# **Biological Considerations in Geotechnical Engineering**

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**Abstract:** The understanding of soil behavior during the last 300 years has centered on mechanical principles, geological processes, and later on, mineralogy and the relevance of colloidal chemistry. More recently, research in biology and earth science has enabled important advances in understanding the crucial involvement of microorganisms in the evolution of the earth, their ubiquitous presence in near surface soils and rocks, and their participation in mediating and facilitating most geochemical reactions. Yet, the effect of biological activity on soil mechanical behavior remains largely underexplored in the geotechnical field. The purposes of this paper are to introduce microbiological concepts, identify and illustrate their potential roles in soils and rocks, and stimulate interest in seeking improved understanding of their importance and potential for advancing the states of knowledge and practice in geotechnical engineering. It is shown that microorganisms play an important part on the formation of many fine grained soils, can alter the behavior of coarse grained soils (including hydraulic conductivity, diffusion and strength), accelerate geochemical reactions by orders of magnitude, promote both weathering and aging, and alter the chemical and mechanical properties of specimens after sampling. While extensive research is needed to delineate the full impact of biomass and biomediated reactions on soil behavior, it is anticipated that a proper understanding of biological principles will lead to improved soil characterization, enhanced understanding of soil behavior, and even alternative geotechnical engineering solutions.

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### Introduction

Pioneering contributions in the 18th and 19th Centuries, such as those by Coulomb and Rankine, used mathematics and physics to explain, quantify, and predict some aspects of behavior relevant to soil mechanics and foundation engineering. Karl Terzaghi, in the early part of the 20th Century, demonstrated the essential role of geology in identifying subsurface materials, their distribution, and many aspects of their behavior. By defining the principle of effective stress, he also provided a fundamental basis for understanding the key elements of stiffness, volume change and strength of soils. However, by the mid-20th Century some phenomena of geotechnical importance, e.g., the unusual behavior of quick clays, clay swelling, collapsing soils, creep and stress relaxation, still required further explanation. This led to elucidation of the important roles of chemistry and mineralogy in determining soil properties and behavior.

Although there is more biomass below the ground surface than above, and references have been made over the years to the relevance of biological factors in relation to some specialized as-

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pects of soil behavior, little effort has been devoted by geotechnical engineers to assess the extent, possible importance, and usefulness of biological considerations in geoengineering.

With the explosion of new knowledge of biology and microbiology in science and technology during the past several years, it is appropriate to critically assess whether developments in these fields may have potential to lead us to better understanding and new directions in geotechnical research and practice. The purposes of this paper are to define, assess, and illustrate the importance of some biological factors and processes in soils and rocks, and to stimulate interest in developing an improved understanding of their roles and potential usefulness in advancing geotechnical knowledge and practice. We do this by:

- Defining and describing biological constituents, characteristics, and processes within a soil. We confine our scope to microbiological phenomena, excluding detailed discussion of organic matter and organic soils, as they have been studied extensively over the years.
- Providing examples of how microbiological conditions and processes may influence engineering properties and behavior of earth materials.
- Reviewing geomicrobiological processes that appear particularly promising for further study.
- Identifying relevant references to aid in obtaining in-depth background and understanding of many of the points, which, owing to length limitations, are only addressed briefly herein.

Our review of microbiology and biochemical processes in the geoenvironment is based on the readily available references that are cited. As for all disciplines, microbiology has its own special terms and definitions; those used herein should be adequate to meet the objectives of the paper. A comprehensive glossary of microbiological terms is given in Ehrlich (1996a).

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#### Bacteria Archea Eukarya Most cell walls contain significant This domain includes: algae (photosynthetic eukarya -· Peptidoglycan in the cell wall is unique to bacteria (attacked by some amount of protein. produce most of the oxygen in the world), fungi (gather energy by decomposing sources of organic carbon antibiotics) They are primarily present in includes tube-like filaments call mold), and protozoa Some bacteria can survive harsh anaerobic subsurface environments conditions by forming spores. and around organic compounds. (complex single cell decomposers). They may not have a cell wall (1). Many cell functions are Common Characteristics not implemented by the membrane (2), but by specialized · Bacteria and archea have different chemical composition but similar cellular membrane-enclosed organelles (3). They always have a structure: a strong cell wall in most cases (1), membrane (2), cytoplasm (3), folded chromosome (4). They have no membrane-enclosed nucleus. membrane-enclosed nucleus (4). Diatoms are eukarya with cell walls. Foraminiferan have • The semi-permeable cell membrane controls the transport of chemicals and electrical charges in-and-out of the cell. In addition, the cell membrane conducts internal shells. Compartmentalization allows eukarya to be larger (diameter many functions including energy generation. • They are spherical, rod and curved shaped, and typically 0.5-to-3 μm long. Size 2-100 µm) than bacteria and archea reflects optimal conditions between volume-related internal functions and Most eukarya are aquatic. The population size is expected surface-related transport of input and waste. to be small in pristine aquifer sediments, but it increases in • There are cells that can live under extreme pH, temperature, and salt sediments contaminated with organic compounds. concentration. Therefore, they are present in most subsurface environments. Most eukarya require oxygen. Therefore, they are less They reproduce by fission into two (reproduction time: from minutes to few common microorganisms in the subsurface.. days). Flagela may protrude from the surface to propel the cell, and pili to attach There are multiple forms of reproduction and motion in the eukarya domain (e.g., flagela, cilia, pseudopods).

Fig. 1. Classes and some characteristics of microorganisms. Complied from Woese et al. (1990); Ehrlich (1996b); Chapelle (2001): and Madigan et al. (2000).

### Microorganism Types and Characteristics

There are from  $\sim 10^9$  to  $10^{12}$  organisms in a kilogram of soil near the ground surface. Some characteristics and properties of these microorganisms are given in Fig. 1. Single cell microorganisms include all types of *bacteria*, *archea*, and *eukarya*. Bacteria and archea have a simple cell structure with no membrane-enclosed nucleus, and are distinguished by their chemical composition rather than by their structures. Their genetic information is contained in the DNA molecule (there may be more than one chromosome). Eukarya cells have a nucleus; they include algae, fungi, protozoa, and slime molds. Noncellular *viruses* are parasites of cellular organisms, and they use these organisms to derive energy and reproduce. Microorganisms can be identified and classified by shape and type of wall, nutrients, type of biochemical transformation, and DNA and RNA sequences.

### Metabolism—Classification of Microorganisms

Cell growth and reproduction require carbon to form the molecules in the cell, and energy to sustain life. These needs allow classification of organisms on the basis of their nutrition ("trophos" in Greek). In terms of energy source, organisms are *phototrophs* (derive energy from light) or *chemotrophs* (obtain energy from either organic or inorganic chemical sources). In terms of cellular carbon source, organisms are *autotrophs*, also known as lithotrophs, when they obtain cellular carbon primarily from biosynthesis of carbon dioxide or *heterotrophs*, a.k.a. organotrophs, when they use organic compounds as the principal source of cellular carbon. Therefore, autotrophs are the "producers" whereas heterotrophs are the "consumers," e.g., decomposers and scavengers. These energy and carbon-source categories combine into four classifications for organisms: photoauto, photohetero, chemoauto, and chemoheterotrophs.

Chemotrophs capture the energy liberated during oxidation, whereby a chemical compound loses an electron. Because of Coulombian attraction and electroneutrality, electrons are not found alone, therefore, liberated electrons must be taken by "electron acceptors" in complementary reduction reactions (gaining electron); hence, oxidation-reduction reactions always occur in pairs.

Although energy may be liberated from chemical reactions (e.g., H<sub>2</sub> and O<sub>2</sub> combine to form water and generate 237 kJ/mol of H<sub>2</sub>O), many coexisting chemical compounds may spontaneously react only at very low rates, because an "activation energy" is required to overcome the energy barrier. Microorganisms often act to reduce the energy barrier through specialized enzymes that adapt to the reactants. This can increase the rate of chemical reactions by as much as  $10^{20}$  times. Enzymes are proteins or macromolecules in the cell membrane, cytoplasm, or excreted outside the cell. They have shapes adaptable to the reactants they process. Hydrogen bonds, van der Waals forces, and hydrophobic interactions are responsible for the attraction and temporary bonding of enzymes to reactants. From the cell perspective, enzymes catalyze the chemical reactions involved in cellular metabolism. From the soil point of view, biological activity mediates geochemical reactions, causing them to proceed at rates that are sometimes orders of magnitude more rapid than would be predicted solely on the basis of the thermo-chemical reactions involved. Therefore, biogeochemical processes can have important geotechnical consequences over relatively short time periods of only weeks to years.

### Bacteria

Bacteria are the dominant microorganisms in soils. They are found even at large depths in the earth's lithosphere, but in smaller numbers (Bartlett 1998). Some bacteria can make spores

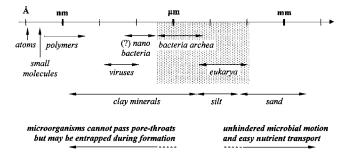


Fig. 2. Comparative sizes of microorganisms and soil particles

to endure adverse environmental changes. Bacteria vary in shape and may be nearly round, rodlike, or spiral. The cell diameter is usually in the range of 0.5–3  $\mu m$ , and spores can be as small as 0.2  $\mu m$ ; the size of bacteria may decrease under stressed conditions. Although the generally accepted size limit for life "as we know it" is  $\sim\!0.2~\mu m$ , self-replicating small entities, 0.05–0.2  $\mu m$  in size, have been identified in blood, rocks, and minerals by Folk and Lynch (1997) using electron microscopy, although the existence of these "nanobacteria" remains controversial (Nealson 1997). A comparative analysis of microorganisms and soil particle sizes is shown in Fig. 2.

Bacteria can survive at pH ranging from less than 2 to greater than 10 and in salinities much greater than that of seawater. They can withstand pressures of many hundreds of bars and temperatures from below freezing to above boiling. Bacteria cells were identified in boiling hot springs in 1967, and bacteria with temperature optimum greater than 100°C were isolated in 1982 (Madigan et al. 2000). On the other hand, samples of ice from a very salty Antarctic lake that had been frozen for more than 2,800 years contained microbial populations that retained metabolic potential after thawing (Doran et al. 2003).

Microorganisms can reproduce very rapidly. Under ideal conditions, the "generation time" for bacterial fission can be as short as 10 min; however, an hour scale is typical. The growth is exponential. For example, starting with one bacteria and a generation time of 1 h, the number of possible cells after 24 h, is  $N=2^{24}\approx 17\times 10^6$ . These high-speed generation rates, mutations, and natural selection lead to very fast adaptation and extraordinary biodiversity. Therefore, microbial activity can be expected everywhere.

Most bacterial cells have a negative surface charge for a ground water pH between 5 and 7, which is typical for near-surface soils; the negative surface charge decreases with increasing concentration and valence of ions in the pore fluid; surface charge also decreases with decreasing pH, reaching the isoelectric point at pH 2–3 in gram-positive and pH 4–5 in gram-negative bacteria (Hattori 1973). Thus there are some similarities between bacteria cells and charged clay particles. The water in most cells has higher ionic concentration than the adjacent pore fluid, so water tends to flow into the cell, driven by an osmotic pressure gradient. If the ionic concentration in the environment increases, the external water activity decreases, and water will tend to flow out of the cell, thereby stressing the cell. Organisms that can accommodate to high NaCl concentration environments such as seawater are called *halophiles*.

Aerobic and anaerobic bacteria are heterotrophic. Aerobic bacteria live in the presence of free or dissolved oxygen and use  $O_2$  as the electron acceptor; anaerobic bacteria can survive in the absence of oxygen, use other compounds besides  $O_2$  as electron acceptors, and are more robust to survive in subsurface environ-

ments. Facultative bacteria can live with or without oxygen. Some bacteria may resort to fermentation to sustain their metabolism under anaerobic conditions, e.g., fermenting bacteria oxidize carbohydrates to produce simple organic acids and  $\rm H_2$  that are used for reduction of ferric iron (Fe<sup>3+</sup> gains electron to become Fe<sup>2+</sup>), sulfate reduction, and the generation of methane, i.e., methanogenesis (see Purves et al. 1997; Chapelle 2001).

# Microbial Activity in the Geoenvironment—Limiting Factors

The maximum bacteria count in soil pore fluid can be estimated as the ratio between the pore fluid volume and the volume of a bacterium; packing restrictions and nutrient transport requirements limit this maxim number. Therefore, the number of 1  $\mu m$  size bacteria could reach  $\sim\!10^8$  bacteria/ml of pore fluid. Likewise, a monolayer of bacteria on a mineral surface would involve  $\sim\!10^6$  bacteria/mm²; however, multilayer colonies can result in higher counts per unit area.

Microbial activity and reproduction rate are restricted by the most critical or "limiting factor," either nutrient availability, water availability, or other environmental factors.

### Nutrients

Nutrients are required by microorganisms for cellular material (carbon and minerals) as well as for an energy source; common nutrients are listed in Table 1. Most aquifer systems are nutrient limited (Chapelle 2001). Unpolluted lakes are oxidizing in most cases; however, the pore water in soils tends to be reducing (Freeze and Cherry 1979). Bacterial growth in most soils is limited by the lack of organic constituents. The lack of nutrients or of substances required to complete redox cycles explains the absence of otherwise viable biochemical redox reactions in old soil deposits. Bacteria may not metabolize nonaqueous phase compounds such as crystalline minerals, organic liquids, and surface-adsorbed materials, until they become desorbed or dissolved. In this case, the rates of desorption or dissolution will control the time scale of metabolism.

Table 1. Common Nutrients for Bacterial Growth

Nutritional requirements		Nutrients: Sources and Compounds
1: Elements needed to form molecules in	Cellular carbon	CO <sub>2</sub> , HCO <sub>3</sub> Organic compounds
the cell	Minerals	N, P, K, Mg, S, Fe, Ca, Mn, Zn, Cu, Co, Mo
2: Energy needed to sustain	Organic compounds <sup>a</sup>	
	Inorganic compounds <sup>a</sup>	
		Sunlight
3: Other growth-conducive	amino acids, vitamins, etc.	

Note: Sources: Alexander (1961), Horn and Meike (1995).

<sup>&</sup>lt;sup>a</sup>Organic and inorganic compounds (O<sub>2</sub>, NO<sub>3</sub>, NO<sub>2</sub>, N<sub>2</sub>O, SO<sub>4</sub><sup>2</sup>CO<sub>2</sub>, Fe<sup>3+</sup>) can act as acceptors of electrons liberated in reactions that generated the required energy.

#### Water

Life cannot exist in the absence of water, which comprises about 70% of the cell. Further, water supports nutrient transport, participates in chemical reactions, determines the type and amount of soluble materials (including salts and minerals), establishes the environment pH, controls aereation (depending on degree of saturation), and contributes to thermal stability (Hattori 1973). Severely water-limited environments distress microorganisms and hinder biological activity in response to factors such as high suction and increased ionic concentration as water evaporates. On the other hand, when the water content approaches saturation conditions, the gaseous phase can no longer provide percolation paths, and the availability of oxygen is restricted by the low diffusion rate of oxygen in water. In this case, subsurface conditions become anaerobic. Aerobic microbial activity is optimal within 60–80% saturation (Horn and Meike 1995).

There is great bacterial activity in the unsaturated organic surface layer of a soil deposit where plant roots are found; this upper layer has been extensively studied, and the relation between soil fertility and the presence of microorganisms is well documented. However, details of bacterial activity in the unsaturated region beneath the organic layer are poorly known. Fierer et al. (2003) observed that bacterial activity decreases by 1 or 2 orders of magnitude by 2 m of depth. Similarly, Colwell (1989) observed a drastic decrease of several orders of magnitude between surface soils and unsaturated soils at a depth of 70 m. These observations contrast with the more moderate reduction in bacterial count with depth in saturated sediments.

### Other Environmental Factors

These factors include pH (affects surface charge, adsorption, and dissolution), redox potential (to gather energy), temperature (affects reactions within cells as well as physicochemical properties such as diffusion and viscosity), the presence of predatory microorganisms which may limit bacterial population, and spacelimitations (Paul and Clark 1996).

Although microorganisms are free to move in the pore space between large soil grains and aggregations, narrow pore throats formed by small soil grains prevent their entry. Therefore, bacteria are not expected to enter through pore throats smaller than  $\sim\!0.4~\mu m$ ; fungi and protozoa require pore throat sizes greater than 6  $\mu m$  for entry (refer to Fig. 2).

In coarse-grained soils, bacteria may adhere to mineral surfaces and form microcolonies or biofilms. Biofilms may create their own local moisture conditions. Further, although some biofilms are made of single-type bacteria, others involve symbiotic communities where two or more bacteria types coexist and environmentally complement each other; for example, biofilms involved in rock weathering may include a sequence of aerobic, facultative, and anaerobic layers (Ehrlich 1998b).

### Microbiology and Geological Processes

### Evolution of the Earth

Autotrophic photosynthetic bacteria, i.e., photoautotrophs, played a crucial role in the geological history of the earth (P. L. McCarty, personal communication, 2004; see also Hattori 1973). The photosynthetic bacteria *cyanobacteria* or "blue–green bacteria" evolved about 3.5 billion years ago (Proterozoic era–

Precambrian) and they are the oldest known fossils. Cyanobacteria use energy from the sun to reduce the carbon in  $\mathrm{CO}_2$  to cellular carbon and to obtain the needed electrons for oxidizing the oxygen in water to molecular oxygen. During the Archaean period (2.5 billion years ago), cyanobacteria converted the atmosphere from reducing to oxidizing, and changed the mineral nature of the earth (today, oxygen comprises about 45% of the earth crust by weight and most minerals contain either oxygen or hydroxide).

Eukaryotic algae evolved later, followed by the multicellular eukaryotes including plants. Photosynthesis is the primary producer of the organic particulate matter in shale, sand, silt, and clay, as well as in coal, petroleum, and methane deposits. Furthermore, cyanobacteria and algae increase the water pH when they consume CO<sub>2</sub> dissolved in water, resulting in carbonate formation and precipitation of magnesium and calcium carbonates, leading to the earth's major carbonate formations. Microbial decomposition of the heavy layers of organic matter in top soils formed through photosynthesis results in oxygen depletion and carbon oxidation back to CO2 which is leached by rainwater that penetrates into the subsurface. The high CO<sub>2</sub> concentration, lowered pH and anaerobic nature of such penetrating waters cause reduction and solubilization of iron and manganese minerals, the reduction of sulfates, and dissolution of carbonate rocks. If the moving waters become comingled with oxygenated water in the ground, or as groundwater emerges into rivers and streams, iron, manganese and sulfide oxidation results, and carbonate precipitation can occur (P. L. McCarty, personal communication, 2004).

Therefore, apart from its impact on life itself, microbial activity (in general, and photosynthesis in particular) has influenced the evolution of the earth's surface, impacted mineral, sediment, and rock formation, accelerated the rate of rock weathering and altered its products, influenced the composition of groundwater, and participated in the formation of gas and petroleum hydrocarbons.

In the remainder of this section some common microbial mediated processes of geotechnical importance are briefly reviewed. They are examples of how nutrients needed for bacterial growth and the end products that are formed affect the geochemistry and properties of soils and rocks. The presence of biomass in the pore space, as well as the formation of slimes and other surface modifications have geotechnical consequences as well, and illustrations are given later in this paper.

### Sulfur and Iron Cycles

Sulfide oxidation and sulfate reduction, often occurring in combination with iron reduction and iron oxidation, have significant geological, geotechnical, and environmental consequences, such as the formation of quick clays, soil property changes after exposure or sampling, production of acid rock drainage, and the swelling and breakdown of pyrite-bearing rocks. Details of the reactions are now reasonably well understood. Brief, simplified descriptions are given here; details can be found in Ehrlich (1996a, b), Madigan et al. (2000), and Chapelle (2001).

Elemental sulfur ( $S^0$ ); sulfides ( $S^{2-}$ ), which are the stable form of sulfur under anaerobic conditions; and sulfates ( $SO_4^{2-}$ ), which are the stable form of sulfur under aerobic conditions, are all found in nature. The different oxidation states of sulfur allow chemical energy to alternately be stored and released, and the series of processes associated with these energy changes is known as the sulfur cycle (Chapelle 2001).

Sulfides form under anaerobic conditions from sulfates already

present in seawater and sediments, from decaying biomass, and they can be introduced by diffusion and groundwater flow. The sulfate ion is not reduced to sulfide at earth surface temperature and pressure unless biologically mediated. Sulfate reducing bacteria are anaerobic and can exist over a broad range of pH and salt content. They are heterotrophic, i.e., the energy for their metabolic processes is derived from organic matter. The number of sulfate reducing bacteria varies greatly depending on local conditions.

When exposed to aerobic conditions, reduced sulfur compounds, hydrogen sulfide  $(H_2S)$  and elemental sulfur are used as energy sources by sulfide-oxidizing bacteria and converted to sulfates.

Iron in the subsurface exists predominantly in the reduced or ferrous Fe<sup>2+</sup> state or the oxidized ferric Fe<sup>3+</sup> state (Chapelle 2001). Ferrous iron is relatively soluble and mobile; whereas, ferric iron forms oxyhydroxides and is relatively immobile. The iron cycle consists of alternate reduction of ferric iron and oxidation of ferrous iron. Several microorganisms mediate the iron reduction phase of the cycle; genus *Thiobacillus* bacteria are important in mediating the iron oxidation reaction. Chapelle (2001) notes that bacteria are able to derive only relatively little energy from oxidizing Fe<sup>2+</sup>, therefore, they must process large amounts of Fe<sup>2+</sup> and produce large amounts of Fe<sup>3+</sup> to obtain sufficient energy to sustain their growth.

### Iron Sulfide Oxidation

In the presence of oxygen, sulfides become unstable and decompose over relatively short time periods, i.e., months to years. Oxygen reduction associated with sulfide oxidation is the controlling reaction. Aerobic respiration of organics, if present, occurs first, followed by the oxidation of inorganic compounds (e.g., sulfide and iron). Both thermodynamic calculations and experiments show that subsequent oxidation of ferrous iron (Fe<sup>2+</sup>) to ferric iron (Fe<sup>3+</sup>) should be very slow at pH below 5.5. However, thermodynamic calculations do not take into account the catalyzing role of microorganisms in iron oxidation, which depends on many factors, especially temperature, accessibility of air, moisture, and microbes to particle surfaces and the neutralizing capacity of other materials in the system (Nordstrom and Alpers 1999), thus complicating the estimation of reaction rates and equilibrium concentrations.

Different species of ferrooxidans exist over different pH ranges. *Thiobacillus ferrooxidans* are rod shaped, about 0.5  $\mu$ m in diameter and up to 2  $\mu$ m long (Bartlett 1998). Oxidation rates in the presence of mediating bacteria can be orders of magnitude faster than in their absence. Although accurate rate comparisons are very difficult, owing to the large number of rate determining factors and multiplicity of physical, chemical, and biological variables, Nordstrom and Alpers (1999) have compared field rates with abiotic oxidation rates of aqueous ferrous iron. At pH=2 and  $T=25\,^{\circ}$ C the field rate is of the order of  $5\times10^{-7}$  mol L<sup>-1</sup> s<sup>-1</sup>, or more than  $10^5$  times greater than the abiotic rate, which is of the order of  $3\times10^{-12}$  mol L<sup>-1</sup> s<sup>-1</sup>. They also report an abiotic rate of oxidation of pyrite by oxygen of  $0.3-3\times10^{-9}$  mol m<sup>-2</sup> s<sup>-1</sup>, or about 100 times slower than the microbial rate of  $8.8\times10^{-8}$  mol m<sup>-2</sup> s<sup>-1</sup>.

One important consequence of the rapid oxidation of iron sulfide in the presence of oxygen is the formation of acid rock drainage. The chemical reactions, beginning with the oxidation of pyrite and the generation of sulfuric acid, are:

$$4FeS_2 + 14O_2 + 4H_2O \rightarrow 4Fe^{2+} + 8H^+ + 8SO_4^{2-}$$

$$16H^+ + 8SO_4^{2-} \rightarrow 8H_2SO_4$$

$$4Fe^{2+} + 4H^+ + O_2 \rightarrow 4Fe^{3+} + 2H_2O$$

$$4Fe^{3+} + 12H_2O \rightarrow 4Fe(OH)_3 + 12H^+$$

One mole of ferric hydroxide and 2 mol of sulfuric acid are produced for every mole of pyrite that is oxidized. However, ferric hydroxide is not a stable end product unless other species are present that can neutralize the acid that is produced, and other ferric compounds may form in addition to iron hydroxide, including jarosite,  $KFe_3(SO_4)_2(OH)_6$ . Jarosite is essentially insoluble and has a greater volume than the pyrite from which it is derived. It has been identified as the cause of heaving of some black shales in Canada (Penner et al. 1970, 1973).

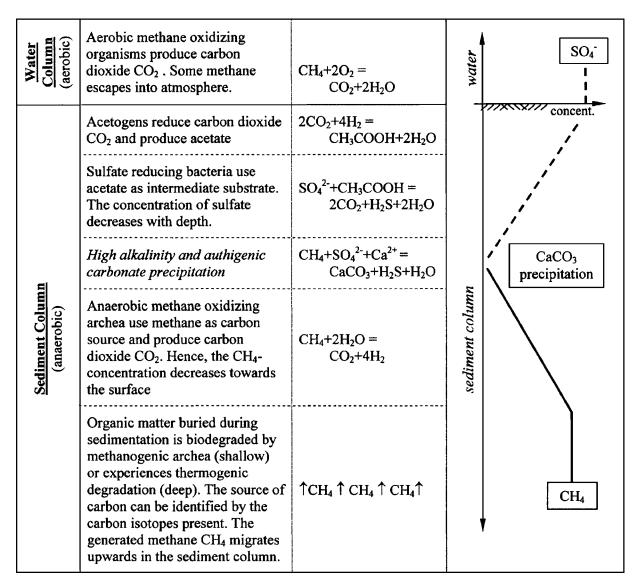
Although  $Fe(OH)_3$  has low solubility, the formation of  $H_2SO_4$  can be the source of important reactions in the solid and pore water phases, including the dissolution of carbonates and the formation of gypsum ( $CaSO_4 \cdot 2H_2O$ ). Precipitated ferric hydroxide is thermodynamically unstable and rapidly transforms to yellow goethite, FeO·OH. Geothite, while stable under wet conditions, will slowly dehydrate to red hematite,  $Fe_2O_3$ , under dry conditions.

### Methane, Carbonates, and Sulfates

Methane, CH<sub>4</sub>, is the most common gas in sediments. It is produced through the biodegradation of organic matter in the seafloor, ponds, marshes, deltaic muds, sludge digesters and municipal waste landfills. Methanogens (archea, Fig. 1) produce methane from carbon dioxide CO<sub>2</sub>, methyl-type compounds (e.g, methanol CH<sub>3</sub>OH), or acetate CH<sub>3</sub>COOH (Johnson and Flores 1998; Madigan et al. 2000). Methane is a greenhouse gas, and if all the methane in sediments were released to the atmosphere, extreme climatic changes would result.

However, the upward migration of methane through sediments and water columns is affected by both anaerobic and aerobic biomediated reactions implemented by methane oxidizing and sulfate reducing bacteria. The depth of sediment involved in these phenomena varies from the upper few meters to  $\sim\!150$  m, depending on the methane flux and the methane bacterial oxidation rate, which can reach 0.005  $\mu mol\ cm^{-3}\ year^{-1}$  (Borowski et al. 2000; Dickens 2001). Methane that escapes from the sediments into the water column is oxidized by methane oxidizing bacteria (methanothrophs), causing a decline in oxygen content in the water column. Eventually, methane may be released into the atmosphere if the flux rate is sufficiently high relative to the water column depth, the availability of oxygen relative to methane, and the extent of microbial activity.

Salient biomediated reactions are outlined in Fig. 3; they highlight the intricate interplay among the multiple processes taking place in the sediment, their impact on the chemistry of the pore fluid in shallow sediments, and the precipitation of authigenic carbonates. Authigenic carbonate formations are found offshore [e.g., Gulf of Mexico (Greinert et al. 2001)] and inland when they have risen above the water level (Fan et al. 1999; Cavagna et al. 1999). Other biomediated mechanisms leading to the formation of carbonate and iron minerals are reviewed in Ehrlich (1998a).



**Fig. 3.** Some biomediated geochemical processes in the seafloor: Methane oxidation, sulfate reduction and authigenic carbonate precipitation. Sources: Harder (1997); Cavagna et al. (1999); Borowski et al. (2000); Elvert et al. (2001); Greinert et al. (2001); and Kastner (2001).

### **Geotechnical Examples**

The preceding review of microorganisms and biogeochemical reactions helps both to identify circumstances where biological activity may affect soil properties and subsurface processes, and to explain a number of findings of geotechnical importance. Several examples are given in this section.

### Biological Effects on Soil Particles

Microorganisms can be important in the formation of fine-grained particles and clay minerals. A continuous bacterial monolayer can form on mineral surfaces within minutes. The ensuing biofilm binds cations in the pore fluid and facilitates nucleation and crystal growth even at low ionic concentrations in the pore fluid (Konhauser and Urrutia 1999). After nucleation is initiated, further mineral growth or precipitation can occur abiotically, including the precipitation of amorphous iron-aluminum silicates and

poorly crystallized clay-like minerals, such as allophane, imogolite and smectite (Urrutia and Beveridge 1995; Ehrlich 1999; Barton et al. 2001).

In some fine-grained soils, microfossils formed by secretions from eukarya cells present during soil formation make up a significant portion of the solid granular mass. These microfossils include (see Fig. 1): diatoms (siliceous cell wall of eukarya cells in either fresh water or marine environments), radiolaria (found in marine environments and consisting mostly of silica), and foraminiferan (calcium carbonate shell in marine eukarya). Examples of soils with microfossils include: diatomaceous earth [see Day (1995) for engineering characteristics], Ariake clay [diatoms in marine clay; Ohtsubo et al. (1995)], Bogota soil [diatoms in lacustrian formation; Moya and Rodriguez (1987)], Charleston Cooper marl [foraminiferans in marine formation: Camp et al. (2002)], and Mexico City soil [diatoms and ostracods in a lacustrine formation; Diaz-Rodriguez et al. (1998)]. The presence of microfossils as soil particles can have a profound effect on the

**Table 2.** Processes, Mechanisms, Particle Arrangements, and Time Scales Associated with the Development of Sediment Microstructures (adapted from Bennett et al. 1991)

Processes	Mechanisms	Predominant particle arrangements <sup>a</sup>	Size Scales	Time Scales	Remarks
Physical	Sedimentation	Single to multigrain	μm to mm	s to years	Soil particle types and sizes and the sedimentary environment determine initial microstructure (see Mitchell 1993)
Physicochemical	Electromechanical	E-F	Atomic and molecular to ~4 μm	μs to ms	Some particles may rotate to F-F association
	Thermomechanical	F-F (some E-F)	Molecular to ≥0.2 mm	ms to min	Initial contacts E-F then rotation to F-F: common in selective environments
	Interface dynamics	F-F and E-F	$\mu$ m to $\sim > 0.5$ mm	S	Some large compound particles may form at high salt and particle concentrations
Biologically influenced (Bioorganic)	Biomechanical	E-F	~0.5 mm to >2.0 mm	s to min	Some F-F possible during bioturbation
	Biophysical	E-E and F-F	μm to mm	s to min	Some very large clay organic complexes possible
	Biochemical	Largely arrangement independent	μm to mm	h to year	New chemicals formed, some altered. Mediating bacteria influence reaction rates
Burial and diagenesis	Mass gravity	F-F localized swirl	cm to km	years	Can operate over large physical scales
	Diagenesis cementation	Arrangement independent	Molecular reactants	years	Altered and new minerals formed, changes in morphology

<sup>&</sup>lt;sup>a</sup>For platy particles: E-F=edge-to-face; E-E=edge-to-edge; and F-F=face-to-face.

behavior of the soil mass, conferring unusual geotechnical properties that deviate from general parameter correlations, including high porosity, high liquid limit, unusual compressibility, and uniquely high friction angle.

Microorganisms have a limited effect on the formation of coarse grains. However, bioactivity can affect diagenetic evolution, promote the precipitation of cementing agents, cause internal weathering, and alter fines migration, filter performance, and drainage in silts and sands. The formation of biofilms on coarse particle surfaces is likely; however, the consequences in terms of surface properties and conditions at interparticle contacts are largely unstudied.

# Biological Contributions to the Development of Sediment Microstructure

Current geotechnical understanding of the structure of fine-grained soils has developed almost entirely in terms of the physical and chemical processes active at the time of formation and during subsequent diagenetic changes. However, there is an increased awareness and understanding of the important role of biological phenomena in soil formation. The abundance of microorganisms, their colloidal size, their net electrical charge, their ability to catalyze chemical reactions, and to secrete substances that can coat and/or bind small mineral grains have major influences on the particle associations in sediments and their changes with time. The following bio-related conditions and processes affect sediment formation (Bennett et al. 1991; Hattori 1973):

 Bacterium-clay complex: The pH and ionic strength of the pore fluid determine the surface charges in bacteria and clay

- particles, and regulate the balance between double layer repulsion and van der Waals attraction. When attraction prevails, bacteria and clay particles in suspension aggregate. The type of aggregation depends on the relative bacteria-to-particle size (details in Hattori 1973).
- Biophysical mechanisms that produce clusters of randomly oriented clay-silt particles that are bound together by sticky organic mucus or polymer bridging.
- Biochemical mechanisms that lead to changes in microfabric as a result of chemical transformations mediated by organisms.
- 4. Biomechanical mechanisms. These include bioturbation by burrower organisms as in Bothkennar Clay (Paul et al. 1992) and the presence of pellets as in marine Bangkok Clay (Tanaka et al. 2001). A random microfabric that is similar to the primary fabric of flocculated clay often reflects biomechanical effects.

Bacteria coated with small size clay minerals such as montmorillonite and allophane exhibit a reduction in metabolic activity (probably because of restricted access to nutrients), and an increased survival rate in arid conditions.

The physical scales over which physicochemical, bioorganic, and postdepositional mechanisms of sediment forming processes act to determine microfabric range from atomic dimensions to kilometers, and time scales from microseconds to years. Table 2 summarizes the processes, fabric characteristics, and scales associated with the different mechanisms. The importance of fabric and soil structure in determining soil properties and behavior is discussed at length in Mitchell (1993). Microbiological influences

on soil fabric and structure must be considered to better understand the current properties and to anticipate the changes in earth materials that may occur in the future.

#### **Bioremediation**

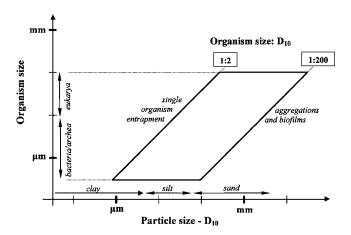
Some carbon-rich crude oil and derivates such as aliphatic and aromatic compounds, may be aerobically degraded. In situ bioremediation generally consists of the addition of inorganic nutrients (e.g., phosphorous and nitrogen) and an electron acceptor, typically oxygen, to the subsurface to stimulate the growth of microorganisms that degrade the hydrocarbon (the primary electron donor). The goal is to produce non-toxic products such as water and CO<sub>2</sub>; however, attention must be given to all the byproducts and end-compounds [various examples are documented in Olguin et al. (2000)]. "Bioventing" is used when aerobically degradable contaminants are present above the water table.

There have been many successful bioremediation efforts, including the clean up of the Exxon Valdez oil spill in 1989. Bioremediation techniques are most applicable to sands. They are less useful in clays owing to the low permeation rate for nutrients and oxygen, the small pore sizes, and because water adsorption on the high specific surface of the clay particles limits its availability for cellular growth.

Currently, the implementation of bioremediation techniques is restricted by the relatively primitive "delivery methods" available to enhance the "limiting factor" that controls microbial growth rates, and by our limited ability to sense the types and magnitudes of ongoing processes (J. B. Hughes, personal communication, 2004).

### Seepage and Bioclogging

Bioactivity involving bacteria and eukarya (algae and fungi) can reduce the hydraulic conductivity of soils and fractured rocks, alter fines migration, clog filters, enhance hydrodynamic dispersion, and increase chemical retardation during flow due to adsorption onto the biomass. Biological activity can develop naturally, or it can be promoted by altering the limiting factors; e.g., by injecting nutrients such as glucose and oxygen and changing the environmental conditions.



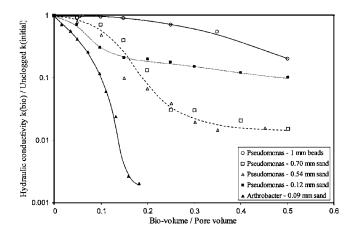
**Fig. 4.** Bioclogging: The bounded region indicates the range of soil sizes that can be treated by bioclogging. The region is determined by the size of microorganisms and the relative size of microorganisms to particle size (it takes into consideration the size of pore throats and the formation of aggregations and biofilms).

Reduction in hydraulic conductivity, or bioclogging, is the result of pore-level phenomena such as (1) bacterial transport and attachment, (2) bacterial growth, multiplication, biomass accumulation, and the development of micro-colonies and biofilms on mineral surfaces, with consequent reduction in pore space, (3) shear detachment and mechanical filtering at pore throats, (4) excreted extracellular polymers, and (5) the effects of poorly soluble biogenic gas bubbles that become trapped and occlude pore throats (see Vandevivere and Baveye 1992; Bonala and Reddi 1998). Bioclogging self-regulates through mechanisms such as the balance between the rate of biomass generation and the rate of removal, which increases with increase in hydraulic gradient across the clogging zone, as well as through decrease in nutrient transport with increased clogging.

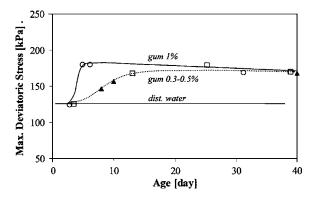
Bacteria are transported at lower velocity than the seepage velocity through the porous network, because of Stokes' drag and interactions with mineral surfaces. Bacterial transport and retardation within the interconnected porous network of a soil mass is controlled by relative size (pore throat relative to a single bacteria or bacterial aggregations), electrical interactions (repulsion and van der Waals attraction), surface roughness and cell shape. Relative size determines the homogeneity of bacterial transport through the spatially varying porous network. Bacteria adsorption to mineral surfaces depends on the pore fluid chemistry [i.e., pH, ion type and concentration, and dissolved organic carbon (Mueller 1996)]. For example, the attachment of pseudomonas fluorescens to silica beads decreases between pH 5.5 and 7 and with decreasing ionic strength [e.g., Deshpande and Shonnard (1999)]. Preferential attachment takes place onto surfaces that are nutritionally advantageous.

The pore-scale processes described above determine the range of soils that can be affected by bioclogging. Fig. 4 identifies the main boundaries and delineates the region where clogging can take place. Because small soil particles pack between large particles reducing the effective pore size, the  $D_{10}$  criterion is selected for this chart.

The decrease in hydraulic conductivity due to bio-clogging in materials with initial hydraulic conductivity in the range of  $10^{-4}$ – $10^{-1}$  cm/s (i.e., silts, sands, sandstones, and fractured rocks) can exceed three orders of magnitude [e.g., Hill and Sleep



**Fig. 5.** Evolution of the saturated hydraulic conductivity (normalized by the unclogged initial value  $k_0$ ) with respect to the bulk volume of biomass (normalized by the pore volume of the unclogged medium). Bacteria: *Pseudomonas aeruginosa* and *Arthrobacter sp.* After Baveye et al. (1998).



**Fig. 6.** Effect of xantham gum and Na-alginate treatment on the strength of clayey silt (adapted from Martin et al. 1996)

(2002); Seki et al. (1998); Dennis and Turner (1998)]. The time scale is 10–40 days to reach asymptotic values in laboratory specimens. Typical data are shown in Fig. 5.

If biotreatments can be shown to be cost competitive with other hydraulic barrier materials, potential applications may range from the creation of waste containment to seepage barriers. Before such systems are deployed, it will be necessary to investigate their durability over time, including the potential for biological and chemical degradation and their mechanical stability.

### Strength and Stiffness

We identified a limited number of studies on the effect of biomediated reactions on the strength and stiffness of soils. A comprehensive investigation was conducted by Villarraga and coworkers [U. Colombia-Medellin (Martínez et al. 2003)] using compacted specimens of inorganic silt. The pore water included selected nutrients and was inoculated with Bacillius Subtilis. The silt and pore water were thoroughly mixed 24 h prior to compaction, at water contents on the dry and wet side of optimum. Compacted specimens were cured for 21 days and tested. Results showed an increase in undrained strength of 20-100%, an increase in undrained stiffness of 50-200%, an increase in drained strength ranging from 10 to 50 kPa, and a decrease in permeability of one-to-two orders of magnitude. The extent of the effects depends on the compaction moisture content relative to the Proctor optimum, and is influenced by gas generation and changes in saturation.

Biopolymers are widely used to form biodegradable muds that are useful for a variety of soil drilling and temporary excavation support applications. Potential applications for biopolymer systems that over time become highly viscous or gel as a result of biological activity include the creation of seepage barriers, strengthening soils and increasing liquefaction resistance. Martin et al. (1996) conducted laboratory hydraulic conductivity and strength tests on biologically treated clayey silt (CL-ML). The soil was treated with up to 2% of three different types of biopolymers: Xanthan gum (a high molecular weight polysaccharide), sodium alginate, and slime forming bacteria. Permeation was continued for six months, and strength measurements using the triaxial test were made on samples aged for 40 days. The permeability was reduced by factors up to about 100 compared to the untreated soil and remained constant with time, and the strength increased up to about 50% during the first week, as shown in Fig. 6.

### Soil Sampling: The Effect of High Replication Rate

Bacteria growth and reproduction in favorable environments exhibit high metabolism, whereas minimum metabolism is detected in dormant and nongrowing bacteria such as most spores. Dormant cells can revive and repopulate the surroundings after growth-conducive conditions are reestablished; this may happen in soil specimens after sampling, leading to potential postsampling changes in geotechnical properties and behavior.

Contamination and exposure to air during sampling, together with changes in water content, fluid chemistry, and temperature, can significantly alter biological activity within soil specimens. The potential impacts of sampling can include: changes in pore fluid chemistry, changes in permeability by clogging, changes in stiffness if mineralization is promoted, and changes in undrained strength by biomass stabilization. Therefore, proper specimen handling practices should involve prompt testing or biological stabilization within a short time after sampling.

### Bacteria Mediated Oxidation of Pyrite

The catalytic role of bacteria in accelerating the oxidation and reduction of sulfur and iron was described earlier in this paper. Some geotechnical examples where these processes are known or suspected to be important are described in the following.

### The Formation and Postsampling Aging of Quick Clays

Some postglacial marine clays of Canada and Scandinavia lose essentially all of their strength and flow as viscous fluids when mechanically disturbed. This "quick clay" behavior is a consequence of geological, physical, and biogeochemical interactions during and after deposition that produce a metastable soil structure. Following undisturbed sampling of quick clays, the disturbed strength may increase substantially over times of weeks to months, thereby making the clay behave more like a moderately sensitive material than one that turns fluid on disturbance. Lessard (1981), Lessard and Mitchell (1985), Mitchell (1986, 1993), and the references cited therein, describe mechanisms of quick clay formation and aging in some detail. It is suspected, but not yet confirmed, that microorganisms play an important role in determining the reaction rates.

### Pyrite Oxidation at Carsington Dam

Geotechnical aspects of the embankment stability failure in 1984 of the Carsington Dam in Derbyshire, England, are well known (Skempton 1988; Rowe 1991; Banyard et al. 1992; Skempton and Vaughan 1993). Perhaps, less well known, however, are the severe consequences of pyrite oxidation during construction following the excavation and exposure to air and water of the Namurian Shale used in the embankment. This shale contains pyrite in concentrations up to 6%. It also contains swelling clay minerals, which facilitate breakdown of the shale on exposure to water. Cripps et al. (1993) hypothesized that autotrophic bacteria greatly accelerated the oxidation rate of the pyrite, with lethal consequences during the subsequent months of construction. The sulfuric acid that formed reacted with drainage blankets constructed of carboniferous limestone, which then resulted in precipitation of gypsum and iron hydroxide and generation of carbon dioxide. The gypsum and iron hydroxide clogged drainage blankets. The heavier than air carbon dioxide accumulated in drains, excavations, and manholes (Cripps et al. 1993). This concentration of CO<sub>2</sub> led to the asphyxiation of four workmen in an inspection chamber.

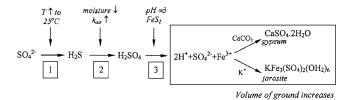
### **Bacterially Generated Heave of House Foundations**

About 1,000 wooden houses founded on mudstone sediments in Iwaki-City, Fukushima Prefecture, Japan were damaged by heaving of their foundations (Oyama et al. 1998; Yohta 1999, 2000). The amount of heave reached a maximum of 480 mm. The cost for repairs was estimated at 10 billion yen (Yohta 2000). The mudstone at the site contained 5% pyrite. Whereas the pH of the sediment was initially 7-8 before heave, the pH of the heaved ground was about 3 and it contained acidophilic iron-oxidizing bacteria (Oyama et al. 1998). Yamanaka et al. (2002) further confirmed the presence and effects of sulfate reducing, sulfur oxidizing, and acidophilic iron oxidizing bacteria by several series of laboratory culture experiments. Test results presented by Yamanaka et al. (2002), which include electron photomicrographs of the bacteria, show consistent variations of hydrogen sulfide concentration, pH, Fe<sup>3+</sup> concentration, Fe<sup>2+</sup>+Fe<sup>3+</sup> concentration, and  $SO_4^{2-}$  concentration over time periods up to 50 days for both the natural mudstone and the mudstone after heat treatment to 121°C. The heat treatment served to prevent or slow greatly the bacterial activity, whereas, very significant changes in concentrations and pH were measured for tests done at 28°C. For example, the concentration of H<sub>2</sub>S increased from 0.3 to 2.2 mM in 20 days, the pH decreased from  $\sim 6.5$  to 1.3 in 47 days, the concentration of Fe<sup>3+</sup> increased from  $\sim$ 6 to 125 in 5 days, and the concentration of SO<sub>4</sub><sup>2-</sup> increased from less than 1 to about 15 mM in 25 days.

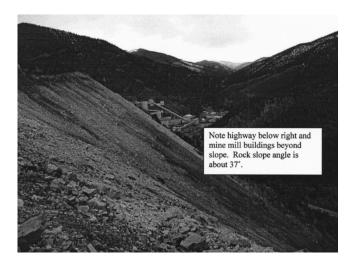
Based on their results and observations, Yamanaka et al. (2002) developed the schematic representation of the processes leading to the heave of foundations shown in Fig. 7. The ground temperature, which had been about 18°C at depth, increased to about 25°C in the summer after excavation. Initial anaerobic, high water content conditions and the stimulation of sulfate reducing bacteria generated  $\rm H_2S$ . As the ground dried and became permeable to air, sulfate oxidizing bacteria grew and stimulated production of  $\rm H_2SO_4$ , the lowering of pH and pyrite oxidation. The reaction of  $\rm H_2SO_4$  with calcium carbonate present in the mudstone led to formation of gypsum and, with potassium and ferric ions, to formation of jarosite. Foundation heave is associated with the volume increase that accompanies the formation of both gypsum and jarosite crystals.

### Long-Term Stability of a High Rock Pile

Open pit mining of molybdenum disulfide ( $MoS_2$ ) ore began at a high elevation site in northern New Mexico in 1965 and continued until 1981. About 350 million tons of overburden rock were excavated and dumped onto mountain slopes and tributary valleys. One of several high rock piles is shown in Fig. 8. The particle sizes range from a small content of fines <#200 mesh, to cobbles and boulders up to 300 mm or more in diameter. The rock piles extend vertically from about 2,400 m (8,000 ft) to 3,230 m (10,600 ft) elevation, at an angle of repose of about 37°. Indi-



**Fig. 7.** Ground heave: Summary of biomediated processes. Sulfate-reducing, sulfur-oxidizing and acidophylic iron-oxidizing bacteria are involved (adapted from Yamanaka et al. 2002).



**Fig. 8.** High open pit mine waste rockpiles on steep slopes at a site in northern New Mexico. Studies of the possible long-term effects of biogeochemical changes in the pyrite-containing rock on the strength of the rock mass are in progress.

vidual piles are up to 105 m (350 ft) thick. State regulations require that a suitable closure plan be developed to assure both environmental and public safety in the future. Among the issues to be addressed is the long-term stability of the rock piles against slope failure.

The rock piles include pyrite-mineralized andesite, aplite porphyry, andesite-to-quartz-latite porphyry flows, and welded rhyolite tuff rock aggregate. Alteration scars are common on the natural mountainsides throughout the area, and have been the source of debris flows into the river valley that borders the mine site on the south. Oxidizing pyrite within the debris flow and mine waste rock has been the source of acid rock drainage. The rate and consequences of pyrite oxidation are crucial as it may impair the stability of one or more of the several rock piles as a result of internal shear strength reduction. The rate of pyrite oxidation is dependent, as we have seen, on the catalyzing effects of microorganisms.

Therefore, some of the questions being addressed are: What mineralogical and physical biomediated or abiotic alterations of the constituent particles are occurring? What effects can these alterations have on the stress deformation and strength properties of the individual particles and on the composite strength of the mass? Can adverse changes occur of magnitude and rate sufficient to impair the overall stability of the piles within a specified post-closure period? If adverse changes are likely, what can be done to prevent them or mitigate their consequences? Answering these questions will require knowledge and understanding at the interfaces between geology, microbiology, geochemistry, and geomechanics.

### Mold

Mold is a current problem area that is much in the public eye. It is biological in nature, involves geotechnical and legal issues of great importance, and appears to be of widening impact. There are more than 1,000 types of indoor mold, some of which are toxic and have been related to various illnesses, from respiratory infections to cancer. Mold spreads and reproduces by making resistant and long-lasting spores, which are light and readily transported in

air and water. Extensive coverage of this topic and its relevance to geotechnical engineering is given in the April 2003 issue of the ASCE GeoInstitute *GeoStrata*.

### **Conclusions**

Soils are ecosystems. Great microbial diversity and the fast reproduction rate of microorganisms are responsible for their ubiquitous presence in the geoenvironment. Microbial activity requires a source of carbon for cell mass, a source of energy to sustain life-activity, water, and a favorable environment (including pore size). Any of these aspects can act as the growth limiting factor.

Until recently, most geochemical and geotechnical processes have been assumed abiotic, in part due to the relatively long time scales under normal subsurface conditions. However, microbial catalysts can drastically increase the rate of geochemical reactions. Harnessing this great potential requires proper engineered control of the limiting factors.

Microbial processes influence rock weathering, mineralization, soil formation and fabric, and soil grain surface properties. They can produce slime, gel, polymer, and biomass, cause pore and filter clogging, and change the deformation and strength properties of soils. This biological activity adds further evidence to the noninert nature of soil behavior.

Inherent pore-size restrictions in relation to the sizes of microorganisms limit the postsedimentation bioengineering of clays and clayey soils (more than  $\sim 12\%$  clay). Therefore, good candidate soils for biomodification include GW, GP, SW, SP, ML, and organic soils.

There is need to reconsider proper sampling and laboratory testing procedures or protocols to account for microbiological activity. Moreover, there is need to rescrutinize some poorly explained laboratory results and field responses, in particular those that exhibit changes in strength, stiffness, conduction and diffusion properties, and pore fluid chemistry that may have occurred in relatively short time scales. To do this properly, geotechnical engineers will need better understanding and appreciation of geochemistry and microbiology.

Reconsideration of "nature's way" and "bioinfallibility" may open unique new opportunities for geotechnical engineering to harness the inherent biodiversity in the subsurface. In addition to the bioremediation of contaminated ground, research at the interface between microbiology and soil mechanics may lead to the development of enhanced ground modification strategies and even new bioinspired foundation methodologies that exploit the potential of microorganisms. The research will require new training; the combined expertise of microbiologists, geochemists, geologists, and geotechnical engineers; and the development of enhanced sensing methods, including non-invasive tools, to assess bio-geochemical activity in the subsurface.

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