ABSTRACT

‘Biogeophysics’ is a rapidly evolving Earth science discipline concerned with the geophysical signatures of microbial interactions with geologic media. It spans the established disciplines of Geomicrobiology, Biogeoscience and Geophysics. Biogeophysics research in the last decade has confirmed the potential for geophysical techniques to measure not simply the physical and chemical properties of the subsurface, as already well established, but also to detect microbes, microbial growth, and microbe-mineral interactions, thus representing a major paradigm shift in geophysical thinking. In this review we begin by defining biogeophysics and provide a historical perspective. We then consider microbial alterations of petrophysical properties; as such alteration is the source of most biogeophysical signals. Our review then focuses on geophysical interrogation of microbial processes, including the direct detection of microbial cells and biofilm formation, microbial metabolic byproducts, microbial mediated redox processes, biogeochemical and microbe-mineral transformations. We conclude by discussing challenges, opportunities and potential new applications of biogeophysics to the exploration of life in extreme environments.
e.g. the deep biosphere, cryosphere and other planets. We find that published biogeophysics studies to date are mostly observation-based, presenting only empirical relationships between microbial and geophysical variables. Future research endeavors must focus on developing theoretical and/or numerical models for predicting geophysical signals arising from microbial activity.

1.0 INTRODUCTION

1.1. What is Biogeophysics?

Microorganisms are ubiquitous and found in almost every conceivable niche of the Earth [Konhauser, 2007], from near surface geologic materials as part of soil biota down to depths of tens of kilometers in the crust and the deep ocean; from hydrothermal vents to polar ice caps, and from alkaline to acidic environments. As such, microorganisms have played an important role in many aspects of the evolution of the Earth and its atmosphere, leaving their mark on the geologic record. Their role in transforming Earth systems, the cycling of elements and formation of minerals, and the powering of Earth’s biogeochemical cycles over the past 4 billion years is well documented in many biogeochemical studies [e.g., Hiebert and Bennett, 1992; Banfield et al., 1998; Ehrlich, 1998; 1999; Konhauser, 2007]. From the initial stages of microbial colonization of mineral surfaces, through the development of biofilms and biomats, the presence of microorganisms within geologic media can have a profound effect on both the micro-organisms themselves as well as the chemical and physical properties of the surrounding environment. Recognition of the important role that microorganisms play in altering Earth systems resulted in the development of geomicrobiology (the study of microbial interactions with geologic media) as a scientific field of study more than three decades ago. However, their role in altering subsurface physical properties is only now being recognized and currently is not completely understood. As
suggested by Knight [2001] “although geophysicists have tended to carefully control the physical and chemical conditions during laboratory measurements of near surface materials, control of biological conditions, such as the level of bacterial activity has been completely overlooked”. Yet it is apparent from decades of groundwater geochemistry studies that microbial metabolism and growth can impact both the chemical and physical properties of aquifers [e.g., Chapelle and Bradley, 1997]. For example, it has been documented from geochemistry studies that microbial metabolism can lead to the development of secondary porosity through the enhanced dissolution of different mineral phases [e.g., Hiebert and Bennett, 1992; McMahon et al., 1992; 1995; Bennett et al., 1996] or to the reduction of porosity [McMahon et al., 1992] by the clogging of pores due to precipitation of secondary mineral phases (e.g., calcite). Furthermore, the growth of microorganisms in the pore space, either in isolated colonies or as continuous biofilms, can lead to clogging of the porous medium with significant changes in the effective porosity and hydraulic conductivity [e.g., Taylor et al., 1990; Cunningham et al., 1991; Vandevivre and Baveye 1992; Baveye et al., 1998; Seifert and Engesgaard, 2007; Brovelli et al., 2009] causing the development of preferential flow paths [e.g., Seifert, 2005; Brovelli et al., 2009]. Porosity is an important aquifer and reservoir property and a key petrophysical parameter that can affect geoelectrical and seismic measurements [Schön, 1996]. Hence, there is a growing interest in the geophysics community to investigate the links/relationships between biological interactions with geologic media and potential changes in geophysical properties in near subsurface environments. Recognition of this potential has resulted in the development of a new sub-discipline in geophysics called ‘Biogeophysics’, which combines the fields of Microbiology, Biogeoscience, and Geophysics. More specifically, Biogeophysics is concerned with the links between dynamic
subsurface microbial processes, microbial-induced alterations to geologic materials, and geophysical signatures. Some of the questions investigated by biogeophysical studies include:

What are the direct geophysical signatures of microbial cells and biofilms?

Can geophysical methods be used to understand microbe-mineral transformations?

How can geophysics be used to improve understanding of biogeochemical processes in natural and anthropogenic environments?

Can geophysics be used to determine rates of metabolic activity in-situ (e.g., rate of biomass production)?

Which geophysical methods are best suited for investigating microbial activity?

Are there unique biosignatures in geophysical data sets?

Can geophysics ultimately contribute to exploration of microbial communities (“hot zones”) in extreme (such as the deep oceans or polar ice caps) or even extraterrestrial environments?

Biogeophysics therefore represents a transformative area of research and provides an exciting new frontier for training the next generation of multidisciplinary research scientists. We therefore recognize here a hitherto untapped potential for the development of geophysical techniques to measure not simply the subsurface physical and chemical properties, as geophysics is conventionally used, but also microbes, microbial processes, and microbe-mineral interactions.

1.2 Historical Background

The early Biogeophysics community primarily developed from the near surface/environmental geophysical community, which recognized that geophysical measurements were indirect
indicators of biodegradation occurring in hydrocarbon contaminated environments [Bermejo et al. 1997, Sauck et al. 1998]. These sites can be considered natural bioreactors because the excess organic substrates stimulate microbial activity. Geophysical evidence suggests that sediments impacted by petroleum hydrocarbons developed higher bulk conductivity over time, in contrast to the expected lower bulk conductivity consistent with the insulating properties of the petroleum compounds [Mázac, 1990]. To explain these conductive anomalies, Sauck et al., [1998] proposed that microbial mediated redox processes produce CO₂ and organic acids that enhance the weathering of aquifer sediments, releasing ions that elevate the concentrations of dissolved ions in groundwater. Since these studies, there has been a growing interest in the use of geophysics to interrogate biological interactions with geologic media that has accelerated greatly as a result of American Geophysical Union (AGU) related activities. As a consequence of these activities, the biogeophysics community has expanded far beyond the environmental arena as researchers have recognized the potential to indirectly observe bacteria and microbial processes occurring in situ. The unique properties of geophysical datasets (e.g. non-invasive data acquisition, spatially continuous properties retrieved) present opportunities to explore geomicrobial processes outside of the laboratory, at spatial scales unachievable with geochemical or microbiological techniques. It is now clear that geophysical technologies have the potential to improve understanding of metal and nutrient cycling driven by aerobic and anaerobic (e.g. iron and sulfate reducing) microbe-mineral transformations [Ntarlagiannis et al. 2005a, Williams et al. 2005; Slater et al., 2007], detect microbial growth and biofilm formation [Davis et al., 2005], assist in biodegradation and microbial ecology studies [Atekwana et al., 2000, 2004a-c; Allen et al., 2007], assist in the bioengineering of soils (e.g., from liquefaction) to minimization of earthquake effects [Dejong et al., 2006; 2009], improve understanding of microbial
transformation of ocean floor magnetization [e.g., Carlut et al., 2007], assess hydrological and biotransformations at remediation sites [e.g., Hubbard et al., 2008; Williams et al., 2009], probe the physical and chemical properties of the cell wall [e.g., Prodan et al. 2004;2008] and may ultimately contribute to the search for extraterrestrial life (e.g. McKay et al., 1996). Although we have seen nearly a decade of research in this area, our understanding of the geophysical consequences of microbial activity is still in its infancy. Nevertheless, we believe that a comprehensive review article that describes the current state of knowledge of biogeophysics is timely in order to increase recognition of the potential of using geophysical methods in understanding geo-microbial processes and their application to microbial studies of diverse environments.

2.0 MICROBIAL ALTERATION OF PETROPHYSICAL PROPERTIES

Figure 1 summarizes our current understanding of how microbial communities and microbial interactions with subsurface geologic media leading to changes in petrophysical properties can be sensed with geophysical techniques. The main factors we identify are (1) the direct response from microbial cells and extracellular cellular structures, (2) growth of the microorganisms (production of biomass) and their potential to form biofilms, (3) the generation of metabolic byproducts and the interactions of these metabolites with the host material, and (4) microbial mediated processes (Fig. 1). Microbial growth and distribution in subsurface environments is dependent on many variables, including temperature, pH, physical and chemical properties of the geologic media (i.e., porosity and permeability), and availability of nutrients, electron donors and acceptors. Table 1 summarizes the geophysical methods that have been used in support of biogeophysics research and provides what we consider to be the primary applications of each technique.
2.1. Microbial Cells & Biofilms

Although microbial cells can be free living (mobile fraction), most exist attached to surfaces [e.g., Hazen et al., 1991] (immobile fraction). The advantages gained by the bacteria in living attached to a surface are thought to include (1) higher concentrations of nutrients close to a surface; (2) minerals sometimes serve as energy sources for microbial metabolism, and (3) protection and opportunities for synergistic relationships with other cells [e.g., Davis and Luttge, 2005]. The attachment of bacterial cells to mineral surfaces is influenced by the electrical properties of the bacterial cells which are related to the chemical composition of the cell wall [Van der Wal et al., 1997]. Because most bacterial walls (gram negative and gram positive bacteria) have higher concentrations of anionic groups, bacterial exhibit a net negative cell wall charge which is compensated by counterions resulting in electrical double layers (EDL) at their surfaces with high charge density (0.5-1.0 C/m²) [Van der Wal et al., 1997]. Hence bacteria are suggested to behave like electric capacitors capable of storing charges in the presence of an electric field [Janknecht and Melo, 2003]. Static conductivity and dispersion studies show that the counterions give rise to considerable surface conductance within the hydrodynamically stagnant layer [Van der Wal et al., 1997] dependent on their concentration and the ionic mobilities of the counterions. Further, the interior of living cells are electrically negative with respect to their environment. The finite membrane potential leads to an enormous low-frequency dielectric response of living cells at low frequency (10 Hz) in the presence of an electrical field but decreases at higher frequencies [Prodan et al., 2004]. Hence low frequency electrical techniques (e.g., dielectric spectroscopy or capacitance measurements) can be used to probe the
electrical properties of living bacterial cells in suspension and in biofilm monitoring (see further discussion below in section 3).

The Bacterial EDL also plays an important role in adhesion/attachment of bacteria to mineral surfaces. There are several factors which potentially influence the adhesion of bacteria onto mineral surfaces, including bacterial species, the chemistry and surface area of the geological media, and the chemistry (ionic strength), pH, flow rate and temperature of the pore water [e.g., van Loosdrecht et al., 1989; Mills et al., 1994; Yee et al., 2000; Jiang et al., 2007]. As stated above, bacteria have a net negative charge on their surfaces and therefore as a result, bacteria experience strong electrical double layer repulsion on approach to negatively charged mineral surfaces. Conversely, the presence of metals at the surfaces of minerals (e.g., iron coatings) results in a greater affinity of bacterial adsorption to these surfaces due to the strong electrostatic attraction [e.g., Jiang et al., 2007; Abdel Aal et al., 2009]. During bacterial adhesion, a heterogeneous redistribution of charges on the bacterial cell surface reduces the electrical double layer (EDL) repulsion enhancing attachment [Poortinga et al., 2002]. Microbial adhesion to mineral surfaces has been the subject of numerous studies investigating microbial transport as this has direct implications for fate and transport of contaminants [Yee et al., 2000; Jiang et al., 2007].

Microorganisms require both electron donors (usually a carbon source such as glucose, lactate, hydrocarbons etc) and electron acceptors (O₂, NO₃, Fe³⁺, Mn⁴⁺, SO₄) for cellular growth, proliferation, and to maintain metabolic functions. Biofilm formation requires the attachment of microbes to solid surfaces. Irreversible attachment occurs through the secretion of extracellular polymers that provide a strong bond to the solid surface that creates a matrix which surrounds the cells and forms the internal architecture of microbial communities. Further, during microbial
growth, the increase in cell numbers and biomass (either as continuous biofilms or as isolated colonies) and associated extracellular polysaccharides (EPS) structures can effectively clog/plug pores, change pore geometry and effective pore radii leading to reductions in porosity, permeability, and hydraulic conductivity. In fact, pore clogging due to biomass production is a widespread phenomena documented in many natural and anthropogenic environments, and engineered systems, and is commonly observed during amendment strategies (i.e., biostimulation or bio-augmentation) for clean up of contaminated sites. The effect of bioclogging on hydraulic properties has been the focus of many experimental and numerical methods studies [e.g., Cunningham et al., 1991; Vandevivere and Baveye, 1992; Baveye et al., 1998; Bouwer et al., 2000; Seifert, 2005; Brovelli et al., 2009]. Attachment of microbial cells to grain surfaces can also lead to an increase in surface roughness as well as surface area. For example, Figure 2a shows an environmental scanning electron microscope (ESEM) image of *Pseudomonas aeruginosa* cells embedded in biofilm and attached to the surface of a mineral grain acquired during a microbial growth experiment (see the Davis et al., [2008] study discussed further in section 3). Figure 2b is the image from the control column. Clearly the surface characteristics of the mineral grain from the bio-stimulated column have been significantly altered compared to the grain from the unstimulated column. Both surface area and surface roughness are known to exert a major control on surface conductivity [e.g. Revil and Glover, 1998]. Thus, the constriction of pores by biomass and the development of biofilms attached to mineral surfaces may have a profound effect on electrical properties, as we will discuss in Section 3. Additionally, biofilms can act as organic precipitates and exhibit linear viscoelastic behavior [Stoodley et al., 1999; Klapper et al., 2002]. The presence of biofilms can fill pores, alter permeability and grain contact coupling thereby changing the sediment matrix stiffness, shear strength, and the elastic
and/or visco-elastic moduli of the media, potentially detectable by acoustic and seismic techniques (see further discussion below in section 3)

A recent discovery suggests that bacteria can produce certain proteinacious extracellular appendages (pili) that are electrically conductive and serve to facilitate long range electron transport [Reguera et al., 2005, 2006, 2007 Gorby et al., 2006]. In addition to serving as electrical conduits for electron transfer to solid state electron acceptors (e.g. Fe (III) oxides), these pili (e.g., of Geobacter sulfurreducens) enable maximum biofilm growth [Ruguera et al., 2007]. Ruguera et al. [2006] also document that the pili of G. sulfurreducens enabled cells at the surface of a thick layer of biofilm to remain viable and connected to cells at the bottom of the biofilm in direct contact with an electrode increasing electricity production by up to 10-fold. The authors suggested that the pili of G. sulfurreducens probably served as an electronic network permeating the biofilm and connecting cells throughout the biofilm, facilitating and promoting long-range electrical transfer in an energy-efficient manner. We speculate from the above study that in natural and anthropogenic environments in sufficient numbers, the permeation of these electrically conductive nanowires through porous media could conceivably increase the bulk electrical conductivity by providing a conductive network through an otherwise resistive geologic media. Conceivably, pili in biofilm across the water table interface may well represent a potential mechanism for electron transfer at organic rich contaminated sites generating large self-potential anomalies [e.g., Naudet et al., 2003] akin to the geobattery effect of Sato and Mooney [1960] observed over sulfide deposits. We will expand on this further in section 4 below. Finally, we note that the action of magnetotactic bacteria can result in the precipitation of magnetosomes which can have a major impact on magnetic properties of the subsurface environment with a direct impact on magnetic geophysical techniques. Although in most field situations it is often
assumed that the ferrimagnetic mineral content is too low to affect GPR wave behavior, a study by Pettinelli et al. [2005] using magnetite/silica mixtures as Martian soil simulants observed that an increase in magnetite concentration affected both the wave velocity and the attenuation of electromagnetic (EM) waves. Therefore, the possibility exists that ferrimagnetic minerals produced by microbial activity if in sufficient concentrations can have an effect on ground-penetrating radar (GPR) signals. We discuss magnetotactic bacteria further in section 3.

2.2 Generation of Metabolic Byproducts

Microbial metabolism is considered to be a major factor enhancing the weathering of minerals through the attachment, growth, and colonization of mineral surfaces by microorganisms [Bennett et al., 1996]. Microbial metabolic byproducts include the production of biogenic gases (e.g., CO₂, H₂S, CH₄ etc.) organic acids and biosurfactants. These byproducts have the potential to affect the physicochemical properties, and thus the electrical properties, of porous media in various ways. For example, microbial production of organic acids and biosurfactants (both cationic and anionic) directly adds ions to solution, increasing the electrolyte concentration of pore fluids [Cassidy et al., 2001, 2002; Davis and Atekwana, 2006], accounting for ~12% of fluid conductivity observed at organic rich contaminated sites [Davis, unpublished data]. In addition, evidence suggests that metabolic byproducts such as organic acids enhance the mobility of sparingly soluble metals and also increase the rate of mineral surface reaction sites thus accelerating the rate of mineral weathering [e.g., Hiebert and Bennett, 1992; McMahon et al., 1995]. The dissolution of minerals will further increase the ionic strength of pore fluids, enhancing the electrolytic conductivity (σₑ). In fact, in our own studies we have documented microbial enhanced mineral weathering that resulted in direct increases in Ca²⁺ concentration,
concomitant with an increase in the electrolytic conductivity and electrical conductivity [e.g.,
*Abdel Aal et al.*, 2004; *Atekwana et al.*, 2004a-c]. Enhanced mineral dissolution catalyzed by
increased organic acid concentration can lead to physical changes in grain surface morphology,
surface area, surface roughness and the generation of secondary porosity and increased
permeability [e.g., *McMahon and Chapelle*, 1991]. Changes in porosity resulting from mineral
dissolution may alter grain contact coupling affecting acoustic wave propagation. In addition, the
generation of biogenic gases can have an effect on both the electrical conductivity (reducing
conductivity) and seismic properties (enhancing attenuation of signal amplitudes) as we discuss
further in Section 4.

### 2.3. Redox Processes

Microbial metabolic activity plays a key-role in redox processes because microbes derive
energy from oxidation-reduction reactions (the transfer of electrons from one reactant to the
other). The chemistry of the fluid within the pores where microbial growth occurs is
significantly different relative to the chemistry of the fluid in pores where microbial growth is
absent [*Atekwana et al.*, 2006]. The abundance of terminal electron acceptors (TEA’s) govern
nutrient utilization by microbes during the breakdown of organic carbon [*Bekins et al.*, 2001;
*Cozzarelli et al.*, 2001]. Terminal electron acceptors are sequentially utilized from O$_2$, NO$_3^-$, Fe
(III), Mn (IV), SO$_4^{2-}$ and CO$_2$. As the TEAs are consumed, changes occur in the pore fluid
chemistry. For example products such as NH$_4$, Fe$^{2+}$, and Mn$^{2+}$ may accumulate in the pore fluid.
As discussed earlier, addition of these ions in solution can cause changes in the pore fluid
conductivity. We have documented in field studies that peaks in the bulk electrical conductivity
are coincident with peaks in redox species such as Fe$^{2+}$ and Mn$^{2+}$ [*Atekwana et al.*, 2005].
Significant changes in the pH and Eh in the sediment environment may also occur as a result of microbial mediated redox reactions. Such pH and Eh changes can result in new mineral stability fields in which some minerals become unstable and are dissolved and mobilized (e.g., Fe$^{2+}$). On the other hand as ions in solution exceed their saturation indices, minerals begin to precipitate from solution [Stumm and Morgan, 1995]. For example, iron sulfide can be precipitated from solution during sulfate reduction by *Disulfurbrio vulgaris*. Therefore, microorganisms and their activity can result in the formation of mineral phases by the process of biomineralization. Biomineralization can occur in two different ways: ‘biologically induced mineralization” in which the mineral precipitation occurs without control by the cell (passive mechanism) usually due to changes in microbial redox state (e.g., precipitation of calcite, magnetite, iron hydroxides etc.) and “biologically controlled biomineralization” in which mineral precipitation occurs by the microorganism precipitating minerals that serve some physiological functions [Konhauser, 2007]. The precipitates usually occur on mineral grains or in the pores between sediment. In some cases the bacteria cells can form the nucleation points of mineral precipitation resulting in a biomineral. Mineral precipitation can result in changes in surface area, formation porosity and pore throat diameter with significant impact on electric current flow and seismic wave propagation as we will show in Section 4. Also, the precipitation of magnetite, for example, can change the magnetic properties of the subsurface contributing to the remanent magnetization of the rocks, while the weathering of iron mineral phases can equally contribute to the demagnetization of rocks [e.g., Carlut et al., 2007].

The conceptual model presented in Figure 1 demonstrates that changes in the geophysical properties of geologic media are the end product of the integrated biogeochemical processes associated with microbial growth and activity. Microbial interactions with geologic media and
their by-products can lead to changes in: (1) the petrophysical properties of the rocks (e.g., changes in porosity, formation factor, tortuosity, mechanical properties etc.) by direct microbial colonization of mineral surfaces and biofilm formation, or indirectly by mineral breakdown and precipitation from metabolic intermediates and end products; (2) the fluid chemistry by release of ions from mineral weathering and from metabolic intermediates (e.g., organic acids, biosurfactants, NH$_4^+$, Fe(II), Mn(II)); (3) the properties at the mineral surface-fluid interface by microbial colonization of the mineral surfaces or by concentrating chemical degradation products at this boundary; (4) the redox state of the groundwater geochemistry with the potential to develop strong electrochemical potentials; (5) the geophysical signatures that arise directly from electrical and magnetic properties of cells themselves (e.g., precipitation of magnetosomes by magnetotactic bacteria). The above figure also illustrates that microbial transformations of geologic media are complex, coupled, and can occur over a wide range of spatial and temporal scales. Thus, the main challenge lies with decoupling the complex microbial-induced physical and chemical transformations inherent during microbial activity, and quantifying the magnitudes of the geophysical response.

3. DIRECT DETECTION OF MICROBIAL CELLS AND BIOFILMS

Microbial cells themselves exhibit distinct electrical properties and certain (magnetotactic) bacteria also display unique magnetic characteristics. In this section we consider further the possibilities of detecting the geophysical signatures produced by bacterial cells themselves as well as those associated with biofilms forming in porous media.

3.1. Microbial Cells
One well-studied biophysical phenomenon is the polarization of living cells and the resulting macro-scale dielectric permittivity of cellular suspensions (e.g. Frohlich, 1975; Foster and Schwan, 1989). Laboratory biophysical studies of cellular suspensions have been primarily conducted with the focus on medical sciences, although more recent work has been conducted in support of national security, as well as fundamental research directed towards searching for evidence of life on other planets. Dielectric spectroscopy methods, analogous to electrical geophysical exploration methods, show that the low-frequency relative dielectric permittivity of suspensions of living cells is huge (in excess of 1000 below 100 Hz).

Broadband dielectric spectroscopy data on suspensions of live cells frequently record two polarization mechanisms, commonly referred to as the lower frequency alpha ($\alpha$) and higher frequency beta ($\beta$) effect (Fig. 3-1) [Prodan et al., 2008]. The membrane potential (the voltage drop across the membrane due to the negatively charged molecules in cells) associated with live cells results in an accumulation of mobile electric charge carriers at the membrane surfaces. When live cells are placed in time-oscillating electric fields, these charges move on the surface of the membrane, giving rise to high polarizations (\(\alpha\) effect). As the mobility of these surface charges is relatively small, this effect is manifest at low frequencies, such that the relative dielectric permittivity of live cell suspensions can be as high as $10^6$ [e.g. Stoy et al., 1982; Raicu et al., 1998; Prodan et al., 2004]. The effect is very similar to the polarization of the electrical double layer forming at the mineral surfaces of an interconnected porous medium, as traditionally measured with the spectral induced polarization (SIP) geophysical method. The $\beta$-relaxation is related to the dielectric structure of the cell’s membrane itself and can enhance the dielectric permittivity at higher frequencies [Prodan and Prodan, 1999].
Theoretical modeling frameworks have been developed to interpret dielectric spectroscopy measurements on cellular suspensions [Prodan and Prodan, 1999; Prodan et al., 2008]. Prodan et al. [2008] present a fully analytical solution for suspensions of spherical cells. The independent input parameters for this model are the outer and inner radius of the cell’s membrane, the dielectric constant and conductivity of the medium, of the membrane and of the inner cell region, the diffusion constants of surface charges accumulated at the outer and inner surfaces of the membrane, and the membrane potential. This model shows that the outer and inner cell radii, diffusion constants and membrane potential all have a very distinct effect on the broadband dielectric spectroscopy data. For example, the membrane potential determines the height of the $\alpha$ plateau (Fig. 3a); the diffusion constants control the width of the plateau whereas the thickness of the cell membrane controls the height of the $\beta$ (Fig. 3b) plateau. Figure 3c illustrates how the low frequency polarization of E Coli in suspension increases with membrane potential of the cells [Prodan et al., 2004].

These well-known dielectric properties of cellular suspensions suggest that it may also be possible to use dielectric spectroscopy to investigate microbial cells in porous media. Ntarlagiannis et al. [2005b] performed the equivalent of a single (10 Hz) frequency dielectric spectroscopy measurement on sand columns injected with varying high concentrations of Shewanella putrefaciens and E. coli and found measurable changes coinciding with changes in cell concentration inferred from optical density measurements. However, the interpretation of dielectric spectroscopy data in porous media is complicated by the additional possible polarization mechanisms that may exist relative to the two mechanisms ($\alpha$ and $\beta$) attributed to the electrical properties of cells themselves. For example, Ntarlagiannis et al. [2005b] observed both increases (at higher cell concentration) and decreases (at lower cell concentration) in
polarization resulting from the introduction of cells to the sand columns. They tentatively attributed the decreases at lower cell concentrations to alteration of the mineral-fluid interface due to cell-mineral interactions and the increases at higher concentrations to the development of a pore throat polarization mechanism resulting from clogging of pores by cells. Assuming this interpretation to be correct, they therefore did not detect the polarization due to the cells themselves, as seen with dielectric spectroscopy of cellular suspensions, but rather changes in the polarization of the porous medium resulting from the addition of cells in suspension.

More recent studies have shed additional light on the polarization mechanism resulting from addition of cells to a porous medium. Abdel Aal et al. [2009] conducted microbial adhesion studies to investigate the effect of cell sorption to mineral surfaces on induced polarization (IP) parameters. Clean quartz sands and varying percentages of iron oxide coated sands were saturated with cell suspensions of *Pseudomonas aeruginosa*. They observed that microbial adsorption to clean quartz sands was gradual and resulted in an increase of 13% in the imaginary conductivity component ($\sigma''$). However, when iron-oxide coated sands (20-100% by weight) were used, a more rapid increase in microbial adsorption was observed (due to the strong electrostatic force between oppositely charged surfaces) with $\sigma''$ reaching a maximum of 37% for the 80-100% iron coated sands. In both experiments, no significant changes were observed in the real conductivity component ($\sigma'$) due to adsorption of cells. A strong power law dependency was observed between the adsorbed cells and $\sigma''$ suggesting that the polarization ($\sigma''$) response resulted from the direct sorption of microbial cells to mineral surfaces. Abdel Aal et al. [2009] interpret these results to suggest that the increase in the surface roughness of the mineral grains due to the progressive sorption of bacteria is the main cause of the polarization and the large surface area of the bacteria accentuates this effect. Since most of the adsorption on cell surfaces
took place within the first 90 minutes of the experiment, it is possible that part of the response observed by Ntarlagiannis et al., [2005a] may have resulted from cell adhesion to mineral surfaces. Thus, the imaginary conductivity component appears a useful parameter for interrogating microbial cells and their interactions with mineral surfaces.

3.2 Magnetotactic bacteria and magnetic signals

It is well known that a diverse group of prokaryotes can exert a significant control over magnetite formation. These microorganisms, known as the magnetotactic bacteria, biomineralize intracellular, membrane-bound, single-magnetic-domain crystals of the magnetic minerals magnetite and/or greigite called magnetosomes [Bazylinski and Frankel, 2004]. In general most magnetotactic bacteria can produce an average of 20 magnetosomes although some bacteria have been known to possess up to 1000 magnetosomes [Vali et al., 1987]. The magnetosomes crystals formed by magnetotactic bacteria have unique morphologies that are distinctly different from magnetite formed by Fe (II) reduction. These magnetosomes are synthesized from soluble forms of iron and are generally arranged as a chain that is anchored within the cell by cellular structural components [Bazylinski, 1999]. Using these magnetosome chains, like a compass needle, the cells are able to passively align along the Earth's geomagnetic field lines while they swim, aiding the cells to locate and maintain optimal position in vertical O$_2$ concentrations and redox gradients. Hence, magnetotactic bacteria are typically found in a wide variety of aquatic environments (e.g., fresh water lakes) with the highest numbers occurring mostly at oxic-anoxic interfaces (typically the sediment-water interface) indicating relatively specific redox and chemical conditions. Their presence in sediments is often used as proxies for paleoenvironmental/climatic conditions. Additionally, their presence in ancient sediments (magnetofossils) suggests that they are important remanence carriers and can significantly
contribute to the environmental and paleomagnetic signals of sediments and rocks, making them useful for studying changes in the secular variation of the Earth’s magnetic field from freshwater aquatic environments and even for the search for early life in ancient sedimentary formations [e.g., Pan et al., 2005]; Studies estimate that the bacterial contribution to remanence in sediments is in the order of $10^{-6} - 10^{-4}$ A/m [Kirschvink and Lowenstam, 1979; Lowrie and Heller, 1982]. However, knowledge of magnetic properties of magnetosomes is still very limited and their identification from bulk environmental samples remains a challenging task and an active area of research [Pan et al., 2005]. While there have been no biogeophysical investigations per se that particularly address the issue of detecting magnetosomes using traditional geophysical techniques, we believe that the presence of magnetosomes in sediments and other secondary magnetic minerals produced by microbial activity can definitely impact the magnetic properties of the subsurface such that that may be measurable using magnetic geophysical techniques. For example, magnetic susceptibility measurements at a hydrocarbon contaminated site undergoing biodegradation showed a zone of enhanced susceptibility at depth interfaces coincident with the presence of contamination [Rijal et al., 2008]. The authors related the higher susceptibility values within the contaminated environment to the biotransformation of iron related minerals within this zone.

3.3. Biofilms

A biofilm refers to an attached state of cell growth, whereby cells are closely packed and firmly attached to each other, usually forming a solid surface. Intuitively, the formation of biofilms might be expected to alter the geophysical properties of soils (see discussion above in section 2). Davis et al. [2006] conducted experiments on bio-stimulated sand packed columns and found significant changes in column polarization coinciding in time with changes in concentration of
cells attached to the sand surface (Fig. 4a and b). They used environmental scanning electron microscopy (ESEM) to show that peaks in polarization (at 2 Hz frequency) coincided with biofilm attachment to mineral surfaces and apparent bridging of soil grains by the biofilms (Fig. 4c). This led Davis et al. [2006] to suggest that low frequency electrical measurements are a proxy indicator of microbial growth/cell attachment and biofilm formation in porous media. They attributed the initial increases in polarization (Fig. 4a) to increasing cell attachment/aggregation into cell micro colonies [e.g. Watnick and Kolter, 2000]. The later decreases in polarization were attributed to increased detachments rates [e.g. Watnick and Kolter, 2000], or death and lyses of cells [Mai-Prochow et al., 2004]. Recent investigations by Abdel Aal et al. [2008] show that live microbial cells impact the magnitude of the phase and the imaginary conductivity components. Dead cells on the other hand have no impact on the phase or imaginary conductivity (this is also true for dielectric spectroscopy as discussed above since lysed cells cannot impact the systems capacitance [Janknecht and Melo, 2003]). These observations are consistent with the Davis et al., [2006] study. Thus the descending limb in the Davis et al. [2006] study is consistent with the death/lyses of cells. Biomass growth is self limiting because the growth of microorganisms either as immobile fractions attached to mineral surfaces or as mobile components within the pores reduces the available pore space and nutrient flux resulting in the eventual death of the microorganisms [Brovelli et al., 2009]. Hence, the Davis et al., [2006] study raises the interesting possibility that the imaginary conductivity component can be used to infer the rate of biofilm formation (growth) and decay (lysis) processes. However, it was unclear from Davis et al. [2006] whether the source of the polarization is the electrical properties of the biofilm itself (i.e. direct detection), or the modification of the polarization associated with the porous medium as a result of cell attachment to mineral surfaces.
(i.e. indirect detection). Ntarlagiannis and Ferguson [2009] attempted to further understand the low-frequency electrical properties of biofilms by conducting controlled experiments in sand columns injected with artificial biofilms created using an alginate gel matrix (alginate is known to simulate microbial exopolymeric substances e.g. Whitham and Gilbert, [1993]). They showed that a low-frequency polarization developed in response to increasing volume concentration of (artificial) biofilm within columns packed with glass beads. They concluded that this experiment, unlike Davis et al, [2006] conducted on a soil undergoing biodegradation, confirmed that a measurable polarization results from the presence of biofilm itself in a porous medium. Ntarlagiannis and Ferguson [2009] attributed this polarization to a pore throat mechanism generated as biofilms filled and blocked pore throats. However, the generation of such a polarization mechanism due to pore blockage by natural biofilms remains unproven but feasible since numerous studies have documented that the growth of microbial biomass in the form of biofilms in pores, plugs the pore space, altering flow paths through porous media, reducing hydraulic conductivity and mass transport properties [e.g., Cunningham et al., 1991; Vandevivere and Baveye, 1992; Baveye et al., 1998; Brovelli et al., 2009].

The utility of using acoustic imaging techniques to investigate biofilm growth has recently been demonstrated by Davis et al. [2008] in controlled column experiments. In this study, microbial growth (Pseudomonas aeruginosa PAO1 wild type bacteria culture) was stimulated in silica sand-packed columns and acoustic (compressional) wave data over a two-dimensional region was collected for 29 days. The results (Fig. 5) showed relatively high spatial variability in the acoustic amplitude (with areas of enhanced and attenuated signals) measured from initial conditions of homogeneous amplitudes in the biostimulated columns (Fig. 5a) while minimal changes were observed in the unstimulated column (Fig. 5b). The bulk of the changes to the
acoustic wave properties in the biostimulated column were imparted between Day 5 and Day 7 of the experiment, concomitant with a peak in the imaginary conductivity values. Davis et al. [2006] had previously demonstrated that peaks in the imaginary conductivity correlate with peaks in biomass production. Hence the commonality of when maximum changes occurred in the electrical and seismic measurements led the authors to suggest that the changes in the acoustic wave amplitude resulted from the direct presence of biomass accumulation. The spatial variability observed in the acoustic amplitude results from this study suggests that enhanced microbial growth has a variable affect on the elastic properties of porous media. While the exact microbial-induced mechanisms for the variations in amplitude are yet unclear, the authors speculated that the differences in amplitude may arise from a non-uniform distribution of microbial activity or possible heterogeneity in the biomass distribution and biofilm morphology (e.g., variations in biofilm thickness, roughness, hydration etc). ESEM imaging qualitatively showed apparent differences in the structure and/or thickness of biofilm on the surface of the sand grains, as well as evidence of bacterial attachment, between areas of increased and decreased acoustic wave amplitude (Fig. 5c). Although significant changes in attenuation were observed, only slight changes in velocity were observed within the biostimulated columns. The small change in velocity observed in the Davis et al. [2008] study is similar to results on biomineralization processes obtained by Williams et al. [2005] discussed below. These results suggest that microbial processes cause significant changes in the amplitude response of acoustic waves but only minimal changes in velocity. The above studies underscore the fact that geophysical techniques are sensitive to the growth of microbes in porous media and represent a significant first step towards the use of geophysical imaging techniques for the validation of bioclogging models, while highlighting the potential for using geophysical methodologies to
provide spatio-temporal changes in biofilm growth and accumulation within porous media at field sites as well as mapping preferential flow paths resulting from this process. We also see some potential applications of geophysics for the assessment of biofilm barriers used for contaminant remediation or as sealants of reservoirs for CO$_2$ sequestration and the remote monitoring of microbial activity during microbial enhanced oil recovery.

4. METABOLIC BYPRODUCTS AND ENHANCED MINERAL WEATHERING

One of the most profound effects of microbial metabolism on the rock record is in the enhancement of mineral weathering either through mineral dissolution or mineral oxidation [Hiebert and Bennett, 1992; Bennett et al., 1996; Konhauser, 2007]. The physical attachment and colonization of indigenous micro-organisms to mineral substrates in their attempt to gain access to nutrients promotes chemical weathering at rates faster than is theoretically predicted [Konhauser, 2007; Hiebert and Bennett, 1992]. In addition, the production of metabolic byproducts such as organic acids enhances the dissolution of minerals leading to significant increases in the total reactive surface area of minerals [Davis and Luttge, 2005]. Thus microorganisms play a fundamental role in the modification of rates of mineral weathering with significant implications for both soil and sediment formation [Banfield et al., 1999] and in the regulation of global climate as a result of their participation in the cycling of carbon through the different carbon reservoirs [Kump et al., 2000].

Hydrocarbon contaminated environments represent one environment where the effect of metabolic byproducts on geophysical properties is well documented [Werkema et al., 2003; Abdel Aal et al., 2004; Atekwana et al., 2004a-c; 2005]. Until recently, it was commonly assumed by many field geophysicists that hydrocarbon impacted sediments can only be
effectively imaged by their higher resistivity (low conductivity) compared to “background” due to a simplified conceptual model based on the partial replacement of conductive ground water by highly resistive petroleum compounds [e.g., Mázac et al., 1990]. However, extensive evidence exists in the literature for enhanced mineral weathering in hydrocarbon contaminated aquifers [e.g., Hiebert and Bennett, 1992; McMahon et al., 1995]. This weathering results primarily from the production of organic acids and CO₂ (carbonic acid). For example during the congruent dissolution of calcite, Ca²⁺ and HCO₃⁻ are produced that go into solution:

\[ \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{Ca}^{2+} + 2\text{HCO}_3^- \]

The net effect is the increase in the concentration of dissolved solutes that results in an increase in the pore fluid conductivity. We also note that elevated pore fluid conductivity can result from the direct addition of organic acids and biosurfactants to solution. Hence, elevated pore fluid conductivity at hydrocarbon contaminated sites appears to be the rule rather than the exception. Changes in pore fluid conductivity directly result in changes in bulk conductivity measurable by electrical resistivity, SIP, electromagnetic induction, GPR, and self potential (diffusion potentials) techniques [Sauck et al., 1998; Atekwana et al, 2004a-c; Abdel Aal et al., 2004, 2006]. Indeed the effect of metabolic byproducts such as organic acid production on electrical properties is well demonstrated by the seminal work of Sauck et al. [1998] at the Fire Training facility (FT-02) at the decommissioned Wurtsmith Air Force Base, Oscoda, Michigan USA. Electrical resistivity and GPR investigations across the hydrocarbon plume at this site showed elevated bulk electrical conductivity and attenuated GPR reflections coincident with the zone of contamination (Fig. 6a and b). Groundwater investigations suggest that intrinsic bioremediation and enhanced mineral weathering is occurring at the site (Fig. 6c and d). In addition, studies by Bradford [2007] at the same site observed a distinct zone of increased dispersion coincident with
the zone of GPR signal attenuation. The increase in the pore water conductivity alters the relaxation characteristics of the bulk formation resulting in the attenuation of the GPR signal amplitudes [Bradford, 2007]. Attenuated GPR reflections resulting from elevated pore fluid conductivity due to biodegradation have also been reported by Cassidy [2007, 2008]. Using GPR signal attribute analysis and dielectric property studies, Cassidy [2007, 2008] documented attenuation related to material property losses of >2-6 dB/m within contaminated zones. The author also noted a slight but observable frequency dependence to the attenuation spectrum with the lowest frequency data (<300 MHz) showing the greatest degree of loss.

In another study through a vertical cross section of a hydrocarbon plume, Allen et al. [2007] documented that zones of higher bulk electrical conductivity were coincident with higher percentages of oil degrading microbial populations. Using molecular microbial techniques determined by construction and evaluation of 16S rRNA clone libraries along a vertical gradient at the contaminated location, Allen et al. [2007] further showed that spatial heterogeneity in the microbial community structure and shifts in the microbial community were concomitant with vertical changes in bulk electrical conductivity (Fig. 7). The shifts in the microbial communities were also related to the partitioning of the different phases of the hydrocarbon: methylo trophs and aromatic hydrocarbon degraders made up the dominant population in the residual phase; syntrophic populations characterized the dissolved phase, while hydrocarbon degrading fermenters and iron and sulfur reducers made up the bulk of the populations within the free phase. More importantly, a multivariate statistical analysis suggested that elevated bulk electrical conductivity may be related to the presence and activities of specific microbial populations (e.g., syntrophic species α- and β-Proteobacteria). The results presented by Allen et al. [2007] suggest that geoelectrical techniques may help guide sampling in microbial ecology studies.
Other metabolic byproducts produced by microorganisms include biogenic gases (e.g., CO₂, CH₄, H₂S, and H₂). These gases can impact geophysical properties. For example, methane production by archaea in anaerobic peat soils can result in extensive free phase gas production in peatlands that has been non-invasively detected using GPR [Comas et al., 2005] and variants of the technique in the laboratory [Comas and Slater, 2007]. The implications for monitoring production and release of this important greenhouse gas from peatlands are further discussed in Section 7.

5. MICROBIAL MEDIATED REDOX PROCESSES

Under aerobic conditions bacteria first utilize oxygen as terminal electron acceptors (TEAs) whereas in anaerobic environments other electron acceptors, such as sulfate in the presence of sulfate reducing bacteria or Fe (III) in the presence of iron reducing bacteria, are utilized. Microbial respiration consequently results in reduced conditions and strong redox gradients in the Earth typically develop in the presence of bacteria. In this section we consider how microbial-induced redox gradients may drive detectable geophysical signatures.

Self potential is the only geophysical method dependent, as shown by theory, on redox gradients and the only method therefore to concern ourselves with in this section. In the self potential (SP) method the electric fields generated by internal electric current sources in the Earth are recorded with a pair of precision electrodes carefully constructed, using a porous pot construction, so as to avoid reactions between the metallic electrode and the electrolyte [e.g. Petiau et al., 2000]. This is achieved by immersing metal electrodes in saturating solutions/gels of the metal (an identical solution being used for both electrodes making up the pair such that the redox state at the two metal surfaces is identical). Interest in the application of the SP method for indirectly detecting
microbial activity from redox gradients produced has been fueled by a number of studies showing strong SP signals (exceeding 0.1 V) at sites where microbial degradation of hydrocarbons is occurring [Sauck et al., 1998; Nyquist and Corry, 2002; Naudet et al., 2003; Minsley et al., 2007]. However, understanding of self potential signals in the presence of microbial activity has been perhaps hindered by a number of factors including (1) interpretation using electrochemical models physically inconsistent with SP measurements (Nyquist and Corry, 2002), and (2) nomenclature, whereby some authors have chosen to refer to measurements of redox chemistry occurring at an electrode (i.e. a reaction between the electrolyte and the metal electrode surface) as a “self potential” [Williams et al., 2007]. We hope to address some of these issues in this section.

There are numerous well-understood mechanisms generating sources of electric current in the Earth e.g. the electrokinetic source term resulting from the transport of excess charge in the electrical double layer at pore surfaces as a result of the viscous drag exerted by fluid flow through the pores. However, there are only two established current-source mechanisms that can potentially generate self potential signals in the presence of microbial induced redox gradients: (1) electro-diffusional effects due to gradients in the chemical potentials of the charge carriers causing the redox gradient [e.g. Mainuelt et al., 2006], and (2) redox reactions and coupled electron transport associated with electronic conductors (geobattery effects) [Sato and Mooney, 1960; Bigalke and Grabner, 1997; Naudet et al., 2003; 2004].

Mainuelt et al. [2006] conducted a detailed laboratory study of electro-diffusional effects in the presence of sharp redox gradients produced by parallel advecting KMnO4 and FeCl2 solutions in contact along a reaction front. One of the incentives of this work was to ascertain whether an SP source mechanism additional to the electro-diffusional and electrokinetic terms was needed to
satisfy SP signals observed across the redox front. They showed that, despite the presence of a redox reaction occurring across this front (visible precipitation of Fe (OH)₃), the self potentials across this redox gradient were small (< 0.01 V) and not significantly greater than those resulting from the combination of electro-diffusional and electrokinetic effects outside of the reaction front. They concluded that the SP signal corresponding to this second front is not related to the variation of the redox potential itself, but rather to electro-diffusional mechanisms associated with changes in composition and concentration of the solution resulting from the redox reaction. This work suggests that (1) an alternative SP source mechanism is needed to explain the SP signals recorded at sites where biodegradation is occurring as the signals are large (>0.01 mV), and (2) this mechanism is absent in the abiotic experiments of Maineult et al. [2006].

The fact that an alternative mechanism is needed is evident from the work of Minsley et al. [2007] who inverted borehole SP signals (approximately normally distributed between 0-0.15 V) from a site of known dense nonaqueous phase liquid contamination for a distribution of current sources that could explain the measured potentials. They showed that the distribution of current sources obtained from the inversion was empirically related to the distribution of redox potential. Minsley et al. [2007] describe a conceptual model for the generation of the current sources associated with the biodegradation of contaminants (primarily PCE, TCE and TCA at their site) by microbial-mediated redox reactions. They suggest that electron transport, via the production of a chain of intermediate compounds until the final electron acceptor is reduced, must be balanced by an external current flow in the Earth such that the total electric current density is divergence free. They present an analogy to the corrosion of two metals in a short-circuited energy-producing electrochemical cell whereby the electron transport via the short circuit is associated with the electron transfer involved in the reduction chain associated with the
biodegradation. The driving force of this electrochemical cell was assumed to be the redox gradients caused by the biodegradation reactions occurring at the site.

Such an energy producing cell is the basis of the traditional ‘geobattery model’ proposed to explain very large (~1.0 V) SP signals repeatedly recorded over electronically conductive ore bodies straddling the large redox gradient provided by the water table [Sato and Mooney, 1960; Sivenas and Beales, 1982; Bigalke and Grabner, 1997; Castermant et al., 2008]. The traditional geobattery for an ore body (Figure 8a) considers both the distribution of the redox potential and the kinetics of the chemical reactions at the surface of the metallic particles [Castermant et al., 2008]. The ore body participates directly in the two half-cell reactions of the electrochemical cell, consisting of anodic (oxidizing) and cathodic (reducing) reactions located at the bottom and the top of the ore body, respectively. The deep anodic reaction corresponds to the galvanic corrosion of the metallic body, whereas the cathodic reaction is the reduction of oxygen radicals at the top of the body [Sivenas and Beales, 1982]. The driving force of the electrochemical cell is atmospheric oxygen dissolved in groundwater and the electronically conductive ore body serves as the short circuit for electron transport. This model has been widely used in mineral exploration to explain SP signals over metallic ore bodies.

In order to explain the large (~0.4 V) SP signals recorded over the Entressen Landfill (France) [Naudet et al., 2003] that could not be explained by electro-diffusional effects, Naudet et al. [2004] devised an alternative bio-geobattery model as shown in Figure 8a and b. Here the distribution of redox potential is associated with a strong redox gradient between highly reducing conditions below the water table within the plume (due to biodegradation and oxygen depletion) and the oxidized zone above the water table. The driving force of this electrochemical cell is dissolved Fe\(^{2+}\) resulting from the oxidation of organic matter by bacteria followed by reduction
of Fe(III) oxides. To complete the geobattery, Naudet et al. [2004] proposed that biofilms forming at the groundwater table could possibly provide the short circuit and required electron transport (Fig. 8b). This fascinating model has generated considerable interest and debate in the field of biogeophysics as it opens intriguing possibilities for monitoring microbial processes from large SP signals associated with geobattery effects.

A weakness of this model is that the ability of biofilms to facilitate electron transport, for example over the scale of the groundwater interface, is currently unproven. However, there is growing evidence that metal reducing organisms, such as *Shewanella* and *Geobacter*, produce electrically conductive appendages called bacterial nanowires that facilitate electron transfer to solid phase electron acceptors [Gorby et al., 2006; Reguera et al., 2005]. In *Geobacter*, nanowires are composed of a unique pilin protein. These “geopili” are electrically conductive, presumably in the absence of traditional electron transport proteins such as cytochromes. In *Shewanella*, nanowires require multiheme cytochromes to be electrically conductive, as evidenced by the fact that mutants lacking genes for a pair of extracellular decaheme cytochromes (MtrC and OmcA) produced non-conductive appendages [Gorby et al., 2006]. However, the complete composition and mechanisms of electron transfer in nanowires produced by either *Shewanella* or *Geobacter* remain ill defined. We therefore consider the assumption that electron transport is occurring at sites of biodegradation via nanowires forming at the groundwater surface [e.g. as made by Arora et al., 2007] uncertain. Nonetheless, as discussed in section 2 above, the pilus of Geobacter have been documented to play an important role in not only enhancing biofilm growth but also in forming an electronically conductive network within biofilm enhancing electricity generation [Ruguera et al., 2006; 2007]. The use of nanowires as a potential mechanism for electron transfer at organic rich sites is not entirely impossible and the
contention is not whether the bacteria can produce a network of nanowires linking cells in a biofilm (as Ruguera et al. [2006] have already documented this as discussed in section 2) but rather, the debate and surrounding controversy is over whether such a network of nanowires within biofilm can allow for direct cell-to-cell long range electron transfer at the spatial (e.g., centimeters) scales suggested by the Naudet et al. [2003] model. There remains a large knowledge gap to be bridged between the scale at which electron transport by geopili in metal reducing bacteria has been proven (nm scale) and the scale of the representative elementary volume sampled with a geophysical measurement (cm-m scale). This should be an important area for future research.

Clearly, controlled laboratory experiments are needed to prove that bio-geobattery effects exist in microbial systems and can generate large SP signals as recorded at field sites. Ntarlagiannis et al. [2007] performed column experiments, where scanning electron microscopy (SEM) revealed geopili linking cells-cells and cells to mineral surfaces, leading them to suggest that the column was effectively electrically “hardwired” (Fig. 9). Given the large redox gradient across the column, the essential elements of a geobattery were produced. Interestingly, they recorded large (~0.6 V) electrical potentials on Ag-AgCl metal electrodes straddling the column, which were interpreted as SP signals arising from a bio-geobattery. Perhaps most importantly, this geophysical data was presented as evidence that geopili could facilitate electron transport (hardwiring) at the column scale. However, Ntarlagiannis et al. [2007] used Ag-AgCl metal electrodes that were in contact with the column electrolyte and thus sensitive to the sum of contributions from (1) self potentials associated with any current source in the column (i.e. the biogeobattery), and (2) open circuit galvanic cell (GC) potentials between the electrodes resulting from the tendency of the electrode surface to react with the electrolyte as a result of any
difference in redox state at the two electrodes [Timm and Moller, 2001]. As such GC potentials due to local reactions at the electrode (henceforth termed electrodic potentials) are very sensitive to changes in redox state between the two electrodes and can exceed 0.6 V in the presence of metal reducing organisms [Williams et al., 2007; Slater et al., 2008; Personna et al., 2008]. The results reported by Ntarlagiannis et al. [2007] therefore warrant further studies and validation.

We note that some geophysicists have intentionally explored the use of electrodic potentials for monitoring activity of sulfate reducing microbial communities under anaerobic conditions [Williams et al. 2007; Slater et al., 2008, Personna et al., 2008]. Metallic electrodes in contact with the pore-filling electrolyte are routinely used for electrochemical applications to measure geochemical parameters, such as Eh and pH, as well as specific chemical concentrations (e.g., Br using a bromide specific electrode). A common target compound is sulfide, which can be accurately monitored using Ag-AgS electrodes [Berner, 1963; Whitfield, 1971]. This GC measurement exploits the known reactivity between the target compound and the electrode used, with the resulting electrodic potential usually being measured relative to an electrode with a fixed potential.

Williams et al. [2007] showed how a pair of Ag-AgCl metal electrodes, deployed in a fashion similar to a conventional geophysical survey, records a GC potential when they straddle a gradient in bisulfide (HS⁻) concentration. Oxidation of the Ag-AgCl anode results from a half-cell reaction in the presence of elevated HS⁻ concentrations relative to the cathode, whereas reduction of the AgCl electrode coating occurs at the cathode. The anode effectively becomes an Ag/Ag₂S electrode with the known sensitivity to HS⁻ [Berner, 1963; Whitfield, 1971]. A voltmeter connected between these electrodes will record the open circuit potential caused by the differences in HS⁻ concentration at the local fluid-electrode interface between the two electrode
locations. This open-circuit potential is a measure of the tendency of a spontaneous reaction between these electrodes to proceed (significant current flow does not occur because of the high input impedance of the voltmeter) and it increases with an increase in the HS concentration difference between the two electrodes [Williams et al., 2007]. These electrodic potentials have been used to observe microbial sulfate reduction in saturated synthetic soils following amendment with organic carbon [Williams et al., 2007], in natural wetland soils [Slater et al., 2008], and to provide auxiliary data in complex resistivity studies of FeS biomineralization [Personna et al., 2008]. Although such measurements are diagnostic of microbial sulfate reduction, they only detect redox chemistry at the electrode surface and are thus an electrochemical technique and not a (bio)geophysical technique, for example they can provide no information on microbial activity occurring in the Earth from sensors placed at the surface. It is perhaps inappropriate then to describe such GC measurements as SP measurements. As stated earlier, and explicitly clarified in Slater et al., [2008], the SP geophysical technique is fundamentally concerned with the measurement of potentials resulting from current sources in the Earth that can only be probed using electrodes carefully designed to eliminate electrochemical effects at the surface of the metallic part of the electrode.

From the material presented in this section, it is clear that there is considerable uncertainty, and perhaps confusion, regarding the source of SP signals recorded at sites of active microbial degradation. Certainly, some of the confusion has been driven by a misunderstanding of the basis of the SP measurement and the associated requirements of an SP electrode. The proposed biogeobattery model presents an elegant explanation for microbial-produced SP signals, but suggestions that geopili in biofilms could facilitate the required electron transport [Arora et al., 2007] require the support of more robust datasets. Arora et al. [2007] did also suggest that
precipitation of metallic particles within the vicinity of the water table could provide the electron transport required to complete the bio-geobattery model of Naudet et al. [2004]). We expect ongoing research on biogeophysical signatures of SP measurements to answer the outstanding questions posed here.

6. BIOGEOCHEMICAL (MICROBE-MINERAL) TRANSFORMATIONS

In this section we turn our attention to how the microbial modification of mineral surfaces impacts geophysical measurements. Microbial processes can result in both precipitation of new minerals onto existing mineral surfaces and dissolution of existing mineral surfaces e.g., by organic acids produced as an end result of biodegradation of organic compounds. Here we assess the significance of geophysical measurements conducted during both microbial-induced precipitation and dissolution of mineral surfaces.

Biogeochemical processes resulting in mineral precipitation fundamentally alter the physicochemical properties of the interface and, when microbial induced precipitation is extensive, may also affect the volumetric physical properties of porous media. Table 2 summarizes three of the most well known microbial enzymatic reactions and the associated precipitation of secondary minerals. We consider here the evidence suggesting that these reactions can be non-invasively detected with geophysical methods.

6.1. Microbe-mediated precipitation of metallic iron minerals

We first consider the microbial precipitation of iron minerals. The biological precipitation of these minerals plays a fundamental role in regulating the cycling of iron, sulfur and carbon on the Earth’s surface. In anaerobic environments, iron reducing bacteria and sulfate reducing bacteria can use Fe(III) and sulfate respectively as terminal electron acceptors. Ferrous iron
produced by iron reducing bacteria can promote the precipitation of secondary minerals such as siderite (FeCO₃), magnetite (Fe₃O₄), and goethite (FeOOH) [Fredrickson et al., 1998; Benner et al., 2002; Hansel et al., 2003]. Sulfide produced during microbial sulfate reduction can react with iron(II) produced by iron reducing bacteria to precipitate iron sulfide minerals. Sedimentary sulfide minerals can act as diagenetic cements and represent an important sink for biogenic sulfide. Sulfate reducing bacteria account for ~50% of total carbon mineralization in active marine sediments [Boshker et al., 1998]. In hydrocarbon contaminated groundwater aquifers, the microbial reduction of Fe(III) and sulfate has been linked to the biological precipitation of siderite (FeCO₃), pyrite (FeS₂) mackinawite (FeS) and amorphous iron sulfide (FeS) [Cozzarelli et al., 1999; Prommer et al., 1999]. These microbial induced precipitation mechanisms regulate the cycling of iron, sulfur and carbon may be essential to organic contaminant biodegradation and the remediation of mine wastes using natural/artificial wetlands [e.g., Boshker et al., 1998].

The need for new technologies capable of remotely monitoring bioremediation processes associated with sulfate reducing communities at contaminated sites stimulated a series of experiments to determine the ability of geophysical measurements to track the evolution of metal sulfide biominerals in laboratory columns [Williams et al., 2005; Ntarlagiannis et al., 2005a; Slater et al., 2007, Personna et al., 2008]. Williams et al. [2005] showed that electrical and seismic signatures were generated as a result of microbe-induced ZnS and FeS precipitation within saturated sand-packed columns using the sulfate reducing bacterium Desulfovibrio vulgaris. Observed decreases in seismic wave amplitude throughout the experiment were attributed to the development of differential elastic moduli resulting from the accumulation of metal sulfide-encrusted microbes within the pore space (Fig. 10). The low-frequency complex electrical conductivity data showed a more complicated response, the column becoming
polarizable in the early stages of the experiment but transitioning to conductive in the later stages of the experiment. Williams et al. [2005] attributed the early response to the initial formation of dispersed metal sulfide encrusted biominerals presenting a net high metal-fluid interfacial area. The subsequent loss of polarizability and conduction was attributed to either (1) the formation of biofilm polymers that could conceivably screen metal sulfides from the pore fluids (rendering them unpolarizable), or (2) formation of electrically conductive short circuits along grain surfaces accompanying the ageing of metal precipitates. Ntarlagiannis et al. [2005a] further analyzed the complex conductivity (at a single frequency) results from a series of measurement points along the column and concluded that the temporal behavior of the electrical signals could be explained in terms of (1) the amount of metal-fluid interfacial area, and (2) the amount of electronic conduction resulting from microbial growth, chemotactic movement and final coagulation of biominerals. Interestingly, they found that the polarization peak correlated closely in time with the peak in the microbial activity rates inferred from lactate concentration data, suggesting a common metal surface area effect. Furthermore, the onset of microbial activity was deduced from the onset of polarization observed with the electrical measurements.

Further experiments on FeS biomineralization produced by Desulfovibrio vulgaris under anaerobic conditions, followed by subsequent biomineral dissolution upon return to an aerobic state, have attempted to quantify changes in pore geometry resulting from biomineral formation by modeling the frequency response of complex conductivity data. Slater et al. [2007] describe a column experiment similar to Williams et al. [2005], whereby parameters extracted from Cole-Cole relaxation modeling of the complex conductivity data exhibit behavior conceptually consistent with increases in FeS surface area and pore-size reduction during biomineral growth, and subsequent decreases in FeS surface area and pore expansion accompanying biomineral
dissolution (Fig. 11). This effort was further extended by Personna et al. [2008] who applied an empirical relation, linking the polarization magnitude to specific surface area per unit pore volume ($S_{por}$) of the sand-metal mixtures, to estimate changes in $S_{por}$ driven by FeS precipitation and subsequent dissolution.

The proven sensitivity of complex conductivity measurements to the formation, movement and dissolution of FeS biominerals is perhaps unsurprising given the profound impact that electronically conductive metallic minerals have on the electrical properties of rocks. In fact, the induced polarization and spectral induced polarization geophysical prospecting methods were largely developed for locating buried disseminated FeS deposits. This biogeophysics research has confirmed the existence of measurable complex conductivity signals associated with the formation of sulfide minerals by sulfate reducing bacteria under anaerobic conditions, suggesting that these established geophysical techniques could potentially be adapted to monitoring of microbe-mineral transformations in the Earth. The results of very recent efforts directed towards this goal are described in Section 7.

6.2. Microbe-mediated precipitation of non-metallic minerals

Microbial processes may also result in the precipitation of semi-metallic or non-metallic minerals. One important microbial enzymatic reaction is the microbial precipitation of calcite, due to its ability to sequester metal contaminants and its impact on the hydraulic properties of the subsurface (Table 2). Numerous microorganisms have been shown to induce the precipitation of calcite [Thompson and Ferris, 1990; Pentecost and Spiro, 1990; Ferris et al., 1995; Stocks-Fisher et al., 1999]. Microbial calcite precipitation is mediated by metabolically active bacteria increasing the saturation state and serving as heterogeneous nucleation substrates for mineral
formation [Fortin et al., 1997; Bosak and Newman, 2003]. One example of metabolically induced calcite precipitation is bacterial hydrolysis of urea [Ferris et al., 1996; Ferris et al., 2004]. The production of ammonia and dissolved inorganic carbon during enzymatic decomposition of urea increases pH and alkalinity (Table 2), both of which promotes calcite precipitation. Calcite precipitation induced by ureolytic bacteria has been shown to sequester mobile radionuclide elements such as Sr$^{90}$ [Warren et al., 2001; Fujita et al., 2004; 2008]. Furthermore, calcite precipitation induced by bacteria has been shown to form cements in porous media and affect subsurface fluid flow [Stocks-Fisher et al., 1999; Ferris et al., 1996]. The effect of microbial calcite precipitation on aquifer flow characteristics has been demonstrated by previous hydrologic studies, e.g. the Black Creek aquifer in South Carolina [McMahon et al., 1992]. Microbial oxidation of organic matter produces dissolved inorganic carbon and lead to the accumulation of carbonate and bicarbonate ions along the fluid flow path. Once the groundwater becomes supersaturated with respect to CaCO$_3$ (s), calcite will precipitate to form intergranular calcite cements.

We hence consider here the possible geophysical detection of microbial mediated precipitation of semi-metallic or non-metallic mineral phases. Whereas the electrical properties of conductive mineral phases support detection using electrical geophysics methods, the electrical geophysical detection of non-metallic mineral phases associated with microbial processes is intuitively less certain. Instead of exploiting the conductivity/polarization enhancement associated with the formation of metallic iron minerals we must rely on the intuitively small electrical signals associated with changes in pore volume/pore tortuosity and/or surface area/surface roughness resulting from the precipitation of non-metallic minerals. In contrast, the profound changes in
the elastic properties of soils and rocks (particularly those affecting shear velocities) during precipitate formation as we discuss here.

Microbial-induced precipitation of calcite is currently being explored as a novel strategy for improving the engineering properties (e.g. soil strength, permeability, compressibility) of soils [DeJong et al., 2006]. Here ureolysis is stimulated to form calcite cement that acts to stiffen the soil matrix. DeJong et al. [2006] examined calcite precipitation by the common soil microorganism, Bacillus pasteurii, using SEM to verify the formation of a cemented sand matrix with a concentration of precipitated calcite forming bonds (verified with X-ray compositional mapping) at particle-particle contacts. Shear waves are very well suited to monitoring changes in the particle soil matrix due to precipitation as shear velocity ($V_s$) is largely unaffected by pore fluid composition and directly dependent on void ratio, coordination number (average number of surrounding particles a given particle is in contact with) and confining stress [DeJong et al., 2008]. DeJong et al. [2008] provide a conceptual model of how changes in $V_s$ will detect biomediated calcite precipitation (Fig. 12 a). The process shows a sequence of discrete injections of urealytic microorganisms. During injection $V_s$ remains constant as the pore fluid is replaced. Biological activity then gradually raises the pH to a level where calcite precipitation occurs. The initial microbial treatment resulted in a strong initial increase in $V_s$ believed to be caused by initial binding of the microbes to the soil matrix [DeJong et al., 2006]. The velocity increases until some limiting condition is reached e.g. no additional calcite is available in solution, $V_s$ then remaining constant until a subsequent injection occurs and the process is repeated. Experimental results of this process are shown in Figure 12b, with each vertical arrow indicating an additional discrete injection. Shear wave velocity shows a total 270% increase due to ureolysis and
formation of calcite cement (Fig. 12), suggesting that the measurement of shear wave velocities is an excellent technology for monitoring microbial ureolysis.

As with metallic minerals, studies of the sensitivity of geophysical methods to microbial-induced calcite precipitation have also been driven by the potential for monitoring novel bioremediation strategies. Ureolysis has been recently promoted as an integral component of a novel remediation approach for divalent trace metal and radionuclide contaminants whereby the contaminants are immobilized by co-precipitation in calcite [Fujita et al., 2004; Fujita et al., 2008]. However, recent work to monitor calcite precipitation by ureolytic bacteria using complex resistivity measurements found that the conduction and polarization signals are too small to be reliably indicative of the precipitation process [Fujita and Redden., 2006; Wu et al., 2008]. In both experiments the authors concluded that the amount of calcite precipitation (estimated to be ~ 1% of the pore space in Wu et al., 2008] was not sufficient to present a detectable complex resistivity signature. Clearly, the compelling results from research on using urealytic soil microorganisms to strengthen soils suggest that shear wave velocity measurements may be a viable approach to monitoring ureolysis during bioremediation. As discussed above DeJong et al. [2006] argue that largest changes in $V_s$ accompany stiffening resulting from initial binding of microbes to the soil matrix, suggesting that the relative small volume of precipitate produced in such experiments would not invalidate the technique.

6.3. Magnetic minerals/metal cycling

In addition to mediating the precipitation of different mineral phases as discussed above, microbial activity can also result in the alteration of mineral phases potentially impacting the magnetic properties of the sediments. A study by Carlut et al. [2007] examined the impact of
bacterial activity on magnetic properties of young ocean floor basalts. Fresh basalt samples obtained from young pillow lavas at different locations along the East Pacific Ridge were exposed to the action of the sulfate reducing bacteria *Desulforvibrio desulfuricans* for a total of 355 days during which time the natural remnant magnetization measurements were monitored. The results showed that most magnetized samples lost ~ 30% of their original magnetization. SEM imaging and electron dispersive analysis (EDS) revealed the cycling of iron mineral phases from titanomagnetites which is responsible for the original magnetization of the ocean floor basalts to less magnetic iron sulfide phases. *Carlut et al.* [2007] concluded that microorganisms could play an important role in the rapid loss of magnetization of near surface oceanic crust at ridge axis and could be responsible for the globally observed central magnetic high along ridge axis. The above results suggest that established magnetic geophysical techniques could be used to investigate the activity of bacteria on the deep ocean floor particularly as it relates to the biotransformation of magnetic mineral phases.

7. FIELD MONITORING OPPORTUNITIES

The realization that geophysical measurements are sensitive to microbial processes in the Earth has already encouraged efforts to apply the measurements for monitoring at the field-scale, even though the linkages between biogeochemistry and geophysical signatures, as described earlier, remain incompletely understood. This work has been largely motivated by the pressing need for new technologies for monitoring biogeochemical transformations at contaminated sites experiencing natural or stimulated bioremediation. Geophysical imaging has advanced rapidly in recent years such that it is now possible to generate three dimensional images of time-lapse changes in these properties, at a scale appropriate for observing microbial processes in the near
there is thus great interest in adopting these imaging techniques to monitoring a wide variety of processes in the Earth, such as biogeochemical transformations of interest here.

*Lane et al.* [2007] report one of the earliest studies to apply geophysical imaging to monitor an engineered bioremediation process at the field scale. They injected a vegetable oil emulsion (VOE) to stimulate microbial degradation of hydrocarbons and monitored this process using cross-borehole GPR. They applied petrophysical models to infer changes in VOE saturation, as well as changes in ionic chemistry inferred to be caused by biodegradation of chlorinated solvent contaminants at the field site and/or oxidation-reduction reactions driving mineral dissolution. In a more recent study, *Hubbard et al.* [2008] collected geophysical datasets during a three year Cr (VI) bioremediation experiment. They obtained thirteen seismic and ground penetrating radar datasets representing a modeled 3 m x 5 m image plane and examined the changes in the data to infer qualitative information on the likely spatial distribution of injected electron donor, evolution of gas bubbles, variations in total dissolved solids (nitrate and sulfate), the formation of precipitates and dissolution of calcites and concomitant changes in porosity. Their interpretation was based upon inferences on how three processes associated with the bioremediation (1) pore water replacement by hydrogen release compounds, (2) pore water release be evolved gas bubbles, and (3) changes in solute concentration and/or structure of solid phase, impact seismic velocity/attenuation and EM velocity/attenuation. *Hubbard et al.* [2008] rightly recognized numerous limitations of their study, including the lack of supporting data to verify mechanisms proposed to cause the alterations observed in the geophysical images, the uncertainty in geophysical images caused by non-uniqueness of the inversion and lack of established petrophysical relations linking the geophysics to microbial alteration. Despite these weaknesses, the study demonstrated the potential for such geophysical monitoring to provide information
about biogeochemical transformations resulting from remedial treatments at high spatial resolution and in a minimally invasive manner.

*Williams et al.* [2009] report on a novel SIP study in support of a short-duration (35 day) acetate amendment experiment at DOE's Rifle Integrated Field Research Challenge site designed to promote uranium removal through stimulation of iron reducing bacteria. Figure 13 shows IP data (0.125 Hz) collected as part of this study, showing the development of a distinct phase anomaly focused on the zone of the stimulation. Surface IP data were collected using 30 non-polarizing Cu/CuSO₄ electrodes spaced at 1m intervals using a modified dipole-dipole configuration, with a dipole spacing of 4 m. The data were acquired before and after acetate amendment began (elapsed time indicated) along a surface transect oriented perpendicular to groundwater flow and located 1.5 m down gradient from the region of injection. One explanation for this phase anomaly is the development of polarizable surfaces as a result of biomineral formation as described for laboratory studies in Section 6 [e.g. *Williams et al.*, 2005]. However, *Williams et al.* [2009] propose an alternative explanation whereby accumulation of Fe(II) accompanying biostimulation drives the observed IP signature. Given the role of Fe(II) in facilitating electron transfer processes across fluid-mineral interfaces [e.g. *Wong*, 1979], they infer that increases in Fe(II) may enhance the electrode-polarization mechanism by lowering the charge transfer resistance across (semi)conductive mineral grains. Fe(II) concentrations increased rapidly over the injection interval and remained elevated over the IP monitoring time period (not shown for brevity). Further work is needed to unravel the respective contributions of the increasing interfacial surface area and changing redox chemistry on the IP signature associated with stimulation of iron reducing bacteria.
Outside of bioremediation studies, geophysical measurements have also been successfully applied for monitoring biogenic methane production and emissions from northern peatlands [Comas et al., 2007; 2008]. Free phase biogenic gas (primarily CH$_4$) production in peatlands can drive +15% changes in moisture content, which are readily detectable with surface and borehole GPR measurements [Comas et al., 2005], as well as resistivity imaging [Slater et al., 2007]. The replacement of the pore fluid ($\varepsilon_r \sim 80$) with CH$_4$ ($\varepsilon_r \sim 1$) results in large increases electromagnetic wave velocity in peat, whereas the replacement of an electrolyte with a non-conducting gas phase increases resistivity of the peat soil. Unlike other methods traditionally used to study methane production and emissions from peatlands, GPR can be performed entirely non-invasively with no disturbance of the in situ gas regime. Seasonal GPR monitoring has led to new insights into methane emissions rates from peatlands, and has also contributed to understanding of mechanisms of methane production, transport and release from peatlands [Comas et al., 2008]. Opportunities also clearly exist to adopt geophysical measurements as tools for monitoring the effects of biological activity on soil mechanical behavior [DeJong et al., 2008]. Mitchell and Santamarina [2005] reviewed this evolving subject and noted the need for methods to monitor hydraulic conductivity reduction by bioclogging, the development of bioengineered sediments (as discussed earlier) and bacterial-induced heave of house foundations. However, the application of geophysics to the study of such microbe-mediated mechanical processes is currently in its infancy.

Although field-scale geophysics presents intriguing possibilities for subsurface imaging of microbial processes, significant questions remain regarding the extent to which geophysical signatures can be reliably related to microbial activity. The limited monitoring studies performed to date have recorded geophysical signals that are only indirectly related to the microbial activity.
e.g. due to gas production, changes in solute chemistry or physical alteration of the pore space. The complexity of the field setting, relative to controlled laboratory studies, therefore makes reliable interpretation of microbial processes exceptionally challenging. In essence, we are limited by two primary factors: (1) the changing subsurface conditions causing geophysical signatures observed at the field scale are only proxies of microbial activity, and (2) we are likely measuring the integrated geophysical response to multiple changing subsurface conditions that are occurring simultaneously. However, as Hubbard et al. [2008] note, geophysical monitoring of biogeochemical processes can provide unique qualitative information on the spatial distribution in the variability of microbial activity. A more quantitative interpretation of such changes may be possible as our understanding of the relationship between microbial processes and geophysical signatures improves.

8. CHALLENGES, POTENTIAL FUTURE OPPORTUNITIES AND NOVEL APPLICATIONS

The emerging field of biogeophysics presents unique opportunities for truly interdisciplinary research collaborations between geophysicists, environmental engineers, geochemists, geobiologists and microbiologists. Our understanding of biogeophysical signals will only improve as partnerships between such scientific communities continue to be forged. There is thus a need for early career scientists that are trained in institutions and programs that proactively bridge these scientific disciplines in order to generate communities of multi-disciplinary scientists. In addition to the obvious research opportunities that have been highlighted in preceding sections (e.g. biodegradation research, CH₄ cycling), we identify here opportunities in three areas of exploration where new technologies are needed to accelerate scientific understanding (1) the deep oceans, (2) the cryosphere, and (3) other planets.
8.1. Biogeophysical applications to studies of the deep oceans

The oceans make up 70% of the Earth’s surface and are host to most bacteria and archaea which constitute about one-third of Earth’s total living biomass [Jørgensen, 2008]. Microbial cells have been found in marine sediments and even in the basaltic crust, in some cases extending to depths of at least 15000 m [Jørgensen, 2008]. Unique biogeochemical processes are known to occur at hydrothermal vents associated with mid-ocean ridges (Luther et al., 2001; Reysenback et al., 2000) where, in the absence of sunlight life is supported by geochemical energy provided by oxidation-reduction reactions that are far from equilibrium (Reysenback and Shock, 2002). These represent one of the most extreme environments on Earth as temperatures can reach up to 300-350°C, yet recent discoveries suggest that hydrothermal vents and black smokers represent a hot bed for microbial activity. Another hotspot for microbial activity in the deep ocean is associated with marine gas hydrates, which trap CH$_4$ mostly produced by microbial breakdown of organic matter. Studies of sediments in and/or proximal to gas hydrate zones (often associated with seafloor seeps) have revealed extensive microbial communities, again highlighting the population diversity of the deep subsurface biosphere.

However, the occurrence and spatial distribution of seafloor microbial ecosystems remain poorly understood in part due to the inherent difficulties of obtaining diagnostic data from these extreme ocean environments that represent the most ancient, continuously habited ecosystems on Earth. The vastness of the ocean floor, the remoteness of the deep ocean basins, the high cost of exploration and the inherent difficulties of obtaining uncompromised samples from these extreme ocean environments are all major obstacles that have inhibited our understanding of this deep subsurface marine biosphere. In the same way that spatially continuous magnetic geophysical data was pivotal to proving sea floor spreading at mid-ocean ridges, thus validating
plate tectonic theory [Vine and Matthews, 1963], geophysical data could assist in unlocking the biogeochemistry of deep ocean ecosystems where rocks and warm water are transformed to habitats.

Although few geophysical investigations have been targeted towards deep subsurface biosphere studies, recent work supports our argument that geophysical measurements could contribute to scientific knowledge in this field. Studies documenting microbial reduction of natural remanent magnetization of mid ocean ridge basalts [Carlut et al., 2007, see section 6] provide one example of how magnetic geophysical methods can be used to assess ocean crust alteration due to microbial mediated processes. In addition, the electrodic potential techniques described in Section 4 have been employed since the 1960’s to explore sulfide chemistry associated with microbial activity in marine sediments [e.g. Berner, 1963]. More complex reactive electrode based techniques, such as voltammetry, have been developed to determine chemical speciation associated with sulfide redox chemistry and have provided fundamental data helping to determine microbial-driven redox chemistry of hydrothermal vents [Luther et al. 2001]. Finally, we note that such electrodic reactions are also the basis of the benthic microbial fuel cell [e.g. Finkelstein et al. 2006] whereby electrical power can be generated in marine sediments by connecting an anode buried in the anaerobic subsurface to a cathode in the overlying oxic water. The strong redox gradient driving the microbial fuel cell in these shallow sediments could also drive large self potential signals if microbial biofilms and/or biomineralization drives electron transfer needed to form a bio-geobattery (see Section 4). This presents exciting research opportunities for biogeophysics.

8.2 Biogeophysical applications to cryosphere studies: looking for microbial hot zones in ice
Subglacial environments remain one of the least explored frontiers on Earth [Christner et al., 2008]. The discovery of liquid water at the base of polar ice sheets more than three decades ago [Ueda and Garfield, 1970] raised the interesting possibility of the existence of life under such extreme cold conditions. Most interesting is the discovery of more than 140 subglacial lakes in Antarctica [Siegert et al., 2005] with subglacial Lake Vostok being the largest [Christner et al., 2008]. Investigations into the geomicrobiology of glaciated environments have identified bacterial populations under temperate valley glaciers [Sharp et al., 1999; Foght et al., 2004; Skidmore et al., 2005], an Arctic polythermal glacier [Skidmore et al., 2000], subglacial lakes [Karl et al., 1999; Priscu et al., 1999; Christner et al., 2001, 2006; Gaidos et al., 2004], and in subglacial discharge from Taylor Glacier, Antarctica [Mikucki and Priscu 2007]. The presence of viable microbial communities at the base of and liquid water beneath polar ice sheets has direct implications for the existence of life on Mars or other icy terrestrial bodies (e.g., Europa and Enceladus). Molecular and biogeochemical analysis of accretion ice and glacial ice collected from above Lake Vostok has revealed the presence of microorganisms from numerous taxa, which implies that the lake may contain a complex microbial community [Priscu et al. 1999, Karl et al., 1999, Christner et al., 2001, 2006; D’Elia et al., 2008]. The discoveries of microbial life in glacier ice have opened new avenues for biological, chemical, and physical based research of the cryosphere. However, the exploration and direct sampling of the base of ice sheets, where liquid water is present, in order to understand the biogeochemistry of these environments poses some significant technological challenges. These challenges raise the possibility of the use geophysical techniques to detect biological signatures in ice.

The attractiveness of geophysical studies again lies in the non-invasive nature of these techniques. Although geophysical techniques are being used in polar environments, most of the
applications have been focused on basal topography, hydrologic conditions at the glacier bed, and studies of ice thickness [e.g., Sharp et al., 1993, Pattyn et al., 2003, Hubbard et al., 2004]. We are not aware of any biogeophysical studies that have been conducted specifically to address issues related to geomicrobiology in subglacial or englacial environments. However, enormous potential exists for the application of geophysical techniques in the search for discrete signatures of in situ microbial activity in subglacial ice systems. For example, bacterial heterotrophic respiration may be an important contributor to excess CO$_2$ concentrations measured in sediment-rich basal ice from a polythermal glacier in Antarctica [Montross et al., unpublished data]. As discussed in section 2, 4 and 7, biogenic gas production can have a significant impact on both seismic and electrical properties. In this scenario, biogeophysical techniques may be employed to directly detect the presence of anomalous gas concentrations. Other potential microbial signatures that may be detected using biogeophysical techniques include the presence of biofilms, or liquid water content, which can then be used to infer microbial activity and direct further studies within these basal ice systems. Such biogeophysical signatures could be exploited to guide high resolution microbial and geochemical sampling of these microbial “hot” zones.

8.3. Biogeophysical applications to the search for extraterrestrial life

We are increasingly probing the more remote and inaccessible parts of our planet, as well as our neighboring planets, in a desire to improve understanding of the origins and functioning of Earth, and whether life could exist beyond our own world. The question of whether or not life exists on Mars has been spurred by recent studies of the Martian meteorite Allan Hills 84001 (ALH84001) which suggested that microbial life existed on Mars 4 billion years ago [McKay et al., 1996]. This conclusion was based on the fact that ALH84001 contained abundant polycyclic aromatic hydrocarbons (PAHs), carbonate globules containing fine-grained, secondary phases of single-
domain magnetite (Fe₃O₄) crystals (tens-of-nanometer-size) and iron sulfides with morphological and chemical properties identical to the single-domain magnetite (magnetosomes) crystals produced by magnetotactic bacteria found on Earth. Since it is known that the magnetosomes produced by magnetotactic bacteria (see Section 3 above) are uniquely biogenic and cannot be produced abiotically, the presence of the single domain magnetite provides strong evidence to suggest that the Martian magnetic magnetites represent magnetofossils and therefore evidence for the oldest life forms known [Thomas-Keprta et al., 2002].

Current techniques that have been deployed on Mars to look for evidence of life are invasive. There is potential therefore for the development of non-invasive biogeophysical sensors that can be deployed on future Martian rovers to search for life. Based on this review, and evidence provided by ALH84001, it seems likely that dielectric and magnetic susceptibility measurements could be exploited in the search for life on Mars. However, a priori information on the geophysical properties of Martian soils may first be needed in order to distinguish unique biosignatures associated with microbial life. Geophysical measurements can detect changes in physical properties due to microbial activity when baseline measurements are available. Such information would not be immediately available for biogeophysical exploration on Mars.

Biogeophysical research focused on the search for life on other planets must then rely on geophysical signals that are uniquely biological. Researchers at the University of Houston (UH) are currently exploring whether unique biological signatures can be extracted from dielectric spectroscopy measurements. As discussed in Section 3, the dielectric response of cellular suspensions suggests that the α-dispersion (observed at the kHz frequency) is unique to living organisms and is correlated with the cellular membrane potential, whereas the β-dispersion observed at higher frequencies (MHz frequencies) results from interfacial polarization mainly
attributed to the plasma membrane surrounding the cell [Prodan et al., 2004]. UH researchers have also discovered a resonant-like behavior in the harmonic responses of live cells that could be exploited as a biomarker. Hence preliminary evidence suggests that dielectric spectroscopy may represent a technique that can be used to search for life on other planets. At present it is not known if there are magnetic signals unique to biogenic magnetite. Such knowledge would be important for the characterization of Martian soils for evidence of life using magnetic methods.

8.4. Limitations and challenges and future research directions

Biogeophysical techniques have the potential to generate datasets with some unique advantages not shared by conventional methods for probing microbiology and geobiology. Strengths of the methods include the possibility of deployment in a non-invasive manner, whereby measurements are made using sensors placed at boundaries of bodies (e.g. the Earth surface, or an experimental tank) from which the distribution of a microbial process within the body can be reconstructed. Furthermore, the support volume of the measurement can be somewhat controlled by the location of the sensors. This is mostly the case for methods that use a controlled source (e.g. resistivity imaging, ground penetrating radar) where the support volume can be increased (at some expense of measurement resolution) by increasing the separation of transmitters and sensors. However, definition of the exact support volume of a geophysical measurement remains a challenging area of geophysical research as it is often inherently controlled by the unknown spatial distribution of the physical properties controlling the measurement. Spatially and temporally rich geophysical datasets are become routine given recent developments of automated, multi-channel instrumentation. From an economics perspective, biogeophysical measurements tend to be relatively inexpensive when compared with methods based on sampling and analytical measurements. However, all the methods rely on detecting a signal that is only a proxy of
microbiological activity. As these signals can arise from numerous other sources, the interpretation of biogeophysical datasets is inherently uncertain, highlighting the primary weakness of the methods.

Further research is needed to improve our understanding of the cause and effect relationships between microbial activity and geophysical signatures. New laboratory studies under controlled conditions are required to address some of the uncertainties in the source mechanisms generating geophysical signals in the presence of microbial activity e.g. the generation of self potentials. To date, the majority of published biogeophysics studies are observation-based, presenting only empirical relations between microbial and geophysical variables. Very little progress has been made towards developing theoretical and/or numerical models for prediction of geophysical signals arising from microbial activity (those that have apply to simple systems such as cellular suspensions e.g. Prodan et al., [2008]). The development of modeling frameworks is clearly needed to advance biogeophysics research, although microbial activity within natural soils and rocks may ultimately prove to be a system too complex to model beyond empirically-based approaches. Despite, these challenges, it is obvious that the opportunities to investigate microbial processes in the Earth are only just being realized. We suggest that continued growth of the emerging biogeophysics discipline will lead to many exciting new discoveries in the biogeosciences.

9. SUMMARY AND CONCLUSIONS

Only a decade ago it seemed inconceivable to suggest that microbial processes could potentially impact geophysical signatures. The role of bacterial activity in driving geophysical signatures was largely overlooked. Since that time, this perception has changed profoundly, in large part due to the early work on contaminated sites undergoing biodegradation. We have reviewed
evidence provided by numerous geophysical investigations that point to the links/relationships between biological interactions with geologic media and potential changes in geophysical properties in near subsurface environments. We end this review by summarizing some of the major research findings, identifying challenges that remain and speculating on novel applications of biogeophysical studies for the future.

Microbial cells exhibit distinct electrical properties and certain (magnetotactic) bacteria also display unique magnetic characteristics suggesting that electrical and magnetic methods can be used to investigate the activity of bacteria. Dielectric spectroscopy is a particularly promising technique for studying the properties of cells. The recognition that $\alpha$-dispersion (observed at the kHz frequency) is unique to living organisms, combined with the fact that magnetosomes found in the Martian Meteorite Allan Hills 84001 are uniquely biogenic (and cannot be produced abiotically) opens up opportunities for application of magnetic and dielectric spectroscopy techniques for the search for life on other planets. Other recent work highlights exciting opportunities to use geophysical data for quantifying rates of biofilm growth/development and decay. Potential applications of this knowledge are in the areas of microbial enhanced oil recovery, bioremediation, validation of bioclogging models, assessment of biobaroriers for CO$_2$ sequestration etc.

Other recent efforts have conclusively shown how geophysical techniques can be used to assess and improve understanding of metal and nutrient cycling driven by aerobic and anaerobic (e.g. iron and sulfate reducing) microbe-mineral transformations, as well as assessing hydrological and biotransformations at remediation sites. Other studies have demonstrated the utility of geophysical studies to assess biodegradation in the field and in microbial ecology studies. The latter studies indicate that electrical properties observed at hydrocarbon contaminated sites might
An unresolved issue in biogeophysics is the role of microbial nanowires in generating electrical potentials. Could a network of such nanowires within biofilms permit for long range electron transfer at the spatial (e.g., centimeters) scales necessary to generate measurable electrical signals in laboratory columns, or even at the field scale? While this idea provides an intriguing and exciting possibility for monitoring microbial processes from large SP signals associated with bio-geobattery effects, there remains a large knowledge gap to be bridged between the scale at which electron transport by bacteria nanowires has been proven to occur (\(\mu\)m scale) and the scale at which geophysical measurements are made (cm-m scale). This should be an important area for future research.

Finally, we reviewed potential novel applications of biogeophysics to aid in the search for life in extreme environments such as the deep biosphere. Studies on ocean floor magnetization highlight the potential use of magnetic techniques to assess microbial alteration of oceanic crust, while SP techniques may be important for understanding biogeochemical transformations at hydrothermal vents. We also point to potential applications of biogeophysical studies to detect microbial active zones (through detection of zones of anomalously high biogenic gas production) in subglacial or englacial environments.

Despite the obvious potential of biogeophysics in studies of geomicrobiology processes, our understanding of the effect of microbial processes on geophysical properties is still limited. Most studies to date have been observational with little quantification. All the methods have relied on detecting a signal that is only a proxy of microbiological activity. Because these signals are often
coupled with other processes, the interpretation of biogeophysical datasets is inherently uncertain, highlighting the primary weakness of the methods. Further research is needed to improve our understanding of the cause and effect relation between microbial activity and geophysical signatures and more work is required towards development of numerical models to better describe microbial-geophysics relationships.

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GLOSSARY OF GEOPHYSICAL TERMS

Geophysical Properties

Bulk conductivity: electrical conductivity of a porous medium accounting for conductivity via the electrolyte within interconnected pores and assuming a resistive mineral phase. Often modeled with the Archie (1942) relation, \( \sigma_{\text{bulk}} = (1/F) \sigma_w = \sigma_w \phi^m \), where \( \phi \) is the porosity, \( m \) is the Archie cementation factor and \( \sigma_w \) is the electrolyte conductivity (defined below).

Compressional wave: an elastic wave in which in which the motion of the medium is in the same direction as the wave propagation.

Dielectric constant: see the relative dielectric permittivity (see below).

Electrical conductivity (\( \sigma \)): the ability of the Earth to conduct electric current (SI units are Siemens per meter). The reciprocal of electrical resistivity (\( \sigma = 1/\rho \)).

Electrical resistivity (\( \rho \)): how strongly the Earth opposes the flow of electric current (SI units are Ohm meters). The reciprocal of electrical conductivity (\( \rho = 1/\sigma \)).

Electrolytic conductivity (\( \sigma_w \)): the ability of the pore-filling electrolyte of an Earth material to conduct electric current. Linearly related to the total dissolved solids (TDS) over a limited range.

Formation factor (\( F \)): quantifies the reduction in electrical conductivity due to the addition of an insulating phase (soil/rock) to an electrolyte: \( F = \sigma_w / \sigma_{\text{bulk}} \)

Imaginary conductivity (\( \sigma'' \)): out of phase charge transport here associated with electrochemical charge storage (polarization) at interfaces.
Magnetic susceptibility: the degree of magnetization of a material in response to an applied electric field.

Normalized chargeability ($m_n$): a parameter computed from induced polarization measurements that is proportional to the imaginary conductivity ($\sigma''$)

Phase angle ($\phi$): $\phi = \tan^{-1}(\omega/\sigma') \approx (\sigma''/\sigma')$ ($\phi < 100$ mrad). Ratio of charge storage to ohmic conduction.

Real conductivity ($\sigma'$): in phase charge transport associated with ohmic loss (conduction).

Relative dielectric permittivity ($\varepsilon_r$): ratio of the amount of stored electrical energy in the Earth when a potential is applied, relative to the permittivity of a vacuum; $\varepsilon_r = \varepsilon/\varepsilon_0$ where $\varepsilon$ is the permittivity of the material and $\varepsilon_0$ is permittivity of free space ($8.854 \times 10^{-12}$ F/m).

Remanent Magnetization: the residual or permanent magnetization left in a material after the magnetizing field is removed.

Self potential (SP): a voltage associated with a gradient in an electric field generated by a current source in the Earth.

Seismic velocity: the speed of propagation of an elastic wave through the Earth ($V_p = $ compressional wave velocity; $V_s = $ shear wave velocity)

Surface conductivity: the ability of the electrical double layer forming at mineral-fluid (or microbe-fluid) interfaces to conduct electric current.
Time constant ($\tau$): A parameter of phenomenological models of spectral induced polarization (SIP) measurements that quantifies the dominant relaxation time, being inversely related to the square of the dominant relaxation length scale.

**Geophysical Methods**

Acoustic technique: laboratory technique to measure velocity and attenuation of an elastic wave across a sample.

Dielectric spectroscopy: laboratory technique to measure the frequency-dependent dielectric properties of an Earth material (typically kHz-MHz).

Electrical resistivity (ER): a four electrode technique used to measure the bulk conductivity ($\sigma_{bulk}$) of a soil by injecting current between one pair of electrodes and measuring voltage on a second pair of electrodes.

Geoelectrical method: generic term to describe any subsurface exploration method based on electrical geophysics.

Ground penetrating radar (GPR): a high-frequency electromagnetic (EM) method whereby the transmission and reflection (from interfaces of $\varepsilon_r$) of EM waves is recorded.

Induced polarization (IP): extension of the electrical resistivity method (ER) whereby the transient voltage due to current storage in the Earth is also measured.

Magnetic method: records the spatial variation in the Earth's magnetic field due to bodies of varying magnetization.
Seismic technique: geophysical method based on measuring speed of propagation of an elastic wave through the Earth. Common operating modes in the near surface include seismic reflection and seismic refraction.

Spectral Induced Polarization (SIP): extension of the IP method whereby the frequency dependence of the current storage is recorded by measuring the phase shift ($\phi$) between current and voltage waveforms over a range of frequencies (typically mHz-kHz).
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61


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TABLE CAPTIONS

Table 1. List of six geophysical methods that have been adopted for biogeophysics research. The focus of this review is the biogeophysical relevance of these methods listed in the fourth column. Only those applications with proven potential, as demonstrated in the peer-reviewed literature, are included in this table.

Table 2. Microbial enzymatic reactions and the precipitation of secondary minerals (table credit, Nathan Yee, Rutgers University)

FIGURE CAPTIONS

Figure 1: Biogeophysics flow chart illustrating the perceived relationships between microbiological/biogeochemical processes and geophysical responses [modified from Atekwana et al., 2006].

Figure 2: ESEM images of (a) Cells of P. aeruginosa attached to a sand grain during a microbial growth experiment and (b) Control (Courtesy of C. Davis Missouri University of Science and Technology).

Figure 3: The effect of (a) cell membrane potential, and (b) cell membrane thickness on dielectric spectroscopy data based on the analytical model of Prodan et al. [2008]. The height of the $\alpha$ plateau is determined by the membrane potential whereas the height of the $\beta$ plateau determines the thickness of the cell membrane. In both cases, all other parameters of the model are constant. (c) Measured dielectric permittivity vs frequency ($\varepsilon_r$) for E. coli for three values of
the membrane potential, showing fit to the Prodan et al. [2008] model [modified from Bot and Prodan, 2008].

Figure 4: Polarization signal (a) associated with density of cells attached to mineral surface (b) as observed by Davis et al. [2006]. Filled red circles are biostimulated column, other symbols are control columns. Also shown are ESEM images from (c) biostimulated column showing cell attachment/biofilms and (d) unstimulated column.

Figure 5: Two dimensional seismic scan image from a biofilm growth experiment at selected times over a 29 day experiment (i) biostimulated sample; (ii) control sample; (iii) environmental scanning electron microscope (ESEM) images from sample locations shown in (i) and (ii) [seismic scan images Courtesy L. Pyrak-Nolte, Purdue University].

Figure 6: (a) GPR profile across a hydrocarbon contaminated site showing a zone of attenuated reflections coincident with the plume, (b) electrical resistivity across the plume (red = high resistivity, blue = low resistivity), (c) calcium ion concentrations from the contaminated aquifer, (d) SEM image showing etching and pitting of the mineral grains (500x magnification).

Figure 7. The vertical LNAPL distribution at a contaminated field site along with the corresponding bulk electrical conductivity measurements. The microbial community structure for samples taken from within the vadose, residual LNAPL, free phase LNAPL, and water saturated zones as determined by 16S rRNA gene sequencing show significant differences [Adapted from Allen et al., 2007]

Figure 8: (a) The Sato and Mooney [1960] geobattery model whereby the redox gradient is attributed to the gradient of dissolved oxygen in groundwater and the ore body serves as the electron conductor (from Castermant et al., [2008]) (b) the bio-geobattery model proposed by
Naudet et al. [2004] whereby the redox gradient is attributed to the gradient in dissolved Fe$^{2+}$ resulting from oxidation of organic matter and biofilms at the water table serve as the electron conductor.

Figure 9: SEM images revealing geopili linking cells-cells and cells to mineral surfaces, suggesting electrical ‘‘hardwiring’’ of a soil column as postulated by Ntarlagiannis et al. [2007].

Figure 10: Experimental evidence of attenuation of seismic waves as a result of biomineral formation from Williams et al. [2005] (a) baseline waveform prior to biomineralization (b) waveform after 20 days showing attenuation towards bottom of column in zone of observed biomineralization (c) time variations in wavepulse at 2 cm from bottom of column

Figure 11: (a) Polarization spectra as a function of time during FeS biomineralization induced by an anaerobic transition (b) This biomineralization response is largely reversible during subsequent dissolution (aerobics transition) as apparent from the behavior of modeled Cole-Cole parameters ($m_n$ and $\tau$) (c) SEM images for samples extracted from the column on termination of experiments showing tube-like, elongate D. vulgaris biominerals (upper image) and quartz sand particle encrusted with biomineralization (lower image).

Figure 12: Schematic (a) and experimental data (b) exemplifying the stages of discrete bioaugmentation treatment injections into sand and the associated changes in shear wave velocity ($V_s$) [from DeJong et al., 2008].

Figure 13: Phase ($\phi$) inversion results for surface IP data (0.125Hz) acquired in support of a short-duration (35 day) acetate amendment experiment at DOE's Rifle Integrated Field Research Challenge site designed to promote uranium removal through stimulation of iron reducing
bacteria (see Williams et al., 2009, for experimental details). Figure credit: Ken Williams, Lawrence Berkeley National Laboratory.
<table>
<thead>
<tr>
<th>Geophysical Method</th>
<th>Measured Properties</th>
<th>Measured Parameters</th>
<th>Biogeophysical Applications/Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrical Resistivity</strong></td>
<td>electrical potential generated by current injection</td>
<td>DC conductivity ($\sigma_{DC}$)</td>
<td>Biodegradation, Biofilms?</td>
</tr>
<tr>
<td><strong>Ground Penetrating Radar (GPR)</strong></td>
<td>amplitude and arrival time of electromagnetic energy (1MHz-1GHz)</td>
<td>dielectric constant ($\epsilon_r$); DC conductivity ($\sigma_{DC}$)</td>
<td>Biogenic gasses; Biodegradation</td>
</tr>
<tr>
<td><strong>Induced Polarization/Spectral</strong></td>
<td>decay in electrical potential following current pulse; frequency dependence of complex impedance (mHz-kHz)</td>
<td>magnitude and relaxation length scale of dispersion caused by interfacial polarization</td>
<td>Biominalization; Cell properties (membrane potential); Biofilms</td>
</tr>
<tr>
<td><strong>Diesel Spectroscopy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Magnetometry</strong></td>
<td>magnitude and/or gradient of Earth’s magnetic field</td>
<td>magnetic susceptibility</td>
<td>Magnetotatic bacteria, Biomineralization?</td>
</tr>
<tr>
<td><strong>Seismic</strong></td>
<td>amplitude and arrival time of elastic energy</td>
<td>elastic wave velocities ($V_p$, $V_s$) and attenuation</td>
<td>Biomineralization; Bio-engineered soils; Biogenic gasses</td>
</tr>
<tr>
<td><strong>Spontaneous Potential</strong></td>
<td>naturally-occurring electrical potential, i.e. self-potential</td>
<td>electrochemical potential, redox potential, streaming potential</td>
<td>Bio-geobatteries; Biodegradation</td>
</tr>
</tbody>
</table>
1. Ureolytic bacteria (e.g. Bacillus pasteurii)
   
a) \( \text{NH}_2\text{CONH}_2 + 2 \text{H}_2\text{O} \leftrightarrow 2 \text{NH}_4^+ + \text{CO}_3^{2-} \)
   
b) \( \text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3(s) \)

2. Sulfate reducing bacteria (e.g. Desulfovibrio desulfuricans)
   
a) \( 2 \text{CH}_3\text{CHOHCOO}^- + \text{SO}_4^{2-} + 2 \text{H}^+ \leftrightarrow 2 \text{CH}_3\text{COO}^- + 2 \text{CO}_2 + 2 \text{H}_2\text{O} + \text{HS}^- \)
   
b) \( \text{Fe}^{2+} + \text{HS}^- \leftrightarrow \text{FeS}(s) + \text{H}^+ \)

3. Iron(III) reducing bacteria (e.g. Shewanella putrefaciens)
   
a) \( \text{CH}_3\text{CHOHCOO}^- + 4\text{Fe(OH)}_3 + 7\text{H}^+ \leftrightarrow 4 \text{Fe}^{2+} + \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 10\text{H}_2\text{O} \)
   
b) \( \text{Fe}^{2+} + \text{HCO}_3^- \leftrightarrow \text{FeCO}_3(s) + \text{H}^+ \)
   
c) \( 2 \text{Fe(OH)}_3 + \text{Fe}^{2+} \leftrightarrow \text{Fe}_3\text{O}_4 + 2\text{H}_2\text{O} + 2\text{H}^+ \)
   
d) \( \text{Fe(OH)}_3(s) \xrightarrow{\text{Fe(III)}} \text{FeOOH}(s) + \text{H}_2\text{O} \)
Microbes + Carbon Source + Nutrients → Microbial Growth and Proliferation

- Biofilm Formation
- Chemical Changes
  - Redox Reactions – Eh and pH changes
  - Metabolic Byproducts
    - Organic Acids and Biosurfactants
  - Biogenic Gas Production
    - CO₂, H₂S
  - Mineral Dissolution
  - Mineral Precipitation
- Physical Changes
- Chemical Changes
- Increasing Cell Population

Changes:
- Direct Action of Biofilms or Pili Serving as Channels for Electron Transport
- Changes:
  - Porosity, Pore Size and Shape, Tortuosity, Surface Roughness, Surface Area, Cementation, Formation Factor, Elastic Moduli, Shear Strength, Stiffness
- Changes:
  - Ionic Strength, Ionic Charge Density, Ionic Mobility, Mineral Phases, Electrical Double Layer, Cation Exchange Capacity
- Changes:
  - Membrane Potential, Membrane Thickness, Cell Concentration

Changes in Petrophysical Properties

Geophysical Response
- Electrical Resistivity
- Induced Polarization
- Spontaneous Potential
- Dielectric Spectroscopy
- Seismic
- Magnetics