other bacteria that reduce nitrate even in the presence of molecular oxygen (Kuenen & Robertson 1988, Blackburn & Blackburn 1992).

METAL-RESPIRING BACTERIA For many years, the profiles of metals shown in Figure 2 were regarded as the result of chemical reactions in sediment, primarily because the substrates involved were solids (Mn and Fe oxides) and thought to be inaccessible to direct bacterial reduction. This view has changed dramatically in the past few years, with the discovery of several groups of bacteria that grow by the dissimilatory reduction of iron and/or manganese (Lovley 1993, Nealson & Saffarini 1994), which results in the oxidation of organic matter and the reduction of Fe(II) or Mn(II). The organisms so far identified consist of both facultative anaerobes in the group *Shewanella* (DiChristina et al 1988, Myers & Nealson 1988a) and obligate anaerobes in organisms closely related to the *Geobacter* group (Lovley & Phillips 1988, Lonergan et al 1996).

SULFUR- AND SULFATE-REDUCING BACTERIA Much has been written about bacteria that live by the dissimilatory reduction of sulfur compounds, and just a few groups are mentioned here. Among the sulfur reducers are both facultative anaerobes like *S. putrefaciens* (Moser & Nealson 1996), as well as many anaerobic bacteria in groups such as *Wollinella*, *Desulfuromonas*, and others (see Balows et al 1991). The sulfate reducers, on the other hand, are all obligate anaerobes, including commonly encountered genera such as *Desulfobacter*, *Desulfovibrio*, and others.

Methanogens and Acetogens

All known methanogenic bacteria are found in the domain of the Archaea. The reactions required to produce methane are unique to this group, and the organisms, in groups such as *Methanobacter*, *Methanococcus*, *Methanobacterium*, etc, are widely distributed in anaerobic niches throughout the world, including many thermophilic environments. Some of these bacteria use hydrogen and CO_2 as their substrates for methane formation; others use acetate.

The acetogenic (or homoacetogenic) bacteria are a heterogeneous group united by the ability to catalyze the reduction of two CO_2 molecules to acetate. Many use hydrogen as the reductant, but others utilize a wide range of other substrates (Diekert 1991). While they share with the methanogens the ability to reduce CO_2, they are much more versatile, perhaps the most versatile of all the strict anaerobes, and are taxonomically diverse, found in many different genera (*Clostridium*, *Acetobacterium*, *Acetogenium*, and others) throughout the domain of the Bacteria. Given the proposed importance of both acetate and hydrogen in anaerobic food chains (Reeburgh 1983, Novelli et al 1988), it is not surprising that this versatile metabolic group is widespread in sediments around the world (Diekert 1991).

Proton Reducers (Syntrophic Bacteria)

One of the major modes of bacterial symbiosis involves the exchange of hydrogen between different organisms (Schink 1991), and some of the bacteria commonly involved occur in anaerobic environments, where they eke out an existence by oxidizing fermentation end products like acetate, propionate, or fatty acids, and by producing H_2 gas. Such reactions are energetically feasible only when the hydrogen remains at very low concentrations, so that these bacteria (in the genera *Syntrophobacter*, *Syntrophomonas*, *Syntrophus*, etc) must live in coculture with H_2-utilizing bacteria such as methanogens, acetogens, and sulfur reducers.

THE FUTURE: WHERE ARE WE GOING, AND HOW WILL WE GET THERE?

Although scientific crystal balls are seldom accurate, speculation as to where and how a given field is evolving is of some value. Where are the emerging areas, and which are likely to be the most exciting in terms of scientific progress in the near future? Given the state of sediment microbiology today, one of the major areas of future work will most likely involve dissecting the diversity and interactions of the microbial populations. This will certainly involve the use of new molecular approaches, which have revolutionized our way of thinking about the phylogeny of microbes. The power of these tools, as outlined below,

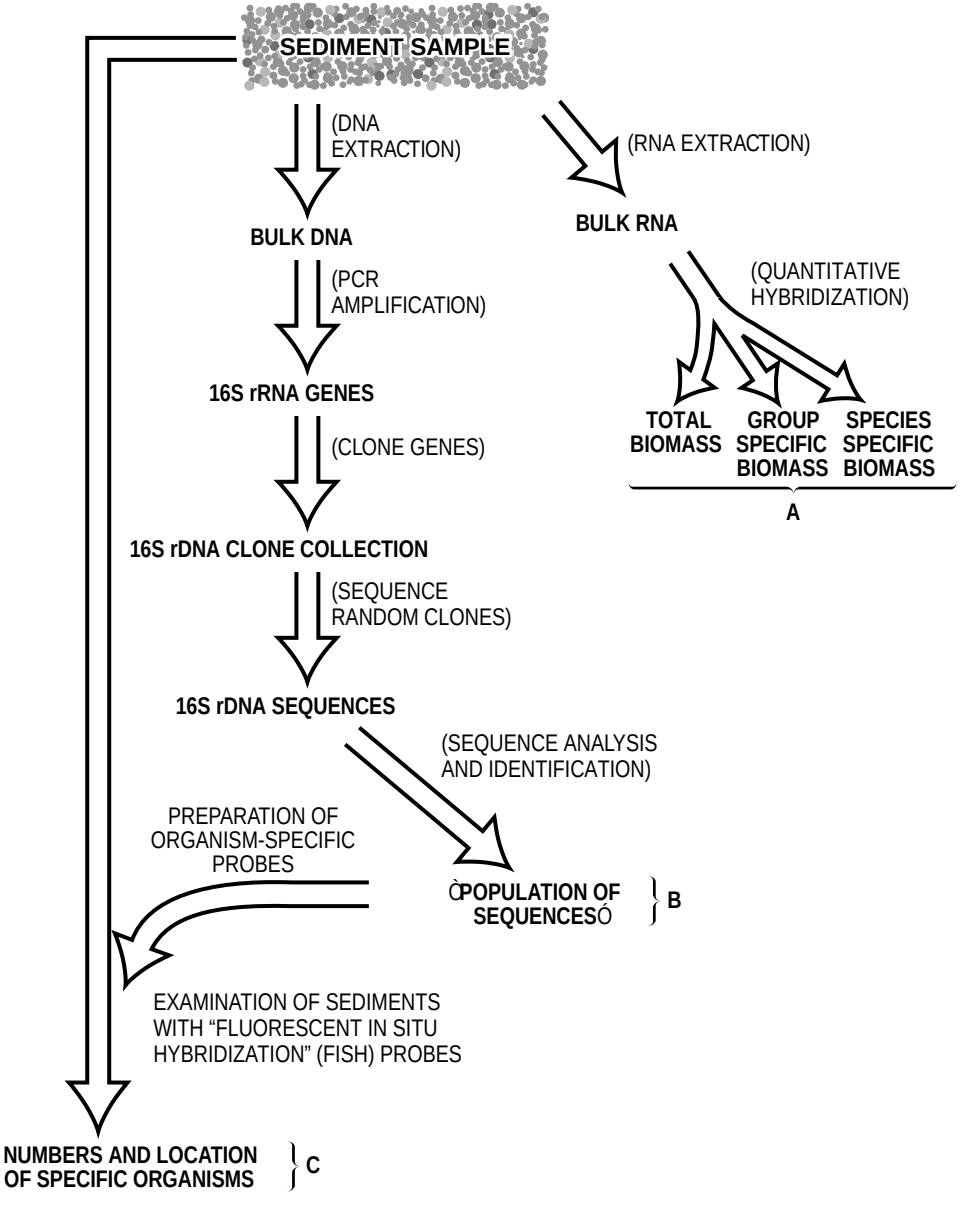
allows us to answer questions that we could not even ask just a few years ago. These tools are already telling us that many new organisms are present that are not yet cultured, and even suggesting what types they are—information that necessitates the study of new isolates with unique metabolic abilities; this point is demonstrated below with a discussion of some new organisms involved in the biogeochemical cycling of metals.

In addition, two emerging areas will involve the nature of the ecosystem itself and will require a particularly interdisciplinary approach. These include the study of the interactions between microbes and minerals and the study of ecosystems where energy is delivered as a result of geological processes. Examples of each of these are presented below. The particular topics I have chosen for discussion are meant to focus on areas that might be less well known to the casual reader and to act as points of departure for further reading and thought.

Molecular Approaches to the Microbiology of Sediments

One of the most exciting recent happenings in microbial ecology has been the introduction of the tools of molecular biology to the field (Amann et al 1992, 1995, Olsen et al 1986, Pace et al 1986, 1993, Stahl 1986, Stahl & Amann 1991, Stahl et al 1988). Molecular approaches (Figure 5) allow one to use informational molecules like rRNA for the indirect characterization of environmental populations, inference of the amounts of total and specific microbial biomass, and even location within a given environment of certain organisms—all this without the need for culturing the microbe(s) of interest. The ribosome is composed of rRNA and specific ribosomal proteins and is the highly complex three-dimensional subcellular structure on which protein synthesis occurs (Lake 1985). Although rRNA is single stranded, it has substantial internal

Figure 5 Molecular methods in environmental analysis. This flow diagram shows one approach that is now being used to exploit molecular methods for the study of microbial ecosystems. The first step is extraction of nucleic acids (both DNA and RNA). The bulk RNA is then interrogated by quantitative hybridization with probes of different specificities to give indications of total biomass, group specific biomass and and species specific biomass (A). This approach gives a view of the population, but is limited in the sense that if organisms are present for which probes are not available, they will not be scored. The next step is amplification (by PCR) of the rRNA genes, using universal primers, followed by cloning and sequencing to give a view of the major components of the population as identified by specific sequences. These sequences can be taxonomically and phylogenetically placed, and a microbial "population" specified according to the sequences obtained (B). Finally, hybridization probes can be synthesized (and labelled with fluorescent dyes), and used to analyze the original sediment sample by fluorescence in situ hybridization (FISH). By this method, it is possible to ask where specific types of organisms reside, and to see if the major groups of microbes have in fact been identified by the method.


SEDIMENT SAMPLE
(DNA EXTRACTION)
(RNA EXTRACTION)
BULK DNA
BULK RNA
(PCR AMPLIFICATION)
(QUANTITATIVE HYBRIDIZATION)
16S rRNA GENES
TOTAL BIOMASS
GROUP SPECIFIC BIOMASS
SPECIES SPECIFIC BIOMASS
A
(CLONE GENES)
16S rDNA CLONE COLLECTION
(SEQUENCE RANDOM CLONES)
16S rDNA SEQUENCES
(SEQUENCE ANALYSIS AND IDENTIFICATION)
PREPARATION OF ORGANISM-SPECIFIC PROBES
POPULATION OF SEQUENCES
B
EXAMINATION OF SEDIMENTS WITH "FLUORESCENT IN SITU HYBRIDIZATION" (FISH) PROBES
NUMBERS AND LOCATION OF SPECIFIC ORGANISMS
C

secondary structure and is thus stable and easily isolated. It can be separated into different size groups (5S, 16S, and 23S), of which the 16S has most been extensively studied by sequence comparison. Analysis of 16S rRNA sequences led to the realization (Woese 1987) that all organisms are related to one another using sequence comparisons, and now, the sequences of more than 5000 different 16S rRNAs have been used to generate molecular phylogenies of the type shown in Figure 1 (Maidak et al 1994). Once such phylogenies are established on the basis of cultured organisms, they can be used to place any new sequence obtained from the environment into this context, so that one can, in principle, identify an organism on the basis of its nucleotide sequence, never having to culture that organism.

Some parts of the 16S rRNA have evolved so slowly that they are identical in all living organisms (universally conserved). Thus it is possible to synthesize an oligonucleotide that is complementary to such a sequence and will anneal with (hybridize to) any known 16S rRNA. Such sites are also used as universal priming sites to initiate DNA synthesis for PCR (polymerase chain reaction) amplification.

Other areas of the 16S rRNA are less well conserved, and these more variable regions can be used for designing hybridization probes that are domain specific, group specific, or even species specific (Devereux et al 1989, 1992, 1994, Kohring et al 1994, Pace et al 1993). Thus, the specific rRNA sequence information provides not only phylogenetic information but allows, at several levels of resolution, the placement of rRNA sequences of microbes to functional groups and taxa (Johnson 1984, Stackebrandt & Liesack 1993, Stahl & Amann 1991, Woese 1987). This latter property is now being exploited for molecular ecological analyses. Through the analysis of rRNA, or the DNA coding for rRNA, it is thus possible to infer a great deal about the populations of microbes in the environment studied. Such approaches have been very successful in many different ecosystems (Amann et al 1992, Barns et al 1994, Giovannoni et al 1990, Liesack & Stackebrandt 1992, Pace et al 1986, Risatti et al 1994, Stahl et al 1988, Teske et al 1996, Ward et al 1992, Weller & Ward 1989), although they have only recently been applied to the study of sediments (Devereux & Mundfrom 1994, MacGregor et al 1997, Rochelle et al 1994).

Though many molecular approaches can be taken to characterize an environment, two that involve 16S rRNA are discussed here in order to demonstrate some of the power of the techniques. Both are outlined in Figure 5. First, one can extract the rRNA directly and interrogate this RNA with specific probes. The probes are labeled with ^{32}P or some other appropriate label that can be quantified, the RNA is hybridized to each probe separately, and the radioactivity used to estimate the relative levels of that population. Thus, universal probes may be used to estimate total rRNA (a proxy of total biomass), and more

specific probes to ask whether more specific groups of bacteria are present [e.g. domain-specific (Archaea-specific)] probes, group-specific probes [e.g. for sulfate reducing bacteria (Devereux et al 1989, 1992], or even species-specific probes. Without culturing organisms, one can thus get a molecular snapshot of the major metabolic groups as represented in the rRNA that is extracted.

Second, one can extract DNA from the samples and amplify the genes for 16S rRNA from these extracts. The amplification is made possible because of the universally conserved areas within the rRNA molecule, which can be used as primers for the sequencing reaction for PCR amplification. Once these molecules are amplified, they can be cloned, random clones sequenced, and these sequences identified as appropriate metabolic groups or taxa, as described above. By this approach, one can get a more specific snapshot of the taxonomic and phylogenetic groups of bacteria within a given environment.

Once specific sequences are determined for a given environment, these can be used to construct oligomeric hybridization probes that will hybridize with the ribosomes of those organisms from which the sequences were obtained. Because actively growing bacteria contain thousands of ribosomes, such probes, when labeled with fluorescent dyes and hybridized to environmental samples, can be used to physically locate the cells by fluorescence in situ hybridization (FISH). Thus, from a nucleic acid extract of the environment, one can return with specific probes and determine the location of specific groups of organisms that have been identified by sequence analysis. This approach also has the advantage that only living (rapidly growing) cells contain enough ribosomes to give a signal, so that dead cells will not be scored. Of course, this is also a disadvantage, in the sense that dormant or slow-growing components of the community may be missed because the number of ribosomes per cell is too low for detection by this method.

Isolation of New Organisms—Metal Active Microbes

Though the techniques outlined above are of tremendous value for determining populations, the environment will never be understood if the organisms are not grown and studied in culture. That is, just knowing who is there is not enough; one must examine the metabolic plasticity and regulation of the population in order to understand how the ecosystem might function under different conditions. Thus, the isolation of new organisms remains an important and guiding part of the field of microbial ecology. One difference, of course, is that on the basis of the molecular data obtained above, it is now possible to suspect the presence of types of organisms that may not have been previously postulated, and to design enrichment cultures and growth media accordingly.

To illustrate this point, I discuss here a few new findings dealing with metal-active microbes, to emphasize that the “art” of isolation and characterization

of new organisms represents an active area with much potential for further development. The examples I present involve bacteria capable of anaerobic iron oxidation and reduction and are used here to demonstrate how a metal cycle, unimagined just a few years ago, might function in nature, interacting either directly or indirectly with organic carbon.

Iron Oxidizing Bacteria

In the past decade, two new groups of iron-oxidizing bacteria have been isolated, and although the product of both these bacteria is oxidized [ferric] iron, the metabolism of the two groups that produce the Fe(III) is decidedly different. The first, described by Ehrenreich & Widdell (1994), are photosynthetic bacteria, which use the energy of sunlight to drive the fixation of CO_2 to organic carbon $(CH_2O)_n$ under anaerobic conditions. The electron donor for this reaction is ferrous iron, so that the product that accumulates during the reaction is ferric iron in a solid form like ferrihydrite [$Fe(OH)_3$].

$$Fe(II) + CO_2 + H_2O \xrightarrow{\text{sunlight}} Fe(III) + (CH_2O)_n.$$

The second group, described by Straub et al (1996), are chemolithotrophic bacteria that utilize ferrous iron as the source of energy, while using nitrate as the oxidant for anaerobic growth. These bacteria use the energy of the $Fe(II)/NO_3^-$ couple to supply energy for carbon fixation and resulting autotrophic growth.

The Iron-Reducing Bacteria

As discussed above, until a few years ago, it was not accepted that bacteria could use solid substrates like iron or manganese oxides for dissimilatory metabolism. This changed in 1988 with the report of two organisms, *S. putrefaciens* and *Geobacter metalloreducens*, that are capable of living anaerobically by coupling their heterotrophic growth to the reduction of iron or manganese oxides. Such bacteria were capable of catalyzing the general reaction shown below:

$$Fe(III) + \text{Organic carbon} \longrightarrow Fe(II) + CO_2 \text{ (or other oxidized organic C)}.$$

ANAEROBIC BIOGEOCHEMICAL CYCLING OF IRON With the knowledge that such organisms exist, it is possible to "construct" a hypothetical biogeochemical cycle of iron, which is driven by light that produces both fixed organic carbon and Fe(III) as an oxidant. These two reactants are then used by the iron-reducing bacteria to complete the cycle (Figure 6). This anaerobic system involves no volatile components as redox members.

Minerals and Microbes

Sediments are dominated by solid phase minerals of many different kinds (carbonates, silicates, clays, metal oxides, metal sulfides, etc), and these minerals are often the reactants and/or products of microbial reactions. They also serve

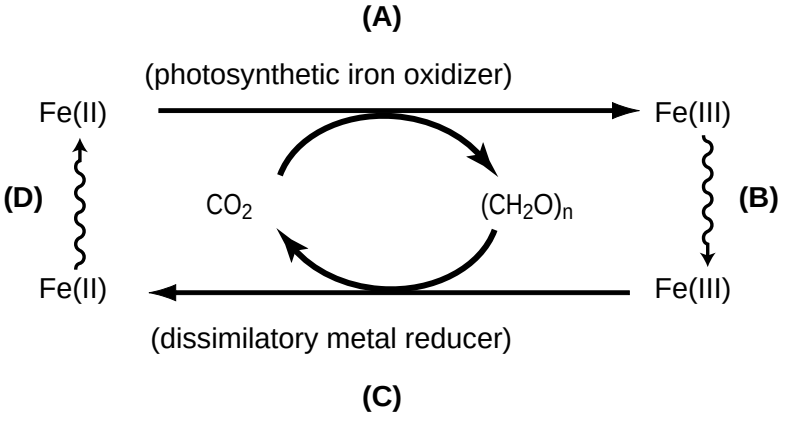


Figure 6 Hypothetical biogeochemical cycle of iron. This figure shows a biogeochemical cycle of iron and carbon, catalyzed by two recently isolated groups of prokaryotes, as described in the text. It is meant only to stimulate thought and to emphasize that many different kinds of elemental cycles are, in principle, possible.

as solid substrates for microbial colonization, irrespective of whether they are directly involved with microbial metabolism. The whole subject of the formation and dissolution of solid mineral phases represents one of the most unknown and uninvestigated areas of microbial ecology, and it is one of central importance to the ultimate understanding of the dynamics of sediments. For example, the magnetic mineral magnetite can be formed (Bell et al 1987) or dissolved (Kostka & Nealson 1995) by iron-reducing bacteria, depending on the conditions. Similarly, although it is well known that microbially reduced iron can end up as any one of a variety of mineral end products (iron sulfide, phosphate, carbonate, etc), very little is known of any specific roles that microbes play in the formation and/or dissolution of most minerals. In terms of understanding past sedimentary deposits, such knowledge would be extremely valuable but is often lacking.

New Microscopic Techniques

The molecular studies outlined above, as well as the study of mineral-microbe interactions, have both benefited from recent advances in microscopy, which, along with advances in image processing, will undoubtedly continue to enhance our ability to do environmental microscopy of sediments. In particular, the confocal laser scanning microscope (CLSM) has allowed imaging of microbes on surfaces, and reconstruction of their three-dimensional environments. Also, the environmental scanning electron microscope (ESEM; Little et al 1991)

(see Figure 7) allows examination of microbes under ambient atmospheric conditions, which obviates the need for vacuum preparations and gold coating of the sample. These two techniques, in consort with fluorescent probes of the type discussed above, will have major impacts on our appreciation of sediment microbiology in the next decade.

New Microbial Ecosystems

Many new microbial ecosystems undoubtedly remain undescribed, and as the diversity of microbial consortia and populations are unraveled, the description of these systems may represent one of the major challenges of the future. The delicate symbioses in which microbes are involved may well explain why many remain uncultured—we may simply need to understand that many organisms are dependent on syntrophs to either supply or remove a given nutrient or product before growth can occur.

Apart from these speculations, some ecosystems have emerged in the recent past that suggest that the interaction between the geology of the Earth and the microbial ecosystems that reside on it might be much more intricate than had been imagined a few decades ago. The two examples I present here illustrate the generation of reduced energy sources by geological processes and the potential development of major bacterial populations in response to these energy sources.

Hydrothermal Energy Inputs

When subsurface seawater is exposed to very high temperatures, and redirected to the ocean via the midoceanic ridges, major changes in its chemistry occur due to high temperature catalysis (see Jannasch 1995). These so-called hydrothermal vent environments have some very exciting characteristics concerning microbiology, and many of these are directly relevant to sediments on Earth. Under hydrothermal conditions, reduction of sulfur, sulfate, and CO_2 are all thermodynamically favored processes (Shock 1996). In fact, not only are H_2, H_2S, and CH_4 predicted under these conditions, so are a series of organic compounds, like fatty acids, alcohols, and ketones (Shock 1996). Thus, under defined hydrothermal conditions, it is possible to use the geothermal energy of the Earth to create both inorganic (chemolithotrophic) and organic (heterotrophic) energy sources that should be easily exploited by thermophilic microbes. That the former occurs on the contemporary Earth is elegantly shown by the rich populations of organisms around the deep sea hydrothermal vents; whether it could occur in other deep sedimentary environments with hydrothermal input is an interesting question.

Hydrogen-Driven Rock-Based Ecosystems

A recent report by Stevens & McKinley (1995) raises the possibility of other types of ecosystems, which are also present in the subsurface environment and driven ultimately by geochemical energy rather than sunlight. They report

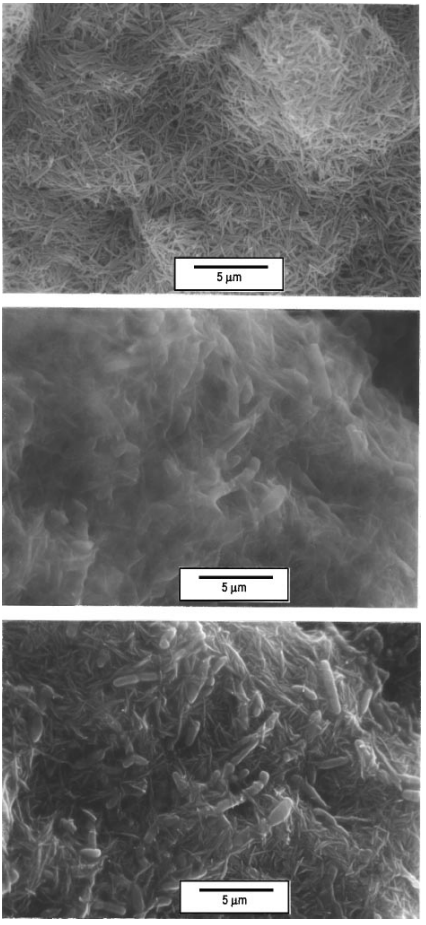


Figure 7 Bacteria on the surface of a metal oxide. This figure shows a metal oxide called manganite (MnOOH) without bacteria (*top panel*) and in a late stage of reduction by a pure culture of the metal reducing bacterium *S. putrefaciens* (*middle* and *bottom panels*). The middle panel shows the image obtained via environmental scanning electron microscopy (ESEM), in which a polymeric layer (probably polysaccharide) is formed and obscures the bacteria. The bottom panel shows the identical field imaged with standard scanning electron microscopy (SEM) in which drying of the samples results in an image of the surface in which the polymeric layer (biofilm) is not seen. ESEM, which allows high-resolution imaging of hydrated samples, thus allows one to eliminate many of the drying and coating artifacts common to SEM analysis of natural samples (Little et al 1991).

hydrogen production as a result of the interaction between anaerobic water and basaltic rocks. These reactions have been verified in the laboratory using rock/water systems, and they are consistent with the presence of rich populations of anaerobic microorganisms in the deep subsurface—populations that exist entirely as a result of the input of hydrogen gas. To find a ready cadre of organisms that might be expected to reside in an anaerobic niche where hydrogen and CO_2 are readily available (methanogens, acetogens, or sulfate reducers), consult Table 1; these organisms have already been found in abundance in such subsurface environments (Stevens & McKinley 1995).

SUMMARY

By presenting my personal view of prokaryotic life in sedimentary environments, I hope to stimulate some new ideas, especially across disciplinary boundaries that must be crossed if progress is to be made. One might note that nearly everything discussed in the final section of this review involves more than one discipline. The spatial and temporal scales involved often require disciplines that range from geology and geochemistry at one end, to microbiology and molecular biology at the other. Microbial populations that cannot be cultured may now be defined. Microbes are also now readily recognized to exploit many energy sources and oxidants that were not known a few years ago, and some environments are now recognized to exist where energy is provided through geological processes rather than sunlight. Despite these advances in knowledge, our understanding of the ways that sedimentary microbes interact with each other and with their mineral rich environment is admittedly only now beginning to emerge.

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