Electrical Geophysical Characterization of Biogeochemical Processes

by

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ABSTRACT OF THE DISSERTATION

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Dissertation Director:

Professor Lee Slater

Biogeochemical processes alter the physical and chemical properties in the subsurface, and efficient monitoring of these changes is important for successful implementation of soil and groundwater remediation. Electrical geophysical methods offer spatially extensive and densely sampled information on variations in the physiochemical properties of subsurface at various scales. The focus of this dissertation is the development of electrical geophysical monitoring approaches sensitive to microbial sulfate reduction in porous media, sulfate-reducing bacteria activity, and mineral precipitation in porous media. This research suggests the feasibility of using electrical geophysical techniques in monitoring environmental contamination remediation.

In the first research topic, electrodic potential (EP), in conjunction with spectral induced polarization (SIP) and self-potential (SP) measurements were used for monitoring microbial sulfate reduction in a silica beads column saturated with natural river water. Significant EP signals were recorded and correlated with HS− near multiple sensing electrodes, no significant SP signals were observed. The SIP responses were linked with
overall microbial activity in the column. Joint use of multiple electrical geophysical methods can be applied to capture the spatiotemporal variability in microbial sulfate reduction in a porous medium.

The second research topic examined the sensitivity of dielectric spectroscopy to sulfate-reducing bacteria (*Desulfovibrio vulgaris*) growth in cellular suspensions. Low frequency dielectric properties of *D. vulgaris* were studied using a high quality two-electrode system over frequencies 20 Hz to 1 MHz. The dielectric permittivity, real and imaginary parts of complex conductivity increased for higher concentrations of bacteria at frequencies <10 kHz. Reduced permittivity and conductivity were found as bacteria grew from earlier growth stage to later growth stage.

The third research topic investigated the evolution of urea mediated calcium carbonate mineral precipitation and ion adsorption using SIP in silica gel column. Both real and imaginary parts of complex conductivity increased with rising hydroxide ion concentration during urea hydrolysis. Distinct decreases in complex conductivity were observed during calcium carbonate precipitation. Significant dependence of imaginary conductivity on pH in silica gel was found in a separate study. The results suggest the control of pH and calcite precipitation on the polarization response in silica gel in.
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Chapter 1. Introduction

1.1 Background and overview

The problem of soil and groundwater contamination by toxic and/or radioactive metals has promoted significant research into the exploitation of microorganism mediated and specific chemical stimulated remediation processes through the reduction and/or sequestration of metals [Rittle et al., 1995; Fujita et al., 2000; Valls and de Lorenzo, 2002; Fujita et al., 2008; N'Guessan et al., 2008]. In situ active mitigation approaches for metals often involves injection of solutions containing chemical or biological agents into the subsurface. The agents are chosen to produce soluble or solid phase reaction products that alter the state or mobility of contaminants. The consequent changes in the physicochemical properties of subsurface are often distributed in the form of transient, migrating chemical or biogeochemical reaction fronts.

The capability to monitor the spatial and temporal distribution of such reaction fronts, and associated chemical or physical changes, is important for successful implementation of such remediation approaches in an engineered application. However, conventional, ‘point’ sampling methods applied at boundaries and selected points (e.g., injection and monitoring wells) are labor and cost intensive, and often have poor resolution at relatively large spatial scale. In addition, the limitations of using conventional measurements are magnified when using such methods for long-term monitoring of the dynamic subsurface processes, which is essential for assessing the sustainability of the treatment. Improved monitoring techniques are necessary that are (1) sensitive to changes
in biogeochemical properties associated with remediation treatments, and (2) can provide high-resolution information about surface properties in a minimally invasive manner.

Near surface geophysical methods represent such a monitoring approach, having proven extremely useful for characterizing hydrological and biogeochemical transformations involving \textit{in situ} bioremediation in subsurface environments \cite{Rubin and Hubbard, 2005; Atekwana and Slater, 2009}. Recent applications of geophysical methods in sensing biogeochemical changes at the solid-fluid and fluid-fluid interfaces in the porous media have gained increasing interests. Among such geophysical techniques, electrical geophysical techniques including electrical resistivity (ER), induced polarization (IP), spectral induced polarization (SIP) or dielectric spectroscopy, and self-potential (SP), as well as reactive electrode (electrodic potential (EP)) measurements have been successfully used in the past and have shown promise for delineating various subsurface conditions in both abiotic and biotic processes \cite{Pellerin, 2002; Ntarlagiannis et al., 2005; Chambers et al., 2006; Slater et al., 2006; Slater, 2007; K. H. Williams et al., 2007; Hamilton and Hattori, 2008; Hubbard et al., 2008; Personna et al., 2008; Atekwana and Slater, 2009; Wu et al., 2010; Zhang et al., 2010]. In particular, recent biogeophysics research have repeatedly shown geophysical signatures to be sensitive to subsurface microbial processes and microbial-induced alterations to geologic materials (see \cite{Atekwana and Slater, 2009} for a review). The frontiers in electrical geophysics research could potentially open the door to track the evolution and position of physical, chemical, and biological reaction fronts in the subsurface.
1.2 Objectives and Dissertation Outline

This thesis aims to provide new information on the electrical geophysical responses of biological and geochemical processes in porous media. To accomplish this goal, a series of laboratory-controlled experiments were performed and three research topics were studied. The three topics will be presented demonstrating the sensitivity of electrical geophysical methods to microbial behavior and subsurface abiotic and/or biostimulated geochemical variations. The objectives of three topics are:

Topic 1. Investigate microbial sulfate reduction processes in porous media.
   a. Capture the spatiotemporal changes in sulfate reduction processes stimulated by sulfate-reducing bacteria (SRB) within natural river water using simultaneous collection of EP, SP, and SIP measurements.
   b. Demonstrate the contributions of SRBs to the electrical geophysical signatures in porous media.
   c. Compare EP measurements with fundamentally different SP measurements.

Topic 2. Examine low frequency complex dielectric properties of SRB suspensions using dielectric spectroscopy.
   a. Obtain direct measurements of high-resolution and accurate dielectric responses of SRB suspensions using two-electrode dielectric spectroscopy over a relatively broader range of frequencies.
   b. Identify variability in SRB bacterial concentrations from dielectric dispersion curves.
   c. Identify variability in SRB metabolism stages from dielectric dispersion curves.
Topic 3. Observe spatiotemporal variability in physiochemical properties of reaction fronts associated with mineral precipitation in porous media.

   a. Test the ability of SIP to monitor evolution of calcite precipitation driven by urea hydrolysis in silica gel column.

   b. Evaluate correlations between local changes in complex conductivity and fluid chemical changes associated with calcite precipitation.

   c. Understand hydroxide adsorption effects on SIP signals in different porous media.

This research presented in this thesis specifically addresses the sensitivity of three geophysical methods, SIP, SP, and EP to processes associated with microbial sulfate reduction environment and mineral precipitation. Chapter 2 introduced the basics of SP, EP, and SIP.

The first research topic was described in Chapter 3. In this research, EP measurements, in conjunction with SP and SIP, have been shown to monitor microbial sulfate reduction occurring in a column of silica beads saturated with natural river water. A dual electrode design (Ag/AgCl metal as sensing/EP electrode, Ag/AgCl metal in KCl gel as reference/SP electrode) was employed to constrain EP interpretation of bisulfide (HS\(^-\)) concentration, as well as to isolate biochemical reactions to a sensing/EP electrode. Significant EP signals (>550 mV) were recorded and correlated with HS\(^-\) near multiple sensing electrodes. The SIP measurements captured imaginary conductivity signals responding to microbial growth and biomass formation in the column.
Chapter 4 (the second research topic) reports a follow-up investigation exploring the direct contribution of SRBs to electrical properties of a porous medium. The complex dielectric properties of *Desulfovibrio vulgaris* (a model SRB) in cellular suspensions were studied from 20 Hz to 1 MHz. Precise dielectric spectroscopy measurements distinguished intrinsic macroscopic dielectric function of bacteria with different concentrations and at varying life stages. Higher dielectric permittivity, real and imaginary parts of complex conductivity were observed for higher concentrations of bacteria at frequencies lower than 10 kHz. However, reduced permittivity and conductivity were found as bacteria grew from earlier growth stage to later growth stage.

The final topic presented in Chapter 5 describing SIP tracking of reaction fronts in mineral precipitation and ion adsorption in silica gel column. The calcite precipitation was driven by urea hydrolysis that catalyzed by extracellular urease enzyme immobilized in a defined region within a matrix of high surface area and highly porous silica gels. Correlations were found between real and imaginary parts of complex conductivity and increased hydroxide ion concentration during urea hydrolysis. Additionally, decreased complex conductivity was observed as calcite precipitation occurred. The spatiotemporal variations in complex conductivity suggest the polarization mechanisms were associated with interfacial properties of a solid-fluid surface. The results suggest the control of pH and calcite precipitation on polarization response in silica gel.

Chapter 6 summarizes the primary scientific findings and contributions of this research,
and discusses the challenges and opportunities for future research.
Chapter 2. Electrical geophysical methods

Electrical geophysical methods including electrical resistivity (ER), self-potential (SP),
electrodic potential (EP), and spectral induced polarization (SIP)/dielectric spectroscopy
(DS) measurements can be used individually or in combination to obtain information
about the subsurface structure as well as the composition in a minimally invasive way.
Electrical geophysical methods are used to determine the spatial distribution of the DC
and/or low-frequency (< $10^6$ Hz) resistive and capacitive characteristics of porous media.
The collection of electrical geophysical data could be ranged from laboratory (point)
scales to field scale. The choice of which acquisition technique to use for a particular
investigation depends on many factors, including site condition, the objective of the
investigation, the sensitivity of different geophysical methods, the desired level of
resolution, and time and money. The three electrical geophysical methods (SP, EP,
SIP/DS) will be introduced in the following sections.

2.1 Self-potential

The SP method is a passive geophysical technique that measures spatial and temporal
changes of spontaneous voltages occurring in on earth. The SP method is non-invasive,
inexpensive, and easy to implement over large area. The SP is commonly applied in
hydrogeological surveys since the signals respond directly to the flowing fluids in earth
materials, and has been proven as a fast mean to characterize groundwater flow.

The SP measurements are recorded using non-polarizing electrodes (e.g., Cu/CuSO$_4$ or
Pb/PbCl$_2$) that form a liquid junction between a fixed (reference) electrode and sensing
electrodes at the ground surface. Both reference and sensing metallic electrodes are removed from contact with in pore fluids by embedding the electrodes in a gel or aqueous solution with identical chemistry at both metallic electrodes. This ensures that any potential recorded on the SP electrodes must result from internal current sources, and not from open-circuit galvanic potentials associated with electrochemical reactions between the pore fluid compounds and the metal electrodes. A high-input impedance voltmeter is applied in the SP measurements.

The SP anomalies could arise from three commonly accepted coupling mechanisms: electrokinetic mechanisms, electrochemical mechanisms, and thermoelectric coupling [Bigalke and Grabner, 1997; Revil et al., 2010]. In electrokinetic coupling, the SP signals are associated with fluid motion [Rizzo et al., 2004] and is called the streaming potential. Stream potentials are recored as fluid low in a pore space displaces excess mobile charge in the electrical double layer at the solid-fluid interface of a porous media resonse to the viscous drag exerted by fluid flow through the pores. This charge collects in the direction of flow, and the ion concentration gradient in the pore fluid creat an electric field. The electrokinetic phenomena are made possible by the presence of an electric double layer (EDL) at the interface between solid and liquid in porous media or fracutred rocks. The general accepted description of the structure of the EDL is the Stern layer, which consists of a zone nearest the solid phase where charge is bound to the mineral grain, and diffuse layer, a zone of mobile excess charge that decays exponentially moving away from the grain surface [Ishido and Mizutani, 1981; Revil et al., 2003]. The strucutre of EDL is illustrated in Figure 2.1. The boundary between the fixed and free zones is
defined as the Helmholtz plane, and the thickness of the EDL is described by the Debye length, which is influenced by electrolyte temperature, ionic strength of electrolyte, and dielectric permittivity [Hunter, 1993].

Figure 2.1 A schematic diagram showing the EDL on the surface of a mineral particle.

An increased ionic strength will cause additional charge screening of the surface and effectively compress the EDL. The diffuse layer is an unstirred layer of water adjacent to the surface, and the bulk solution is the free moving fluid.

For electrochemical coupling, the SP signals are associated with (1) ion concentration gradients in the pore fluid create a potential as these ions migrate through the geological media, and (2) oxidation – reduction reactions [Sato and Mooney, 1960]. Strong SP anomalies have been generated over conductive metal sulfide ore bodys and interpreted by so-called ‘geobattery’ model [Sato and Mooney, 1960; Bigalke and Grabner, 1997].
Geobatteries can develop when an electron conductor bridges electron donors and electron acceptors, resulting in current flow in response to the redox gradient (Figure 2.2). Based on the geobattery model, a so-called ‘biogeobattery’ model has been proposed to explain the SP signals recorded over a contaminant plume from a landfill undergoing biodegradation [Naudet et al., 2003; Naudet et al., 2004] (Figure 2.3). In thermoelectric coupling, different thermal diffusion of ions can also generate differences in local voltage.

Considerable volume of literatures have documented the application of SP to monitor hydrological processes, and have suggested the potential for indirect detection of microbial activity from redox gradients produced using SP methods, with strong SP signals (+ 200 mV) observed at sites where microbial degradation of hydrocarbons is occurring [Nyquist and Corry, 2002; Naudet et al., 2003; Naudet et al., 2004; Minsley et al., 2007].
2.2 Electrode potential (EP)

Point metallic electrodes are commonly used to measure geochemical parameters (e.g. Eh and pH), as well as specific ion concentrations (e.g. halide ion concentration determination using commercially available ion selective electrode, and titration of selenite using a silver/sulfide ion selective electrode) [Selig, 1985]. The mechanism for such passive measurement is called ‘potentiometry’, which relies on the measurement of open-circuit potential difference (high input impedance of the recording device impedes significant current flow) of a galvanic cell with quantitatively known reactions between the target component and the electrodes used. When the measurement electrode and the reference electrodes are bridged through a voltmeter, a spontaneous galvanic cell is created in which electrons will flow from the anode (oxidation reaction) to cathode (couple with reduction reaction). The electrode engages in two the half-cell reactions, and the surface reaction of electrode should be accounted for when interpreting open-circuit
The observed potential between the electrodes can be related to the concentration of redox species by Nernst equation:

$$E_{cell} = E^0_{cell} - \frac{RT}{zF} \ln \frac{[\text{Red}]}{[\text{Oxid}]}$$  \hspace{1cm} (2.1)

where $E^0_{cell}$ is the standard cell potential, which is

$$E^0_{cell} = E^0_{cathode} + E^0_{anode}$$  \hspace{1cm} (2.2)

A common target for such point electrode is sulfide concentration, which can be estimated using Ag-Ag$_2$S electrodes [Berner, 1963; Mirna, 1971; Whitfield, 1971; Revsbech et al., 1983]. Williams et al. [2007], Personna et al. [2008], and Slater et al. [2008] showed how a pair of physically separated Ag-AgCl metal electrodes, as used in biogeophysics measurements, similarly record an open circuit potential between two points in a porous medium when the electrodes straddle a microbe-induced gradient in HS$^-$ concentration. Being a simple approach, the EP measurements have inherent uncertainties discussed later, and are unlikely to give precise estimates of HS$^-$ concentration. However, Williams et al. [2007] did show a positive correlation between sulfide concentration and measured EP response, supporting the concept of using these simple in construction and inexpensive electrodes, physically spaced over large distances, to capture spatiotemporal variability in HS$^-$ concentration.

In order to distinguish between SP measurements and the EP measurement, here the SP measurement is defined as the potential measurement which avoid electrochemical
reactions on the electrode surface, whereas the EP measurements refers to spontaneous galvanic cell potential measurement which exists when the electrodes straddle a gradient in electro-active species (e.g. HS\textsuperscript{-}) concentration. The EP measurements involve electrochemical reaction at the electrode surface.

### 2.3 Spectral induced polarization (SIP)/dielectric spectroscopy (DS)

Both SIP and DS describes the frequency dependent complex electrical behavior of a sample (porous medium or biological samples). SIP measures the impedance magnitude and phase shift (\(\varphi\)) of a received sinusoidal voltage across a sample relative to the current waveform over a range of frequencies (typically < 1 kHz). The magnitude and phase (\(\varphi\)) are converted to a complex conductivity (\(\sigma^*(\omega)\)) using the physical geometry of the sample. The dependence of \(\sigma^*(\omega)\) on geologic matrix properties such as pore/grain size, permeability, and surface area has been extensively explored [Börner and Schön, 1991; Lesmes and Morgan, 2001; Slater and Lesmes, 2002a; Slater and Glaser, 2003].

The measured complex conductivity \(\sigma^*(\omega)\) of a system can be expressed as

\[
\sigma^*(\omega) = \sigma'(\omega) + i\sigma''(\omega)
\]

(2.3)

The real conductivity (\(\sigma'\)) represents conduction (energy loss), whereas imaginary conductivity (\(\sigma''\)) represents polarization (energy storage).

\[
\sigma' = |\sigma| \cos \varphi
\]

(2.4)

\[
\sigma'' = |\sigma| \sin \varphi
\]

(2.5)

where \(|\sigma|\) is the conductivity magnitude calculated using the impedance magnitude and appropriate geometric factors.
At frequencies < 1 kHz, $\sigma^*(\omega)$ of a saturated porous material in nonmetallic environments can be modeled as the sum of an electrolytic conduction ($\sigma_{ele}$) within the fluid-filled interconnected pore space and a surface conduction ($\sigma^*_{surf}$) occurring within an electrical double layer (EDL) at the grain-fluid interface [Lesmes and Frye, 2001]. It is usually assumed [Waxman and Smits, 1968; Vinegar and Waxman, 1984] that the electrolytic and surface conduction paths add in parallel such that

$$\sigma' = \sigma_{ele} + \sigma'_{surf}$$ \hspace{1cm} (2.6)

and

$$\sigma'' = \sigma''_{surf}$$ \hspace{1cm} (2.7)

The $\sigma'_{surf}$ represents the real component of the surface conductivity assuming that $\omega \varepsilon_{el} \ll \sigma''_{surf}$, $\omega \varepsilon_{el}$ is equal to high frequency dielectric permittivity $\varepsilon_\infty$ [Slater and Lesmes, 2002b]. The measured $\sigma'$ therefore is a function of electrolytic and interfacial conduction, and the $\sigma''$ is attributed solely to interfacial processes.

The electrolytic conductivity $\sigma_{ele}$ is expressed as $\sigma_{ele} = (1/F) \times \sigma_f$, where $\sigma_f$ is fluid conductivity, and $F$ is the electrical formation factor. Assuming electrical conduction is only through electrolyte-filled pores, then $F$ describes the decrease in conductivity of the rock due to the presence of the insulating mineral grains [Archie, 1942]. In high conductivity solutions, in the absence of metallic minerals, $\sigma_{ele} \gg \sigma'_{surf}$ at low frequencies [Slater and Lesmes, 2002b], and $\sigma' \approx \sigma'_{surf}$.

There are several polarization mechanisms associated with the SIP/DS signals. In the
absence of metallic minerals, the polarization mechanism is often attributed to the diffusion of ions in the EDL along the mineral-fluid interfaces [Lesmes and Morgan, 2001]. The ions in the diffuse layer of the EDL are easily exchanges with other ions in the solution. The polarization of the EDL is primarily caused by the movement of ions and their possible chemical reactions, which could generate an ohmic and a diffusion-produced conduction through a transport current. The EDL polarization mechanism is shown in Figure 2.4. The magnitude of $\sigma''$ is a function of the interfacial parameters, such as surface area, surface charge density, and ionic mobility [Lesmes and Morgan, 2001]. Besides the EDL polarization mechanism, there are other polarization mechanisms associated with ion accumulation and reduced ion mobility at pore throats [Vinegar and Waxman, 1984] and the formation of field-induced free charge distributions near the interface between the phases of the medium, giving rise to a Maxwell-Wagner effect.

![Figure 2.4 The possible EDL polarization mechanism at a non metallic mineral grain – fluid surface. Ion migration occurs tangentially to the interface.](image)
Mechanistic models describing $\sigma_{surf}^*$ have recently been developed, and relationships between microgeometry (grain-size distributions, pore diameter, and pore-size distributions) and SIP signals are reported, mostly single rock formation [Scott and Barker, 2003; Binley et al., 2005; Scott and Barker, 2005]. However, the role of fluid chemistry was until recently overlooked. Vaudelet et al. [2011] recently examined the SIP signals measured during advective flow of a CuSO$_4$ solution in a sand column, and developed a speciation model for adsorption of sodium and copper onto the silica grain surface. Skold et al., [2011] introduced a similar model, but included a proton hopping process, which allowed predictions of SIP measurements made with varying pH and salinities. Such developments enable quantitative interpretation of specific sorption reactions from SIP datasets. However, these models are challenging to calibrate for complex geochemical systems.

Phenomenological models, such as the Cole-Cole model [Cole and Cole, 1941], can instead be applied to observed SIP responses of porous media. Although these models are not directly parameterized in terms of microgeometric and/or geochemical parameters, parameters such as the chargeability and relaxation time constant are related to microgeometry [Titov et al., 2002; Binley et al., 2005] and pore fluid chemistry [Lesmes and Frye, 2001]. A popular variant of the Cole-Cole model can be written as [Cole and Cole, 1941; Pelton et al., 1978]

$$\sigma^*(\omega) = \sigma_0 \left[ 1 + m \left( \frac{(i\omega \tau)^c}{1+(i\omega \tau)^c(1-m)} \right) \right]$$

(2.8)

where $\sigma_0$ is the DC-conductivity, $m$ is the chargeability describing the magnitude of polarization associated with interfacial charge storage, $\tau$ is the characteristic relaxation
time constant related to characteristic pore or grain size [Kruschwitz et al., 2010], and $c$ is a shape parameter (0.2-0.8 for unconsolidated soils). Integrating parameters from a Debye decomposition have also been studied to quantify the dependence of polarizability on pore fluid composition in sandstone samples [Weller et al., 2011]. The $\tau$ for polarization of the EDL surrounding a spherical grain of diameter $d$ (m) is defined by Schwarz [Schwarz, 1962] as

$$\tau = \frac{d^2}{8D_s} = \frac{d^2}{8\beta_s k T / e}$$  \hspace{2cm} (2.9)$$

where $\beta_s$ is the effective ion mobility (in m$^2$s$^{-1}$V$^{-1}$), $k$ is Boltzmann’s constant ($1.381 \times 10^{-23}$ JK$^{-1}$), $T$ is absolute temperature (K), and $D_s = \beta_s k T / e$ is the surface diffusion coefficient (in m$^2$s$^{-1}$). This model predicts that $\tau$ is proportional to grain size and inversely proportional to ion mobility.
Chapter 3. Monitoring microbial sulfate reduction in porous media using multi-purpose electrodes

Abstract

There is growing interest in the application of electrode-based measurements for monitoring microbial processes in the Earth using biogeophysical methods. In this study, reactive electrode measurements were combined with electrical geophysics measurements during microbial sulfate reduction occurring in a column of silica beads saturated with natural river water. Electrode potential (EP), self-potential (SP) and complex conductivity signals were recorded using a dual electrode design (Ag/AgCl metal as sensing/EP electrode, Ag/AgCl metal in KCl gel as reference/SP electrode). Open-circuit potentials, representing the tendency for electrochemical reactions to occur on the electrode surfaces, were recorded between sensing/EP electrode and reference/SP electrode and showed significant spatiotemporal variability associated with microbial activity. The dual electrode design isolates the microbial driven sulfide reactions to the sensing electrode and permits removal of any SP signal from the EP measurement. Based on the known sensitivity of a Ag electrode to dissolved sulfide, we interpret EP signals exceeding 550 mV recorded in this experiment in terms of bisulfide (HS⁻) concentration near multiple sensing electrodes. Complex conductivity measurements capture an imaginary conductivity (σ”) signal interpreted as the response of microbial growth and biomass formation in the column. Our results suggest that the implementation of multi-

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1 This work has been published should be cited as: Zhang, C., D. Ntarlagiannis, L. Slater, and R. Doherty (2010), Monitoring microbial sulfate reduction in porous media using multipurpose electrodes, *J. Geophys. Res.*, 115, G00G09, doi:10.1029/2009JG001157.
purpose electrodes, combining reactive measurements with electrical geophysical measurements, could improve efforts to monitor microbial processes in the Earth using electrodes.

3.1 Introduction

Biogeophysics is a rapidly evolving Earth science discipline concerned with the links between dynamic subsurface microbial processes, microbial-induced alterations to geologic materials, and geophysical signatures [Atekwana and Slater, 2009]. There is growing interest in applying electrical geophysical techniques (resistivity, complex conductivity, and self potential (SP)) for biogeophysical projects as they have repeatedly been shown to be sensitive to bacterial cells, microbial growth and microbe-mineral alterations [Atekwana et al., 2004a; Atekwana et al., 2004b; Naudet et al., 2004; Personna et al., 2008; Slater et al., 2008]. Previous studies have confirmed the potential of electrical geophysics for characterizing microbial production of metabolic byproducts and resulting alteration of mineral surfaces [Werkema et al., 2003; Atekwana et al., 2004a; Atekwana et al., 2004b; Allen et al., 2007; Che-Alota et al., 2009], evaluating hydrological and biotransformations due to microbial reduction of heavy metals [Hubbard et al., 2008], improving understanding of metal and nutrient cycling driven by microbe-mineral transformations [Ntarlagiannis et al., 2005; Kenneth H. Williams et al., 2005; Personna et al., 2008], and detection of microbes, microbial growth and biofilm formation [Abdel Aal et al., 2004; C Prodan et al., 2004; Ntarlagiannis et al., 2005; Davis et al., 2006; Abdel Aal et al., 2009; Ntarlagiannis and Ferguson, 2009; Slater et al., 2009].
Biogeophysical signals are inherently non-unique, as complex and coupled biogeochemical processes alter subsurface physical properties in many ways and can drive multiple geophysical signatures over a wide range of spatial and temporal scales [Atekwana and Slater, 2009]. Additional biological, chemical, and physical information is typically required to reliably constrain interpretation of biogeophysical signals. One approach to constrain the interpretation is to simultaneously collect and analyze multiple electrical geophysical measurements (e.g., joint acquisition of complex conductivity and SP), along with any available aqueous geochemistry data (e.g., pH, Eh, and ion concentration). However, direct sampling of aqueous geochemistry is invasive, time consuming and expensive. Real-time monitoring of aqueous geochemistry using novel sensors offers promise in capturing temporal evolution of biogeochemical processes [Taillefert et al., 2000; Viollier et al., 2003], but is usually impractical and cost-prohibitive to perform at high sampling density over large spatial domains.

Recent studies have shown that electrodic potential (EP) measurements, using simple in construction and inexpensive electrodes, can be utilized to capture temporal and spatial variability in sulfide chemistry associated with microbial sulfate reduction under anaerobic conditions [K. H. Williams et al., 2007; Personna et al., 2008; Slater et al., 2008]. The EP measurements record the tendency for spontaneous redox reactions to occur on the surfaces of metal and/or metal-metal salt electrodes. Williams et al. [2007], Personna et al. [2008] and Slater et al. [2008] showed that, in the presence of a bisulfide (HS\(^{-}\)) concentration gradient across two Ag-AgCl metal electrodes, a galvanic cell (GC)
potential is recorded, being the potential of an electrochemical cell with a known reactivity between the target compound and the electrodes used. We stress here that this EP method may provide only an approximation of HS\(^-\) concentration, as the accurate interpretation of HS\(^-\) may be limited due to possible complex electrochemical reactions in the system. More precise and diagnostic reactive electrode-based techniques, such as voltammetry, have been used with great success to determine chemical speciation associated with sulfide redox chemistry in the deep-sea [Luther et al., 2001]. However, the anode and cathode in EP measurements are physically separated, and rely on very simple in construction and inexpensive electrodes that could potentially be deployed over large distances to provide spatially rich datasets collected in tandem with geophysical datasets.

In this paper, we investigate the application of simultaneous EP/electrical geophysical measurements for monitoring microbial sulfate reduction. Unlike earlier studies [K. H. Williams et al., 2007; Personna et al., 2008; Slater et al., 2008], we employ a dual electrode (Ag-AgCl metal as sensing/EP electrode; Ag-AgCl metal in KCl gel as reference/SP electrode) technique that constrains EP interpretation of HS\(^-\) concentration by using a non polarizing (SP) electrode as the reference cathode. This dual electrode strategy results in EP measurements with a constant stable reference potential, facilitating HS\(^-\) concentration predictions at the active electrode locations. In addition, this approach overcomes the overlooked limitations of earlier studies in that EP signals would be in error in the presence of significant SP signals, as have been repeatedly postulated to result from microbial activity [Naudet et al., 2003; Naudet et al., 2004; Minsley et al., 2007;
Slater et al., 2007]. Finally, we demonstrate how complex conductivity, SP, and EP measurements can be jointly recorded in order to improve the understanding of microbial processes relative to electrical geophysics measurements alone.

We describe a column experiment where EP, SP, complex conductivity, and resistivity measurements were made during microbial sulfate reduction in a column of silica beads saturated with natural river water amended with lactate. We show how EP measurements using geophysical instrumentation capture spatiotemporal evolution of sulfide production driven by the microbial activity. We also record a complex conductivity response consistent with previous studies [Davis et al., 2006], further supporting the concept that complex conductivity can be used as a non-invasive indicator of microbial growth and biomass formation in porous media, even in silica beads and in the absence of biodegradation. We discuss how the simultaneous collection of EP and complex conductivity data helps to better understand the processes in our microbial active system.

3.2 Experimental Methods
3.2.1 Electrodic potential (EP) measurements

Point electrodes are commonly 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). An open-circuit potential difference (high input impedance of the recording device impedes significant current flow) is recorded between a metal electrode in contact with the pore-filling electrolyte and a reference electrode in close proximity and connected to the metal electrode by a salt bridge. These
open circuit potentials represent the tendency for an electrochemical (galvanic cell) reaction, associated with reactive compounds at the metal electrode, to proceed. A common target of such point electrodes is sulfide concentration, which can be estimated using Ag-Ag$_2$S electrodes [Berner, 1963; Mirna, 1971; Whitfield, 1971; Revsbech et al., 1983]. Williams et al. [2007], Personna et al. [2008], and Slater et al. [2008] showed how a pair of physically separated Ag-AgCl metal electrodes, as used in biogeophysics measurements, similarly record an open circuit potential between two points in a porous medium when the electrodes straddle a microbe-induced gradient in HS$^-$ concentration. Being a simple approach, the EP measurements have inherent uncertainties discussed later, and are unlikely to give precise estimates of HS$^-$ concentration. However, Williams et al. [2007] did show a positive correlation between sulfide concentration and measured EP response, supporting the concept of using these simple in construction and inexpensive electrodes, physically spaced over large distances, to capture spatiotemporal variability in HS$^-$ concentration.

Here we modify the EP method to improve (relative to prior EP studies) monitoring of spatiotemporal variations in microbial-driven aqueous sulfide chemistry. Similar to previous work, metal Ag-AgCl electrodes serve as sensing electrodes (anodic reaction). However, we use a non-polarizing (Ag-AgCl metal in KCl gel) electrode as reference electrode, such that the cathodic reaction occurs under known chemical conditions. The reference electrode therefore maintains a constant potential that is independent of changes in fluid properties in the column. The approach is therefore more akin to the measurement obtained with a point electrode (Figure 3.1b), except that the active
electrode is distant from the reference electrode, and multiple active electrodes can be referenced to a single reference (Figure 3.1a). The assumed cathodic and anodic reactions, as well as

![Diagram of biochemical reactions](image)

**Figure 3.1 Diagram of biochemical reactions**

(a) Summary of biochemical reactions in a natural water system in the presence of sulfate-reducing bacteria (SRB) and two half-cell reactions for a Ag-AgCl (EP)/Ag-AgCl, KCl (SP) electrode pair. In the column, SRB utilize sulfate as electron acceptor, reducing it to HS\(^{-}\), while organic molecules (such as lactate) are used as the carbon source and oxidized to acetate. The Ag-AgCl in KCl gel (SP) electrode serves as a reference electrode whereby a cathodic reaction involves the reduction of AgCl coating. The anodic reaction at the sensing Ag-AgCl (EP) electrode is an oxidation of Ag\(^{0}\) as a result of a reaction with sulfide. The EP/SP electrode pair serves as a redox probe sensitive to HS\(^{-}\) concentration. (b) The dash frame area represents simple schematic of point electrode used in electrochemical-based techniques for determination of aqueous ion concentration for comparison.

overall galvanic cell reactions are summarized in Table 3.1, along with the individual
standard potentials calculated from standard free energies of formation [Stumm and Morgan, 1996].

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Reaction</th>
<th>Standard Potential ($E^0$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathode</td>
<td>KCl (1M) (reference electrode)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{AgCl}<em>{(s)} + e^- \leftrightarrow \text{Ag}</em>{(s)} + \text{Cl}^-$ (Reduction of AgCl)</td>
<td>0.222 V</td>
</tr>
<tr>
<td>Anode</td>
<td>Ag/AgCl (sensing electrode)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$2\text{Ag}_{(s)} + \text{HS}^- \leftrightarrow \text{Ag}<em>2\text{S}</em>{(s)} + \text{H}^+ + 2e^-$ (Oxidation of Ag$^0$)</td>
<td>0.273 V</td>
</tr>
<tr>
<td></td>
<td>$2\text{Ag}_{(s)} + \text{H}<em>2\text{S}</em>{(aq)} \leftrightarrow \text{Ag}<em>2\text{S}</em>{(s)} + 2\text{H}^+ + 2e^-$ (E$^0$=0.036V)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$2\text{Ag}_{(s)} + \text{S}^2^- \leftrightarrow \text{Ag}<em>2\text{S}</em>{(s)} + 2e^-$ (E$^0$=0.69V)</td>
<td></td>
</tr>
<tr>
<td>Overall Galvanic Cell</td>
<td>$2\text{AgCl}_{(s)} + \text{HS}^- \leftrightarrow \text{Ag}<em>2\text{S}</em>{(s)} + \text{H}^+ + 2\text{Cl}^-$</td>
<td>0.495 V</td>
</tr>
</tbody>
</table>

*Anodic reactions with other major possible sulfate reduction products (H$_2$S and S$^2$-), which are not the predicted reactions in our study.

Table 3.1 Half-cell electrochemical reactions and standard potentials

Half-cell electrochemical reactions and standard potentials on cathode and anode, and overall reactions and standard potential of the galvanic cell. Reactions are thermodynamically favorable ($E^0>0$), and standard potentials (versus standard hydrogen electrode) are calculated from standard free energies of formation at 25 °C [Stumm and Morgan, 1996].

3.2.2 Complex conductivity

Complex conductivity measures the frequency ($\omega$) dependent electrical behavior of a sample (e.g., a porous medium or a suspension of cells). The measured complex conductivity $\sigma^*(\omega)$ of a sample can be expressed as

$$\sigma^*(\omega) = \sigma'(\omega) + i\sigma''(\omega)$$ (3.1)

where $\sigma'$ is the measured real part of $\sigma^*(\omega)$, being the conduction (energy loss) term, $\sigma''$
is the measured imaginary part of $\sigma^*(\omega)$, being the polarization (energy storage) term, and $i = \sqrt{-1}$. At low frequencies (<1000 Hz), $\sigma^*(\omega)$ of a saturated porous material can be modeled as the sum of an electrolytic conductivity $\sigma_{el}$ resulting from ohmic conduction within the interconnected pore space, combined with complex mechanisms ($\sigma^*_{surf}$) associated with grain-fluid surfaces. Two electrochemical interfacial polarization mechanisms assumed to occur in porous media are (1) polarization of ions in the electrical double layer at the mineral-fluid interface, and (2) polarization resulting from differences in mobility of ions in the electrolyte driven by variable pore throat diameters.

The sensitivity of complex conductivity measurements to subtle changes in the surface physical properties of geologic media makes it a suitable technique for investigating microbial growth and biofilm formation [Abdel Aal et al., 2004; Ntarlagiannis et al., 2005; Abdel Aal et al., 2009; Slater et al., 2009], and detecting microbial growth and biominalization transformations in porous media [Ntarlagiannis et al., 2005; Kenneth H. Williams et al., 2005; Personna et al., 2008].

### 3.2.3 Self-Potential (SP) method

Self-potential signals are voltages associated with a gradient in an electric field generated by internal current sources in the Earth. Self-potentials are recorded using non-polarizing electrodes where both metallic electrodes are removed (by embedding in a gel or aqueous solution with identical chemistry at both metallic electrodes) from contact with the pore fluids. This ensures that any potential recorded on the SP electrodes must result from internal current sources and not from galvanic potentials associated with reactions.
between compounds in the pore fluid and the metal electrodes.

Self-potential signals arise from multiple mechanisms. Streaming potentials are recorded in the presence of a streaming current source term resulting from the transport of excess charge in the electrical double layer at the solid-fluid interface of a porous medium in response to the viscous drag exerted by fluid flow through the pores. Streaming potentials have been employed with considerable success to monitor hydrological processes [Revil et al., 2002; Rizzo et al., 2004; Linde et al., 2007]. Another SP source mechanism is electrodiffusion, arising due to gradients in the chemical potentials of charge carriers. More important in biogeophysics research is the geobattery mechanism [Sato and Mooney, 1960]. Geo-batteries can develop when an electron conductor bridges electron donors and electron acceptors, resulting in current flow in response to the redox gradient (the thermodynamic driving force) [Sato and Mooney, 1960; Bigalke and Grabner, 1997]. Previous studies have suggested the potential for indirect detection of microbial activity from redox gradients produced using SP methods, with strong SP signals (+ 200 mV) observed at sites where microbial degradation of hydrocarbons is occurring [Nyquist and Corry, 2002; Naudet et al., 2003; Naudet et al., 2004; Minsley et al., 2007].

3.3 Experimental Procedures

3.3.1 Sample preparation and column setup

The objective of our design was to capture EP and electrical geophysical signatures in response to sulfate reduction occurring in a porous medium composed of glass beads. We utilized two identical columns for this experiment; one was used as the experimental
(biological active) column while the second served as a control. Lexan columns were constructed with inner diameter of 3.17 cm and a length of 20.3 cm. Both columns were dry packed with 3 mm glass beads (SiLibeads-Type M) with a measured porosity of 0.38 ± 0.02 and density of 2.5 kg/m³. Silica beads were chosen, in favor of quartzitic sand, since they offer high chemical resistance, excellent roundness, constant size and maintain the pore geometry before and after saturation by dry packing [Niarlagiannis and Ferguson, 2009]. The experimental design is schematically illustrated in Figure 3.2.

Figure 3.2 Schematic diagram of column setup

Schematic diagram of column setup showing flow through configuration, location of Ag-AgCl (EP) electrodes, Ag-AgCl in KCl gel (reference/SP) electrodes, and Ag-AgCl coil (complex conductivity current injection) electrodes. Lagan River water was pumped through the column via a peristaltic pump; pH and fluid conductivity (σ) were monitored at the outflow.
We used water from River Lagan (Belfast, Northern Ireland, UK) as the saturating fluid. The River Lagan, which feeds into Belfast Lough, is the recipient of numerous sewage and industrial discharges. The water sample contained suspended sediments and particles, known to contain sulfate-reducing bacteria (SRB) [K.P. Singh, Queen’s University-Belfast, unpublished data]. Chemical analysis of the collected river water involved ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS) for the determination of major ions and metals respectively. This analysis showed the water contains 29 mg/L Cl⁻, 17 mg/L of SO₄²⁻, 9.3 mg/L of NO₃⁻, 0.60 mg/L of PO₄³⁻, 0.40 mg/L of NO₂⁻, 0.06 mg/L of F⁻, 8.4 µg/L of aqueous Cu, 7 µg/L of aqueous Ni, Zn, and Cr, 5.8 µg/L of aqueous Se, 0.7 µg/L of aqueous As, and trace amounts of other metals. Two days prior to the experiment, the river water sample was spiked with 2.24 g/L sodium lactate solution, and then placed in a dark airproof container to decrease oxygen penetration [Ramsing et al., 1993] and promote SRB growth.

The river water, amended with lactate to stimulate microbial growth, was used to saturate the experimental column. The control column was saturated with the same treated river water, but autoclaved to prevent biological activity. Both columns were positioned vertically and attached to separate closed circulation systems with 1L treated river water (experimental column) and 1L autoclaved treated river water (control column) respectively as the flow-through medium. A multichannel peristaltic pump was used to maintain a steady flow rate of ~2 pore volumes/day (~64 ml/day) for both columns. All tubing and column components were sterilized before the experiment by autoclaving or rinsing with 70% ethanol to minimize contamination.
3.3.2 Electrodic Potential (EP) and Self-Potential (SP) measurements

The experiment was performed at room temperature for a period of ~22 Days, although measurements for Days 10-18 were lost as a result of hardware problems. Measurements on the control column started with a two-day delay relative to the active column.

The EP measurements were recorded on six metal Ag-AgCl electrodes installed in electrolyte filled chambers, in electrolytic contact with the column, placed 2 cm apart along one column side (Figure 3.2). These Ag-AgCl electrodes were made from high grade Ag wire (99.999%) that was immersed in a strong chlorine solution (6.5% NaOCl) until a continuous solid gray AgCl coating formed on the Ag surface. The SP signals were recorded using electrodes placed on the other side of each column, one of which also served as the reference electrode for the EP measurements (Figure 3.2). These electrodes were constructed by immersing a single Ag-AgCl metal electrode into 1M KCl agar gel and housed in a 15 ml pipette tip. The agar gel was prepared by dissolving 14 g/L agar powder and 74.5 g/L KCl into heated DIW water. The solution was then stirred until it became clear, when 0.27 g/L HgCl₂ of biocide was added to inhibit the erosion of agar gel by microbes. The final solution was left to cool in the pipette tips. The fine open end of the pipette tip ensured electrolytic contact between the electrode and the electrolyte in the column.

The EP and SP measurements were obtained with a high-impedance (> 10 MOhm ± 1%) Keithley 2701 digital multimeter (DMM) data logging system. We collected
measurement every 15 minutes during the experimental period, with the exception of 30-minute intervals when complex conductivity measurements were performed. For all measurements we use the same reference electrode, S1 (Figure 3.2). The EP measurements were recorded between S1 and E1, E3, E4, and E6, whereas SP measurements were recorded between S1 and S2. In both EP and SP measurements the reference electrode was connected to the negative terminal of the digital multimeter by convention.

### 3.3.3 Complex conductivity measurements

Complex conductivity measurements were obtained daily (from Day 0 to Day 10) with a two-channel dynamic signal analyzer (DSA, National Instrument 4551) for frequencies between 0.1 and 1000 Hz at 40 logarithmic intervals [Slater and Lesmes, 2002b]. The commonly used four-electrode configuration technique was utilized; two coiled Ag-AgCl electrodes located at each end of the column were used for current injection (Figure 3.2). The resulting potential was recorded between SP electrode pairs S1 and S2. The magnitude ($|\sigma|$) and the phase shift ($\phi$) were measured relative to a known high precision resistor on channel 1. The real ($\sigma' = |\sigma|\cos \phi$) and imaginary ($\sigma'' = |\sigma|\sin \phi$) components of the sample complex conductivity were than calculated. The instrumentation and setup applied in this study is similar to previous studies [Ntarlagiannis et al., 2005], with a phase accuracy of ~0.06 mrad.

### 3.3.4 Sampling and geochemical analysis

Aqueous geochemical measurements of fluid conductivity ($\sigma$), Eh, and pH of circulating
fluid were taken daily for both experimental (from Day 1) and control columns (from Day 4). A 1.5 ml fluid sample was extracted and anaerobically sealed using a sterile syringe inserted into the tubing, and Eh/pH point probes were immediately immersed in fluid to minimize the contact of sample and ambient air. The $\sigma_f$ was subsequently measured using a conductivity probe. We chose not to determine sulfide concentrations (e.g., colorimetrically) in this experiment because of potential sampling errors from a closed anaerobic system of limited volume. Sulfide is unstable and is easily chemically oxidized under aerobic conditions, leading to sulfate production. In addition, previous studies have already proven the positive correlation between measured EP response and sulfide concentration [Williams et al., 2007] as we discussed in Section 3.1, and this was not our objective here.

### 3.3.5 Bisulfide (HS⁻) concentration estimation from EP

The SRB can utilize H₂ and/or low molecular weight organic (e.g., lactate and pyruvate) matter as an electron donor and sulfate as an electron acceptor under anaerobic conditions while producing sulfide [Maier et al., 2000]. Taking lactate for instance, the reaction could be summarized in equation (2), whereby dissimilatory sulfate reduction by bacteria is the most kinetically favorable mechanism by which sulfide is produced [Ledin and Pedersen, 1996],

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow_{SRB} ^{2CH_3COO^- + HS^- + 2HCO_3^- + H^+} (3.2)$$

At circumneutral-pH, HS⁻ is the predominant sulfide species in aqueous system, although other major sulfide species are H₂S and S²⁻. In our experiment with the presence of HS⁻, oxidation of silver occurs at the anode (metal Ag-AgCl electrode) along with the
concurrent conversion of AgCl to Ag$_2$S due to the much smaller stability constant ($K_{sp}$) of Ag$_2$S. Reduction of the AgCl electrode coating occurs at the non-polarizing reference electrode. These reactions and the associated half-cell potentials are summarized in Table 3.1. The processes of microbial sulfate reduction, along with subsequent electrochemical reactions on the electrodes, are depicted schematically in Figure 3.1.

Utilizing the Nernst equation, the HS$^-$ concentration ([HS$^-$]) can be estimated given EP and pH measurements from,

$$\Delta E_{\text{cell}} = E_{\text{cell}}^0 - \frac{RT}{nF} \log \frac{[H^+][Cl^-]^2}{[HS^-]} \quad (3.3)$$

where $E_{\text{cell}}^0$ is the standard potential of the overall galvanic cell reactions determined from two half-cell potentials (Table 3.1), and $\Delta E_{\text{cell}}$ is the EP of our system. However, the potential recorded between the Ag-AgCl metal electrode and the reference electrode is actually the sum of the EP and any SP signal in the system (a fact that was overlooked in previous studies using EP [K. H. Williams et al., 2007; Personna et al., 2008; Slater et al., 2008]). In our setup, the $\Delta E_{\text{cell}}$ can be determined by subtracting any SP signal (as recorded between the non-polarizing electrodes S1 and S2 in Figure 3.2) from the total potential recorded between any EP electrode (E1-E6) and S1. The hydrogen concentration ([H$^+$]) is known from pH, which was measured daily and linearly interpolated to predicted values at 15 min intervals (recording interval for EP measurements). As the chloride concentration ([Cl$^-$]) is known (1M KCl in the cathode), [HS$^-$] is the only unknown in the equation 3. It is important to emphasize that the [HS$^-$] calculated here is the localized concentration immediately adjacent to the sensing EP electrode, and it does not necessarily represent [HS$^-$] of the bulk fluid. Furthermore, the
approach is based on numerous assumptions that we discuss in detail later.

3.4 Results

3.4.1 Visual Observation

A darkening of the fluid in the column and tubing was visually observed, being first noted at the bottom of the column (inflow) (Figure 3.2) at Day 6, and covered the entire active column after Day 8. At the beginning of experiment, both aerobic and anaerobic microorganisms (including SRB) likely co-existed. The observed darkening is indicative of microbial sulfate reduction, expected as the river water contained a significant amount of sulfate (17 mg/L) and SRB communities. In the presence of available metal cations (e.g., Fe^{2+}, Cu^{2+}, and Zn^{2+}), S^{2-}, the main product of sulfate reduction, binds to form insoluble precipitates (MeS). In our experiment the observed darkening was attributed to possible precipitation of such metal-sulfide compounds. The gradual transition from bottom to top may represent the higher availability of nutrients for SRB closer to the inflow. Alternatively, this darkening could also conceivably arise simply from the settling and/or filtration of MeS within the column. This visual evidence of SRB activity was backed up by the characteristic sulfurous smell noted during syringe extraction of fluid from the experimental column. In the control column no change of color or sulfurous smell was observed over the duration of the experiment, indicating no significant SRB activity as expected.

3.4.2 EP and SP measurements

Electrodic potentials recorded at E1, E3, E4, and E6 on the experimental column relative
to the reference electrode are shown in solid color lines in Figure 3.3. The measurements taken at the same pairs on the control column are also shown in the same figure in color dash lines.

![Graph showing EP and SP measurements](image)

**Figure 3.3 EP and SP measurements**

Electrodic potential (EP) and self-potential (SP) measurements over the experiment duration. Solid color lines denote EP signals at different locations in experimental column referenced to S1, and color dash lines indicate EP signals at the same locations in control column. Black and grey lines show the SP signals at pair S2_S1 in experimental and control column, respectively.

At the beginning of experiment, steady (~75mV) EP measurements were observed for both the experimental and control column until Day 6 when strong negative potentials in the experimental column developed. The EP readings in the control column (color dash lines) remained the same thereafter, suggesting that no changes in redox state occurred in the natural waters in the absence of biological mediated redox reactions. In contrast, peak
negative EP values (-550 ~ -560 mV) were measured in the experimental column after Day 7 and were maintained until ~Day 10. Between Day 19 (446 hrs) and the end of the experiment, the EP signals of all pairs in the experimental column slowly decreased in magnitude. During this time, the bottom electrode E1 (pair E1_S1) showed the least change, followed by pair E3, with E4 and E6 behaving similarly and exhibiting the greatest decrease.

Figure 3.3 also shows SP data at pair S2_S1 for both experimental (magenta solid line) and control (bright green solid line) columns. The measurements were steady (+3 ± 4 mV) for the duration of the experiment. The SP in the control column was slightly (~5 mV) higher than the SP in the experimental column.

3.4.3 HS⁻ concentration

The calculated [HS⁻] (in µM) in the experimental column is showed in Figure 3.4 for pairs E1_S1, E3_S1, E4_S1, and E6_S1. Reflecting the relative small increase in EP early in the experiment, [HS⁻] slightly increased from Day 0 to Day 5, the response being nearly the same for each pair. Starting from Day 6, [HS⁻] dramatically increased for all pairs. Pair E1_S1 was the first to develop peak values (14.279 µM), followed by pair E4_S1 (peak value 18.15 µM), E3_S1 (peak value 9.96 µM), and E6_S1 (4.13 µM). These peak values maintained a plateau from Day 7 to ~Day 10. During Day ~19 to Day 22, [HS⁻] decreased, showing a gradual decline from pair E1_S1 to E6_S1. At the end of this experiment, pair E1_S1 had the highest estimated [HS⁻], followed by pair E3_S1, whilst pairs E4_S1 and E6_S1 had the lowest estimated concentration.
Figure 3.4 Estimated hydrogen sulfide concentration

Estimated hydrogen sulfide concentration ([HS\(^-\)]) at different locations in the experimental column over the experiment duration. The [HS\(^-\)] was determined by the Nernst equation as a function of theoretical cell potential (\(E^0_{\text{cell}}\)), cell potential (\(\Delta E_{\text{cell}}\), equivalent to measured EP corrected by eliminating SP), and pH, while holding [Cl\(^-\)] (1 M) and temperature (25 °C) constant. The concentrations shown here are in \(\mu\)M.

### 3.4.4 Complex conductivity and fluid conductivity

The complex conductivity measured between electrode pair S1_S2 from both columns was used to calculate \(\sigma'\) (Figure 3.5a) and \(\sigma''\) (Figure 3.5b) components, respectively (34 Hz values shown). The \(\sigma'\) (Figure 3.5a) increased from 0.085 S/m to 0.27 S/m from Day 0 to Day 4 and remained steady in the experimental column, while \(\sigma'\) remained at 0.07 S/m in the control column and showed no significant changes throughout the experiment.
The $\sigma^\prime$ (Figure 3.5b) in the experimental column increased from $1.16 \times 10^{-5}$ S/m (Day 0) to $4.29 \times 10^{-4}$ S/m (Day 5), and then decreased to $4.76 \times 10^{-5}$ S/m on Day 10. The magnitude of the $\sigma^\prime$ response in the control column was constant at $1.1 \times 10^{-5}$ S/m over the duration of the experiment.

![Complex conductivity data](image)

**Figure 3.5 Complex conductivity data**

(a) Fluid conductivity $\sigma_f$ (dash lines) and results of measured real conductivity $\sigma'$ (solid lines) for pair S1_S2 in both experimental (closed black circles) and control (open circles) columns. (b) Imaginary conductivity ($\sigma^\prime$) for pair S1_S2 in both experimental (closed black circles) and control (open circles) columns. Both $\sigma'$ and $\sigma^\prime$ shown here are at 34 Hz from Day 0 to Day 10.

Aqueous geochemical analysis of fluid samples showed $\sigma_f$ in the experimental column was the same as in the control column at the beginning of experiment, but later increased to ~5 times higher than it in control column. The $\sigma_f$ value remained steady at 0.23 S/m in the control column, whereas $\sigma_f$ in the experimental column increased with slight fluctuations. Normalizing the real conductivity by fluid conductivity ($\sigma' / \sigma_f$), resulted in a flat response, indicating that increases in $\sigma'$ in the experimental column result from associated increases in $\sigma_f$. 
The pH in the experimental column decreased from 7.81 to 7.29, and it dropped from 8.98 to 7.42 in control column throughout the experimental period. This drop was likely due to the existence of CO$_2$ and organic acids, generated from the degradation of complex organic material and biological respiration in the column, dissociated to form protons. The Eh (data not shown) recorded at the fluid sample varied considerably, probably reflecting problems with sampling and exposure to ambient air prior to Eh probe measurements.

### 3.5 Discussion

#### 3.5.1 Sulfide chemistry and reactive electrodes

Our results show how microbial-driven sulfide chemistry can be monitored using EP measurements made on simple in construction and inexpensive metal electrodes, that can also be used to make electrical geophysical measurements. We note that the EP response curves recorded in this experiment are quite similar in shape and magnitude when compared to those previously recorded on other experiments using mixed SRB cultures [Slater et al., 2008], as well as those recorded for a pure *D. vulgaris* culture [Personna et al., 2008]. However, we do not expect identical EP responses across these systems as the mineral matrix, growth media and cultures differ significantly. These differences could result in different onsets of SRB activity (e.g., the EP response curves in Slater et al., 2008 develop later than in this study) and differences in total sulfide produced. Although laboratory experiments using pure cultures are more easily quantifiable, they do not represent the real world. Our laboratory experiment suggests that we can bridge the gap
between laboratory and field scale applications and this technology is transferable to the field scale.

We recorded an open-circuit potential, as in previous work [Personna et al., 2008; Slater et al., 2008], which increases with the difference in HS\(^{-}\) concentration between the cathode and anode. However, in our study we utilize two types of electrodes, allowing for the correction of any SP signals during the EP measurement. We note that the presence of conductive metals between S1 and S2 could facilitate a geobattery SP mechanism [Sato and Mooney, 1960; Bigalke and Grabner, 1997] within the column given a sufficient redox gradient between these two locations. Even though, we only observe very small SP signals (few mV in both columns), suggesting there is no apparent mechanism associated with this microbial sulfate reduction process that could generate current sources. This result is expected given that (1) the redox gradient between S1 and S2 is small in this closed circulation experiment, and (2) it is unlikely that the MeS precipitates were extensive enough to form continuous conduction paths.

Instead, the SP signal probably represents the streaming potential due to the flow of the saturating fluid through the glass beads. In our case then, the SP correction needed to determine EP from the potentials recorded on the metal electrodes was minor. However, recent experiments suggest that this may not be the case in field-scale studies. Previous studies have identified large (+200 mV) SP readings, postulated to be due to biogeobatteries [Naudet et al., 2003; Naudet et al., 2004; Minsley et al., 2007], in the presence of strong microbial-driven redox potential gradients. In such field studies, it
would be critical to remove the SP signals from the EP responses prior to estimation of sulfide concentration. The previous EP studies [K. H. Williams et al., 2007; Slater et al., 2008] employed only reactive metallic electrodes (Ag-AgCl electrodes), therefore making it impossible to determine the contribution of any SP signal to the potential recorded on the metallic electrodes. The fact that SP was not quantified in these earlier studies has implications for the accuracy of the estimated sulfide concentration and adopted interpretation of the mechanism driving the measured potentials in general.

The use of a constant potential SP electrode in the EP measurement isolates the sulfide chemistry information to a single reactive electrode. In contrast, previous studies [K. H. Williams et al., 2007; Personna et al., 2008; Slater et al., 2008] using two reactive electrodes being located in a stable/quiescent, non-reactive environment in order to be isolated from confounding effects of reactive fluid chemistry (e.g., on the column influent for flow-through experiments). The use of a constant potential SP electrode for the reference enhances our confidence in the possible quantitative estimation of HS\(^-\) concentration at the anode, as the concentration at the cathode is known to be constant. Our approach requires a reference SP electrode (Ag-AgCl immersed in a KCl gel), where the electrodic reaction is fixed and known. When two reference electrodes are available, SP measurements can also be performed. These permit correction of the measurements made on metallic electrodes for SP signals and estimation of EP.

Our approach to biogeophysics monitoring, couples electrical geophysical measurements with a simple measurement sensitive to pore fluid redox chemistry at the electrodes. The
interpretation of biogeophysical signals is inherently uncertain due to the multiple possible sources for the recorded electrical signals; additional measurements, such as aqueous geochemistry measurements, are clearly advantageous for constraining the interpretation. In our experiment we demonstrate a quick and inexpensive means to capture spatial and temporal variability in microbial-driven sulfide chemistry using the same electrodes applied for the electrical geophysical measurements. Geophysical monitoring of microbial processes using large numbers of electrodes is increasingly being conducted [Daily and Ramirez, 1995; Werkema et al., 2003; Atekwana et al., 2004b; Allen et al., 2007; Minsley et al., 2007], and our approach could be adopted to determine variations in sulfide concentration at hundreds of locations as defined by the placement of electrodes. Such a sampling density would be prohibitively costly and invasive if on site extraction and sampling was conducted. The suggested EP electrode design is sensitive to other electrochemical reactive species and could be used as a qualitative Eh indicator in environments where no additional information is available; in certain cases where the chemistry is known and the dominant electrochemical species are identified or assumed (e.g., sulfide due to SRB activity in our experiment), EP measurements can be used to quantitatively estimate the concentration of the species of interest.

The quantitative interpretation of the EP signals in terms of sulfide concentration is, however, based on a number of assumptions. First, we assume that the reactions on the anode effectively alter the anode from Ag-AgCl to Ag-Ag\textsubscript{2}S, which is a thermodynamically favorable but unverified reaction here. Second, we treat this system as a single-potential system (although it is probably a mixed potential in reality), and we
attribute the EP signals only to HS\(^-\), without accounting for any other possible sulfate reduction products in the sulfide family (e.g., SO\(_3^{2-}\), S\(^2-\) ions, dissolved H\(_2\)S, and etc.) or any transformation between different sulfide species. Third, we assume the reactions describe an ideal Nernstian response over the whole range of HS\(^-\) concentrations. In fact, a linear Nernstian slope is only obtained for concentrations from \(10^{-6}\) to \(~10^{-4}\) mol/L of total sulfur, the range depending on pH and ion concentration of the aqueous solution [Revsbech et al., 1983; Jeroschewski et al., 1993; Lawrence et al., 2000]. These problems are not unique to our system, but are recognized difficulties in using point Ag/Ag\(_2\)S electrodes to determine sulfide. In addition to deviation from ideal Nernstian behavior, other problems include long response times and poisoning of the reference electrode [Jeroschewski et al., 1996; Lawrence et al., 2000].

Despite these limitations, the Ag/Ag\(_2\)S electrode has for a long time offered the only reasonable solution to estimate sulfide at high spatial resolution at the aerobic and anaerobic interface in aquatic sediments and biofilms, where distinct gradients of chemical and physical parameters exist [Revsbech et al., 1986; Kuhl and Jorgensen, 1992]. Electrode-based electrochemical determination of sulfide in aqueous system has a lower determination limit of a few ppb and an upper limit of hundreds of ppm, depending on the methods and electrodes [Lawrence et al., 2000]. Sulfide concentration in environmental systems varies from a few ppb (e.g., in oxic sea water) to thousands of ppm (e.g., in pore waters from salt marshes). In the latter case, the use of simple in construction and inexpensive multi-purpose electrodes, as proposed here, could conceivably capture spatiotemporal changes calibrated in terms of actual sulfide
concentration. However, in many environments it will likely only be possible to use EP measurements to infer patterns of sulfide variation, but not quantify absolute concentrations.

Even if the determination of HS\(^{-}\) concentration is not entirely accurate in our system, the EP signals still capture the onset of SRB activity, along with spatiotemporal variability in the processes. Such information is very valuable from a monitoring standpoint, and can also improve understanding of biogeophysical signals associated with microbial processes. In fact, validation of field studies often requires accredited chemical and biological analysis techniques, and the application of EP potentially provides a cost effective real time monitoring method that could supplement expensive chemical and biological analysis. In future studies, the estimation of HS\(^{-}\) using our approach might be improved by recording the real time temperature, pH, and ion concentration of the fluid, or constructing electrodes with a constant reactive area [Barrett et al., 1988]. It may also be possible to calibrate these electrodes for variations in pH and ion concentration.

### 3.5.2 Electrical geophysics signals

Our complex conductivity measurements revealed temporal increases in \(\sigma'\) in the experimental column resulting from increases in fluid conductivity (as supported by normalizing the \(\sigma'\) data by \(\sigma_f\)). Fluid conductivity is largely controlled by the total dissolved solids representing the number of ions present in the solution. Organic acids (acetic acid) produced as metabolic by-products of degradation of larger molecular organic matter (e.g., lactate) have been shown to cause mineral dissolution resulting in
the release of ions into the pore space, thus increasing the conductivity of the pore fluid [Abdel Aal et al., 2004; Allen et al., 2007]. In addition, biomass and decay of bacterial cells may contribute to higher electrical conductivity (e.g., Slater et al., 2009).

More significantly, our complex conductivity measurements also reveal a $\sigma''$ signal that is similar to that observed in work of Davis et al. [2006] and attributed to microbial growth/attachment followed by subsequent death/detachment. Given that no signals were observed in the control column, we are confident that the source is microbial in origin. The possible presence of metal-sulfide precipitates in the experimental column, which might be the reason for the observed darkening of fluid, cannot explain the $\sigma''$ response, as $\sigma''$ declined after reaching the peak value at Day 5. Although previous work has shown similar $\sigma''$ curves that were attributed to changes in mineral aggregation and variation in the spatial position of sulfide-encrusted cells [Ntarlagiannis et al., 2005b; Williams et al., 2005], these studies were conducted on columns with continuous supply of metals. In contrast, any $\sigma''$ response associated with metal-sulfide precipitates in our metal-limited system might be expected to show just the early time response observed in these previous studies, whereby $\sigma''$ increases in response to dispersed metal precipitates. This is consistent with elevated $\sigma''$ values throughout the experiment and dark fluid being observed until the end of experiment.

We suggest that our $\sigma''$ response in the experimental column reflects changes of microbial population in our system. Davis et al. [2006] showed a correlation between $\sigma''$ and microbial growth in their diesel fuel and nutrient broth saturated sand column.
experiment. The model of *Davis et al.* [2006] seems plausible in our case, although our study was conducted under much simpler experimental conditions. More recently, *Slater et al.* [2009] demonstrated an electrical conductivity response exhibiting the characteristics of microbial growth models. Our results suggest that a signal associated with microbial growth/attachment to death/detachment cycle can be captured during microbial sulfate reduction in the absence of hydrocarbon biodegradation. In our experiment, \( \sigma'' \) reached peak values at Day 5, although the largest EP signals developed on Day 6. This delay in the EP signals relative to the \( \sigma'' \) peak may suggest that 1) sulfate reduction by SRB was not initiated until oxygen is depleted, and/or 2) significant time is needed for electrochemical conditions at the electrode to develop for EP signal generation. The microbial activity between Days 2-8 captured with \( \sigma'' \) is likely the response of both aerobic and anaerobic microorganisms (including SRB) existing in the natural fluid. The peak in SRB activity occurred later as captured in the EP response. In this case, \( \sigma'' \) detects the growth of all microbial cells in the column whereas the EP signals are associated only with SRB activity. Our work thus lends strength to the notion that the signal recorded in the work of *Davis et al.* [2006] is an important biogeophysical signal that is not specific to the conditions of the *Davis et al.*[2006] experiment.

### 3.6 Conclusion

Here we have shown how EP measurements, in conjunction with SP and complex conductivity measurements, can be used to monitor spatial and temporal variability in microbial sulfate reduction within a porous medium saturated with natural river water using dual electrode sensors. The dual electrode approach permits an SP correction to the
EP measurement, and isolates the microbial driven sulfide chemistry to a sensing/EP electrode while holding the reference/SP electrode constant. We assume these open-circuit EP signals are associated with electrochemical reactions that occur on the Ag-AgCl (sensing/EP) electrode in the presence of dissolved sulfide, although it is possible that other microbial driven redox reactions may also have contributed to the observed signal. The HS⁻ concentration near the EP electrode surface could be estimated from EP signals based on certain assumptions. Our complex conductivity results are consistent with previous biogeophysics research, showing sensitivity to microbial activity in porous media. Thus EP measurements using Ag-AgCl electrodes offer a simple approach to capture spatial variability in microbial driven sulfide chemistry that can be collected using the same instrumentation and hardware as used for electrical geophysics measurements. The joint use of EP and complex conductivity measurements could facilitate simultaneous monitoring of individual microbial communities (e.g., sulfate reducers), whilst also monitoring net microbial growth. Our work may lead to the application of multi-purpose electrodes to improve the geophysical and geochemical monitoring in biogeophysics research.
Chapter 4. Complex Dielectric Properties of Sulfate-Reducing Bacteria Suspension

Abstract

Sulfate-reducing bacteria (SRB) can potentially enhance the remediation of heavy metals in the subsurface. Previous geophysical research has demonstrated the sensitivity of electrical measurements to SRB mediated mineral transformation in porous media. However, the inherent dielectric properties of SRBs and their direct contribution to the electrical properties of porous media are poorly understood. We studied the complex dielectric properties of SRB (Desulfovibrio vulgaris) suspensions at different concentrations and at different growth stages using a two-electrode dielectric spectroscopy measurement over the frequency range of 20 Hz to 1 MHz. Our results show higher dielectric responses (relative dielectric permittivity, real and imaginary conductivity) occurred with higher bacteria concentration at frequencies <10 kHz. Additionally, permittivity and conductivity both decreased as cell aged from mid-log phase to late stationary phase. Our results thus suggest that dielectric spectroscopy measurements can be used to non-invasively monitor biomass and various growth stages of SRBs. Our work advances the interpretation of electrical signals associated with SRBs observed in the subsurface.

4.1 Introduction

The problem of metal (e.g., iron) and radionuclide (e.g., uranium) contamination in the subsurface has promoted the exploitation of microbial mediated reduction of redox-sensitive metals [Valls and de Lorenzo, 2002]. Sulfate-reducing bacteria (SRB) have been extensively studied for their ability to reduce and accumulate heavy metals and radionuclides either enzymatically [Lovley et al., 1993] or through precipitation of metallic sulfide. Stimulated SRB activity and associated immobilization of heavy metals is a promising remediation approach as SRBs regulate the cycling of essential elements such as iron and sulfur. To validate successful in situ bioremediation by SRBs, long-term and non-invasively monitoring of SRB activity and associated biomineralization at sufficiently high spatial resolution over large domains is required.

Recent biogeophysics research has demonstrated the sensitivity of electrical geophysics techniques to subsurface microbial processes and microbial-induced alterations to geologic media [Atekwana and Slater, 2009]. Among these techniques, spectral induced polarization (SIP) has shown potential for (1) detection of bacterial cells and microbial growth [Ntarlagiannis et al., 2005; Abdel Aal et al., 2010], (2) characterizing microbial-mineral alterations at mineral-fluid interfaces [Kenneth H. Williams et al., 2005; Personna et al., 2008], and (3) improving understanding of metal and nutrient cycling driven by SRBs [Ntarlagiannis et al., 2005; Slater, 2007; Personna et al., 2008]. These studies suggest that electrical method could potentially be used to monitor bioremediation by SRBs at the field-scale.
Both SIP and dielectric spectroscopy (DS) measure the complex dielectric permittivity (or complex electrical conductivity) of a material over a frequency range. Whereas SIP is a geophysical technique for studying porous media in both laboratory and field settings, DS is a bench-top technique widely used in many areas including biophysics and pharmacology. Previous geophysical studies focused on sand columns inoculated with SRBs that were monitored using SIP at frequencies less than a kilohertz [Ntarlagiannis et al., 2005; Kenneth H. Williams et al., 2005; Personna et al., 2008]. However, the intrinsic broadband dielectric properties of SRBs and their direct contribution to the electrical properties of a porous medium are not well studied. Such decomposition of electrical signals from complex microbial sulfate reduction processes in porous media is required to better constrain interpretation of SIP data from laboratory and field settings [William et al., 2009].

In this study, we investigated the complex dielectric properties of *Desulfovibrio vulgaris*, a model SRB well studied for its bioremediation capacity, in cellular suspensions with varying concentrations and at different growth stages from 20 Hz to 1 MHz. We show that DS can precisely capture the intrinsic macroscopic dielectric function of the bacterial suspensions, and distinguish the variations in bacterial concentrations and life stages. Both dielectric permittivity and complex conductivity were enhanced at low frequencies with increased bacteria concentration, but were reduced when bacteria aged from mid-log to late stationary growth stages.
4.2 Materials and Methods:

4.2.1 Bacteria and growth conditions

Subculturing of *Desulfovibrio vulgaris* (strain Hildenborough/ATCC #35115) was minimized by using -80°C *D. vulgaris* stocks as 9% inocula for 5 ml of a defined lactate sulfate medium (LS4D medium [Mukhopadhyay et al., 2006]). These starter cultures were then used as 9% inocula for 5 ml production cultures, and incubated in centrifuge tubes in an anaerobic chamber. During *D. vulgaris* growth, increasing cell numbers and precipitation of metal sulfides (mainly iron sulfide) will increase optical density (OD). The bacterial concentration of cultures was therefore determined by OD measurements at 600 nm with a Genesys 10vis spectrophotometer. When the desired ODs for DS measurements were reached, the cultures were pelleted at 1500 ×g for 5 min. After removal of the supernatant, the pellet was resuspended and homogenized by pipetting into 20 ml of 5 mM HEPES solutions (pH = 7.2 and conductivity = 0.01 S/m at 24°C), which is commonly used to maintain physiological pH of cells and has no interference with metal substrates [Panitz et al., 1985]. Each DS measurement lasted less than 7 min, ensuring the viability of *D. vulgaris* cells in the buffer during the measurement [Fournier et al., 2003]. The cultures with ODs of 0.28 and 0.37 grown in LS4D for 48 hours were prepared for DS measurements to evaluate the effect of cell concentrations on dielectric signals, and the cultures with ODs of 0.38 at 26, 49, and 73 hours of growth were prepared in the same way for testing the impact of cell aging on the dielectric response.

Samples grown for 48 hours were collected for scanning electron microscopy (SEM) and X-ray absorption spectroscopy, and the preparation of samples followed previous studies.
For SEM analysis, centrifuged pellets were immersed overnight in 2.5% glutaraldehyde in phosphate buffer solution in the anaerobic chamber, rinsed three times in phosphate buffer, dehydrated in an ethanol-water mixture series (10%, 25%, 50%, 75%, 90%, and subsequently in triplicate in 100% solution for 1 h each), and dried in ambient air. The morphology of *D. vulgaris* cells was characterized with a LEO 1530 VP SEM system under operating voltage of 2 kV. For X-ray adsorption spectroscopy, the cells were preserved with 0.25% glutaraldehyde immediately prior to centrifugation. The centrifuged pellets were then rinsed with Milli-Q water and were mounted on Au grids coated with a thin film and the samples were kept in anaerobic chamber prior to transfer to X-ray sample chambers. X-ray absorption spectra were measured in fluorescence mode at the National Synchrotron Light Source beamlines X3B (Fe K-edge, 19 K to 300 K) at Brookhaven National Laboratory.

### 4.2.2 Dielectric Spectroscopy (DS) measurement

DS measurements were performed with a Solartron 1260 impedance/gain-phase analyzer from 20 Hz to 1 MHz. The experimental setup is described in *C Prodan et al., 2004; C Prodan and Bot, 2009* and illustrated in Figure 4.1. The suspension to be measured was placed between two parallel circular steel electrodes (radius $R = 28.5$ mm) enclosed in a glass cylinder. The distance between the two electrode plates was controlled by a micrometer, and was set to be 3 mm. The applied voltage was 0.15 V. The complex dielectric function $\varepsilon^*$ is expressed as

$$\varepsilon^* = \varepsilon + \sigma' / j\omega \varepsilon_0$$  \hspace{1cm} (4.1)

where $\varepsilon$ and $\sigma'$ represent measured relative dielectric permittivity and electrical
conductivity (real part) of the sample. These two terms are calculated from the impedance measurements,

\[ \varepsilon = \frac{-Z'}{Z'^2 + Z''^2} \frac{d}{\omega \varepsilon_0 \pi R^2} \]  
\[ \sigma' = \frac{Z'}{Z'^2 + Z''^2} \frac{d}{\pi R^2} \]  

where \( Z' \) and \( Z'' \) are real and imaginary part of measured complex impedance \( Z^* \), \( \varepsilon_0 \) is the vacuum permittivity, and \( d \) is the length of the sample. The measured imaginary conductivity (\( \sigma'' \)) can be calculated from

\[ \sigma'' = \varepsilon \varepsilon_0 \omega \]  

Therefore, measured complex conductivity \( \sigma^* \) is given by,

\[ \sigma^* = \sigma' + \sigma'' = \sigma' + i \varepsilon \varepsilon_0 \omega \]  

Two electrode DS measurements can reliably record the complex dielectric properties when the phase angle (\( \varphi = \tan^{-1}(\sigma'/\sigma'') \)) is small. The phase for the measured suspensions was always lower than 0.2 mrad at frequencies < 10 kHz, being below the noise threshold of four electrode SIP measurements. The two electrode DS drastically increase the resolution and accuracy of current SIP, and this advantage of DS could make it ideal for examining the polarization characteristics of SRB.
The experimental set-up and the electronic circuitry used in this experiment. The bacterial suspensions were placed in a glass cylinder with two parallel round stainless steel electrodes. The distance between the electrodes was controlled by a micrometer. A Solartron impedance analyzer provided a voltage on the top electrode while the bottom was kept at virtual ground through the negative input of a preamplifier AD797.

### 4.2.3 Electrode polarization error removal

In two-electrode DS measurements, electrode polarization appears at the interface between electrolyte and electrode, and can cause large errors at low frequencies. The true sample impedance $(Z_c^*)$ and electrode impedance errors $(Z_p^*)$ add in series and vary with frequency, i.e. the measured impedance of bacterial suspensions $(Z_s^*)$ is the sum of $Z_c^*$ and $Z_p^*$. Prodan and Bot [2009] proposed a substitution method to reduce $Z_p^*$ for colloidal suspensions, in which the cells are removed by gentle centrifugation and the resulting supernatant is used to measure $Z_p^*$. Following their approach, we corrected $Z_p^*$.
at low frequencies to obtain \( Z_c^* \). Figure 4.2 illustrates how \( Z_p^* \) was corrected using the imaginary part of impedance. The imaginary impedance for the bacterial suspensions \((Z_s^*)\) is close to the imaginary impedance for the medium or supernatant \((Z_m^*)\), except that the former is higher at low frequencies due to SRBs polarization. The imaginary part of \( Z_p^* \) \((Z_p^*)\) becomes negligible > 10 kHz, and can be calculated by fitting \( Z_m^* \) with high frequency theoretical values. The true imaginary impedance of the sample \((Z_c^*)\) was then determined by subtracting \( Z_p^* \) from \( Z_s^* \). Such error analysis and calibration is important for resolving subtle differences in \( \varepsilon \) and \( \sigma^* \) responses of a solution due to the presence of \textit{D. vulgaris} bacteria.

**Figure 4.2 Electrode polarization removal**

Imaginary parts of complex impedances as a function of frequency: \textit{D. vulgaris} cell suspensions (red line, \( Z_s^* \)), supernatant (green line, \( Z_m^* \)), polarization error (dashed line, \( Z_p^* \)), and corrected cell suspensions after polarization removal (purple open circles, \( Z_c^* \)) for different concentrations of \textit{D. vulgaris} cell suspensions OD 0.28 (subplot a) and OD 0.37 (subplot b).
4.3 Results

Figure 4.3a and 4.3b show the measured and intrinsic $\varepsilon$ of *D. vulgaris* bacterial suspensions at two different concentrations (OD 0.28 and OD 0.37), each obtained by averaging three independent measurements as indicated by error bars. After removing the effects of $Z_p^*$, the subtle differences in $\varepsilon$ curves between the two suspensions became more obvious. At low frequencies (<10 kHz), $\varepsilon$ increases as the OD increases. However, $\varepsilon$ decreases as the concentration of bacteria increases at higher frequencies, as expected.

**Figure 4.3** Dielectric permittivity of *D. vulgaris* cell suspensions with different concentrations.

(a). Relative dielectric permittivity of *D. vulgaris* cell suspensions with different cell concentration – OD 0.28 (red line) and OD 0.37 (blue line); (b). Corrected relative dielectric permittivity of different concentrations of *D. vulgaris* cell suspensions after electrode polarization removal.
For direct comparison with SIP data, Figure 4.4 shows $\sigma'$ and $\sigma''$ for *D. vulgaris* bacterial suspensions of ODs at 0.28 and 0.37 after $Z_p'$ is removed. The $\sigma'$ increased from 0.0075 S/m to 0.013 S/m as ODs increased from 0.28 to 0.37. The $\sigma''$ in OD 0.37 is one order of magnitude larger than it in the OD 0.28 cultures at low frequencies (<10 kHz). However, at higher frequencies, the $\sigma''$ decreases slightly with increased OD.

The variations in the dielectric responses of bacterial suspensions for different growth stages are shown in Figure 4.5. The *D. vulgaris* growth response to LS4D indicates that 26 hours of growth represents the mid-log phase, 49 hours of growth represents the early log phase, and 72 hours of growth represents the late log phase.

**Figure 4.4 Real and imaginary conductivity of *D.vulgaris* cell suspensions with different cell concentration.**

(a) Real conductivity ($\sigma'$) and (b) imaginary conductivity ($\sigma''$) of *D. vulgaris* cell suspensions with different cell concentration – OD 0.28 (red line) and OD 0.37 (blue line).
stationary phase, and 73 hours of growth represents the late stationary phase [Mukhopadhyay et al., 2006; He et al., 2010]. The $\varepsilon$ for bacteria suspensions (OD=0.38) decreased as the bacteria aged from 26 hours to 73 hours of growth at frequencies <10 kHz. At higher frequencies, suspensions at earlier growth stage show lower $\varepsilon$. Figure 4.5b shows $\sigma'$ decreased from 0.017 S/m to 0.011 S/m as bacteria continuously grew over the entire measurement frequency range.

![Figure 4.5 Dielectric responses of cell’s aging effect](image)

Relative dielectric permittivity (a) and real conductivity (b) of suspensions with the same *D. vulgaris* cell concentration (OD=0.38) but harvested at different growth period: 26 hours of growth (red), 49 hours of growth (green), and 73 hours of growth (blue). As the cell ages, the relative dielectric permittivity decreases and the conductivity decreases.

### 4.4 Discussion

By varying *D. vulgaris* bacterial concentrations in suspensions and growing them at
different stages, we have demonstrated that DS measurements can sense changes in cell concentration and different growth stages. Unlike previous biogeophysical studies [Ntarlagiannis et al., 2005; Kenneth H. Williams et al., 2005; Abdel Aal et al., 2010], we investigated the polarization properties of the microbes themselves and associated metabolic activities. We excluded the effects of stimulated sulfate reduction in growth solution and/or sulfide bio-mineral transformations in porous media. The dielectric response of live cells is known to result from movement of accumulated mobile charges at the membrane surfaces under an oscillating electric field at low frequencies (typically <10 kHz, known as $\alpha$-dispersion), or as a consequence of polarization at the interface between the cell membrane and the external medium, giving rise to a Maxwell-Wagner effect (known as $\beta$-dispersion) [Foster and Schwan, 1989]. Most experimental dielectric analysis of biological cell suspensions is limited to the $\beta$ – dispersion in biophysical studies, and almost all biogeophysical research has been restricted to frequencies lower than 10 kHz. In this study, we are the first to explore the dielectric properties of SRB bacteria suspensions over a relatively broader range of frequencies, and determine the dependence of dielectric responses on SRB bacteria concentration and metabolism stages. The recorded changes in $\varepsilon$ and $\sigma^*$ reflect variations in not only bacteria concentration, but also bacteria metabolism stages.

Examining Figure 4.3b and Figure 4.5a, we identify a very high plateau in $\varepsilon$ curves at frequencies <10 kHz. In this range, the $\varepsilon$ of live $D. vulgaris$ suspensions is as high as $10^6$, being 6 to 10 folds higher in the OD 0.37 suspensions relative to the OD 0.28 suspensions. The significant $\alpha$-dispersion depends on not only on the conductivities of the
extracellular medium and cell cytoplasm, but also on the cell size, shape, and biomass concentration [Schwan, 1957; Yang et al., 1999; Di Biasio and Cametti, 2007]. Previous work [Bot and Prodan, 2009] shows that living E. coli cells in pure water have $\varepsilon$ of 10$^5$ and increasing with cell concentration from 10 Hz to 10 kHz. Thus the polarization of D. vulgaris is significantly greater than E. coli. Both D. vulgaris and E. coli are curved rod-shape bacteria with similar cell dimensions (D. vulgaris is ~2 $\mu$m long from our SEM images, whereas the average length of E. coli is reported to be 2 $\mu$m [Trueba and Woldringh, 1980]). Therefore, the morphological difference between the two cells may not explain our observations. Nanostructured FeS$_2$ was found to assemble on the membranes of D. vulgaris from Fe K-edge X-ray near edge absorption spectra. This metal sulfide could contribute to larger polarization of D. vulgaris cells relative to E. coli cells.

The reversal in the dependence of $\varepsilon$ on OD at frequencies >10 kHz was also observed in studies on E. coli [Bot and Prodan, 2009]. This observation was theoretically verified for prolate spheroids [Di Biasio et al., 2010]. The same study showed the higher frequency dispersion could be affected by different strengths of $\alpha$-dispersions resulting from variations in the surface conductivity. The $\alpha$ and $\beta$-dispersions are not clearly separated in our dielectric curves as the two dispersions influence each other heavily and both are very sensitive to the conductivity of the medium. The $\beta$-dispersion would shift to lower frequencies as conductivity of the medium decreases [E Prodan et al., 2008]. In our experiment, we used 5 mM HEPES as the medium for the DS measurements, which has a much lower conductivity compared to other experimental and/or theoretical work where
the $\beta$-dispersion occurred from 10 kHz to 1 MHz [Bai et al., 2006]. Therefore, the $\beta$-dispersion should be shifted to lower frequencies in our study, thereby being integrated with the $\alpha$-dispersion.

We clearly found a significant increase in $\varepsilon$, as well as in both $\sigma'$ and $\sigma''$, due to higher SRB concentrations at frequencies <10 kHz. The SIP study by Abdel Aal et al. [2010] showed no effect on $\sigma'$ with varying concentrations of both live and dead cells of P. aeruginosa in sand columns, but a linear increase in $\sigma''$ (maximum 40%) as live cell concentration increased. In contrast, we found a maximum 10 fold increase in both $\sigma'$ and $\sigma''$ as D. vulgaris concentration increased by 30%. The dielectric signal has previously been reported to be linear with biomass concentration and attributable to the increased number of polarized membranes [Davey et al., 1996]. The DS measurement could therefore serve as a non-intrusive SRB biomass sensor that could be applied in more complex environments.

The polarization of individual microbial cell surfaces is related to the dynamic chemical composition of the cell membrane. The intracellular cation concentrations are functions of the age of the culture, the pH of the medium, and the accumulation of metabolic products. We observed that as bacteria aged from earlier growth stage (26 hours) to later growth stage (73 hours), the $\varepsilon$ decreased at frequencies <10 kHz, while the $\sigma'$ also decreased over the entire measured frequency range. The metabolism of living biological cells has been monitored by DS [Gómez et al., 2002; Sarrafzadeh et al., 2005; Bot and Prodan, 2010], and it shows that the transfer of ions across the cell membrane of living
cells results in a higher conductivity in the suspension surrounding the cells than in an equivalent suspension containing dead cells from which all loosely bound ionic species were removed. Various mechanisms exist for live cells to control osmotic pressure of the cytoplasm, such as the release or uptake of K\(^+\), which is attracted by the negative charges inside the cell \([Gómez et al., 2002]\). However, the cell membrane becomes more permeable when the cell ages, resulting in the decrease of the membrane potential \([Bot and Prodan, 2010]\). Ions (e.g., K\(^+\)) that are forced to concentrate inside of the viable cell by this potential should be able to diffuse back to the surrounding medium, but the concentration of such ions decreases as the cell ages, so the \(\sigma'\) of bacteria suspended in buffer consequently decreases. The decrease in both \(\varepsilon\) and \(\sigma'\) as cell ages is consistent with recent observations in \(Bot and Prodan [2010]\).

4.5 Conclusions

DS can provide an accurate and effective way to distinguish between SRB suspensions with different volume fractions, as well as at different growth stages. The \(\varepsilon\), as well as \(\sigma'\) and \(\sigma''\) were shown to rise with increased \(D. vulgaris\) concentrations, but decrease at frequencies <10 kHz as cells aged. Our results suggest that the electrical signatures obtained in previous biogeophysical studies on SRBs are not only due to the bacterial modification of the geological media, but may also be directly due to SRBs and underlying SRB metabolism processes. Furthermore, DS measurements may afford unique opportunities for non-invasively monitoring SRB bacteria in column-scale experiments on porous media.
Chapter 5. Spectral induced polarization signatures of hydroxide adsorption and mineral precipitation in porous media

Abstract

The spectral induced polarization (SIP) technique is a promising approach for delineating subsurface physical and chemical property changes in a minimally invasive manner. To facilitate the understanding of position and chemical properties of reaction fronts that involve mineral precipitation in porous media, we investigated spatiotemporal variations in complex conductivity during evolution of urea hydrolysis and calcite precipitation reaction fronts within a silica gel column. The real and imaginary parts of complex conductivity were shown to be sensitive to changes in both solution chemistry and calcium carbonate precipitation. Distinct changes in imaginary conductivity coincided with increased hydroxide ion concentration during urea hydrolysis. In a separate experiment focused on the effect of hydroxide concentration on interfacial polarization of silica gel and well-sorted sand, we found a significant dependence of the polarization response on pH changes of the solution. We propose a conceptual model describing hydroxide ion adsorption behavior in silica gel and its control on interfacial polarizability. Our results demonstrate the utility of SIP for non-invasive monitoring of reaction fronts, and indicate its potential for quantifying geochemical processes that control the polarization responses of porous media at larger spatial scales in the natural environment.

3 This work has been published as: Zhang, C., L. Slater, G. Redden, Y. Fujita, T. Johnson, and D. Fox (2012), Spectral induced polarization signatures of hydroxide adsorption and mineral precipitation in porous media, *Environmental Science & Technology*. 10.1021/es204404e.
5.1 Introduction

Proposed approaches for in situ remediation of groundwater contamination include induced mineral precipitation schemes where contaminants are immobilized via coprecipitation or retarded by flowpath diversion. Successful application of such remediation strategies requires understanding of evolution of the relevant reaction fronts, and of changes in media properties due to precipitation. Given the transience of precipitation reaction fronts, the complexity of the reaction networks, and the challenge of accurately capturing potentially widely dispersed changes using traditional point sampling, novel monitoring approaches that sense both media property changes and temporal reaction fronts are needed to support both laboratory and field investigations.

Spectral induced polarization (SIP) is a promising geophysical technique for delineating variations in the properties of solid-fluid and fluid-fluid interfaces in porous media in a minimally invasively manner [Atekwana et al., 2000; Personna et al., 2008; Atekwana and Slater, 2009; William et al., 2009]. Recently SIP has been applied for spatiotemporal monitoring of biogeochemical processes in both laboratory and field settings [Ntarlagiannis et al., 2005; Kenneth H. Williams et al., 2005; Davis et al., 2006; Personna et al., 2008; William et al., 2009]. Applications include monitoring of metal and nutrient cycling by sulfate reducing microbes [Ntarlagiannis et al., 2005; Slater et al., 2007; Personna et al., 2008], iron corrosion [Wu et al., 2005; Wu et al., 2006], and calcite precipitation [Wu et al., 2009; Wu et al., 2010; Wu et al., 2011]. A recent review [Atekwana and Slater, 2009] describes how SIP has the potential to provide information on microbial growth, biofilm formation, and biomineralization in porous media. The
strong polarization of the metal/electrolyte interface results in large SIP signatures when metallic mineral phases (e.g., FeS) are present [Ntarlagiannis et al., 2005; Personna et al., 2008; William et al., 2009]. However, the electrical properties of nonmetallic minerals and associated reaction fronts have been less studied. In one recent experiment [Wu et al., 2010], significant SIP signals were recorded during calcite precipitation on glass beads induced by mixing of CaCl₂ and Na₂CO₃ solutions. These previous studies suggest that SIP may be a valuable technique for non-invasive monitoring of chemical reaction fronts associated with in situ mineral precipitation.

We describe an experiment to evaluate the ability of SIP to monitor the spatiotemporal evolution of processes associated with calcite precipitation driven by urea hydrolysis within a silica gel column, catalyzed by immobilized extracellular urease enzyme. This model system is an abiotic analog to an approach that has been proposed for remediation of contaminants that can be co-precipitated in calcite, where ureolysis is mediated by indigenous subsurface microorganisms [Fujita et al., 2000; Warren et al., 2001; Fujita et al., 2008]. The two primary reactions capable of changing interfacial electrical properties in this system are

\[
(NH₂)₂CO(urea) + 3H₂O \xrightleftharpoons{urease} 2NH₄⁺ + HCO₃⁻ + OH⁻ \quad (5.1)
\]

and

\[
HCO₃⁻ + Ca^{2+} + OH⁻ \rightarrow H₂O + CaCO₃ \quad (5.2)
\]

To aid interpretation of the urease column experimental results, we conducted an additional study focused on the reactivity of hydroxide with silica gel, and specifically to examine whether (1) hydroxide adsorption affects SIP signals, and/or (2) changes in SIP
signals can be used to discern between varying reaction fronts at solid-solution interfaces. Our study could improve interpretation of geophysical data collected for monitoring of in situ mineral precipitation.

5.2 Spectral Induced Polarization

SIP measures the impedance magnitude and phase shift (φ) of a received sinusoidal voltage across a sample relative to the current waveform over a range of frequencies (typically < 1 kHz). The magnitude and phase (φ) are converted to a complex conductivity (σ*(ω)) using the physical geometry of the sample. The dependence of σ*(ω) on geologic matrix properties such as pore/grain size, permeability, and surface area has been extensively explored [Börner and Schön, 1991; Lesmes and Morgan, 2001; Slater and Lesmes, 2002a; Slater and Glaser, 2003].

The measured complex conductivity σ*(ω) of a system can be expressed as

\[ \sigma^*(\omega) = \sigma'(\omega) + i\sigma''(\omega) \] (5.3)

The real conductivity (σ') represents conduction (energy loss), whereas imaginary conductivity (σ'') represents polarization (energy storage).

\[ \sigma' = |\sigma| \cos \phi \] (5.4)

\[ \sigma'' = |\sigma| \sin \phi \] (5.5)

where |σ| is the conductivity magnitude calculated using the impedance magnitude and appropriate geometric factors.

At frequencies < 1 kHz, σ*(ω) of a saturated porous material in nonmetallic
environments can be modeled as the sum of an electrolytic conduction \( \sigma_{\text{ele}} \) within the fluid-filled interconnected pore space and a surface conduction \( \sigma_{\text{surf}}^* \) occurring within an electrical double layer (EDL) at the grain-fluid interface [Lesmes and Frye, 2001]. It is usually assumed [Waxman and Smits, 1968; Vinegar and Waxman, 1984] that the electrolytic and surface conduction paths add in parallel such that

\[
\sigma' = \sigma_{\text{ele}} + \sigma_{\text{surf}}^*
\]  

(5.6)

and

\[
\sigma'' = \sigma_{\text{surf}}^*
\]  

(5.7)

The electrolytic conductivity \( \sigma_{\text{ele}} \) is expressed as \( \sigma_{\text{ele}} = (1/F) \times \sigma_f \), where \( \sigma_f \) is fluid conductivity, and \( F \) is the electrical formation factor. Assuming electrical conduction is only through electrolyte-filled pores, then \( F \) describes the decrease in conductivity of the rock due to the presence of the insulating mineral grains [Archie, 1942].

Mechanistic models describing \( \sigma_{\text{surf}}^* \) have recently been developed, and relationships between microgeometry (grain-size distributions, pore diameter, and pore-size distributions) and SIP signals are reported, mostly single rock formation [Scott and Barker, 2003; Binley et al., 2005; Scott and Barker, 2005]. However, the role of fluid chemistry was until recently overlooked. Vaudelet et al. [2011] recently examined the SIP signals measured during advective flow of a CuSO₄ solution in a sand column, and developed a speciation model for adsorption of sodium and copper onto the silica grain surface. Skold et al., 2011 introduced a similar model, but included a proton hopping process, which allowed predictions of SIP measurements made with varying pH and salinities. Such developments enable quantitative interpretation of specific sorption
Phenomenological models, such as the Cole-Cole model \cite{Cole1941}, can instead be applied to observed SIP responses of porous media. Although these models are not directly parameterized in terms of microgeometric and/or geochemical parameters, parameters such as the chargeability and relaxation time constant are related to microgeometry \cite{Titov2002, Binley2005} and pore fluid chemistry \cite{Lesmes2001}. A popular variant of the Cole-Cole model can be written as \cite{Cole1941, Pelton1978}

\[
\sigma^*(\omega) = \sigma_0 \left[ 1 + m \left( \frac{(i\omega \tau)^c}{1 + (i\omega \tau)^c(1-m)} \right) \right]
\] (5.8)

where \(\sigma_0\) is the DC-conductivity, \(m\) is the chargeability describing the magnitude of polarization associated with interfacial charge storage, \(\tau\) is the characteristic relaxation time constant related to characteristic pore or grain size \cite{Kruschwitz2010}, and \(c\) is a shape parameter (0.2-0.8 for unconsolidated soils). Integrating parameters from a Debye decomposition have also been studied to quantify the dependence of polarizability on pore fluid composition in sandstone samples \cite{Weller2011}. The \(\tau\) for polarization of the EDL surrounding a spherical grain of diameter \(d\) (m) is defined by Schwarz \cite{Schwarz1962} as

\[
\tau = \frac{d^2}{8D_s} = \frac{d^2}{8\beta_s kT/e}
\] (5.9)

where \(\beta_s\) is the effective ion mobility (in \(\text{m}^2\text{s}^{-1}\text{V}^{-1}\)), \(k\) is Boltzmann’s constant \((1.381 \times 10^{-23} \text{JK}^{-1})\), \(T\) is absolute temperature (K), and \(D_s = \beta_s kT/e\) is the surface diffusion coefficient (in \(\text{m}^2\text{s}^{-1}\)). This model predicts that \(\tau\) is proportional to grain size and inversely
proportional to ion mobility.

5.3 Materials and Methods

5.3.1 Experiment 1: Urease mediated mineral precipitation

Silica gel (Sigma-Aldrich 236802, Grade636) was used as the porous media due to its ability to immobilize urease enzyme on its external surface. The particle size was 250-500 \( \mu \text{m} \), surface area 480 \( \text{m}^2/\text{g} \), intragranular porosity 38\%, and the internal pore diameter 60 Å. Prior to packing, the silica gel was combined with 50 mM NaCl in a slurry, degassed under vacuum, and titrated with NaOH to neutral pH. Jackbean urease (Sigma-Aldrich, 091K7046) was adsorbed onto the silica gel by slowly stirring enzyme dissolved in 50 mM NaCl with the silica gel slurry for one hour. The enzyme was expected to be at least twice the pore size \cite{Benini1998} of the silica gel and therefore assumed to reside only on the external surface. The silica gel was packed into a polycarbonate (Lexan) column (48cm, inner diameter 5.79 cm), with the urease-coated section restricted to a 16.2 cm interval midway through the column (Figure 5.1).

The column was operated in an upflow configuration. It was first flushed with 50 mM NaCl background solution (all influent solutions mentioned hence contain 50 mM NaCl) for two pore volumes (PV; 1 PV = 916 mL). This was followed by a bromide tracer test (22.5 mM LiBr; 20 ml/min). After removal of the bromide tracer, a 10 mM urea solution was introduced into the column at 10 ml/min for 544 minutes (Stage 1). Next a solution containing urea (10 mM), CaCl\(_2\) (10 mM), and SrCl\(_2\) (0.1 mM) was injected at 10 ml/min for 4400 minutes (Stage 2). The pH indicator phenol red (0.005 mM) was added to all
influent solutions to facilitate visual observation of pH changes using an automated image capture system. The dye transitions from yellow to red over pH 6.8 to 8.2, and becomes bright pink at pH >8.2.

![Figure 5.1 Photograph of urea hydrolysis mediated mineral precipitation experiment](image)

The urease enzyme has been immobilized 6 mm upstream of Zone 3 to 4 mm downstream of Zone 5. As the inflow carried urea into the column, the urea was hydrolyzed by urease and hydroxide ions were produced. The pH indicator turned pink where the pH was >8.2 in the urease and post-urease zones.
Local solution chemistry was monitored by extracting fluid samples from ports (P1-P9) installed at 5 cm intervals along the column (Figure 5.1). The pH and fluid conductivity ($\sigma_f$) were measured immediately in collected samples. The Na$^+$, NH$_4^+$, and urea concentrations in acid-preserved samples were measured by ion chromatography, and the distribution of solid Ca and Sr at the end of Stage 2 was determined by inductively coupled plasma mass spectrometry (ICP-MS) analysis of excavated samples (digested in 10% HNO$_3$).

Nine Ag-AgCl potential electrodes were installed in electrolyte-filled chambers on the side of the column opposite the sampling ports. Spiral Ag-AgCl current electrodes were installed on both end caps with the same inner diameter as the column to facilitate homogeneous electric flow. Real time SIP measurements were performed with a full waveform, multi-channel broadband complex resistivity instrument, with an error of 0.1 mrad for $\varphi$ and less than 1% for $|\sigma|$ [Wu et al., 2008; Wu et al., 2009]. Nine measurement channels were available; one was designated to record the current waveform by measuring the response across a precision reference resistor in series with the column, and the remaining channels recorded the potential response on adjacent electrode pairs (Zone 1 to Zone 8; Figure 5.1). Zones 3, 4, and 5 were within the urease interval. SIP measurements were collected every two minutes without flow interruption using acquisition software described in [Versteeg et al., 2006]. Measurements were acquired at 100 Hz for Stage 1, and over the frequency range 0.1 to 1 kHz for Stage 2, over a total duration of 1390 minutes, spanning Stage 1 and early Stage 2.
5.3.2 Experiment 2: Hydroxide ion adsorption experiment

The silica gel section was centered between Ottawa sands, 17 Ag/AgCl electrodes were installed on one side of the column with intervals of 2.5 cm, and 9 sampling ports (5 cm apart) were installed on the other side of the column. A two-channel dynamic signal analyzer (DSA) from National Instruments (NI 4461) was employed here to perform the SIP measurements over a range of frequencies. Two AD620 preamplifiers were used separately across the sample channel as well as the reference channel to increase the input impedance, minimizing spurious effects that may arise from the impedance of the potential electrodes. The current was injected through the coiled Ag-AgCl electrodes at both ends of the column with 1 V applied voltage, and the resulting potential was recorded between Ag-AgCl electrodes spanning both Ottawa sand zones (E1-E3, E3-E5, E13-E15, E15-E17) and the silica gel zone (E6-E9 and E9-E12).

Figure 5.2 Sketch of the setup of hydroxide ion adsorption experiment
A similar Lexan column design was used in this experiment as for Experiment 1, except that 17 non-polarizing Ag-AgCl electrodes (E1-E17) were installed at 2.5 cm intervals (Figure 5.2). The first and last 15.7 cm of the column were packed with Ottawa sand (0.300-0.212 mm), whereas the intervening section (16.6 cm) was packed with the same type of silica gel (prepared as described previously; no urease) used in Experiment 1. The Ottawa sand/silica gel interfaces were centered on electrodes 6 and 12 (Figure 5.2). The column was again operated in upflow mode and flushed with 50 mM NaCl (pH=7, $\sigma_f = 0.51$ S/m) until stable pH and $\sigma_f$ was achieved. Porosity of the sand was calculated to be 39.5% from wet and dry weight measurements. The effective porosity of the silica gel was estimated at 72.6% based on the Experiment 1 tracer test.

This experiment was conducted over 9 days with intermittent injection of 50 mM NaCl (constant $\sigma_f = 0.51$ S/m) at 10 mL/min. For the first 13 hours on both Day 0 and Day 1, the injected solution was adjusted to pH 7. It was then replaced by the same solution adjusted to pH 8 on Day 2 for 8 hours. From Day 3 until the end of the experiment (Day 8), the solution was adjusted to pH 10 and flowed through the column for a total of 40 hours (6 ~ 8 hours per day). Phenol red (0.005 mM) was included in all solutions, and pH adjustments were made using 0.3 M NaOH (all influent solutions were unbuffered). The influent solutions were housed in pyrex vessels closed with a rubber stopper fitted with ascarite® traps to mitigate acidification by atmospheric CO$_2$. All tubing was wrapped with parafilm to reduce CO$_2$ diffusion. This experiment was performed at room temperature (22.6 ± 0.6 °C).
The $\sigma_f$ and pH were monitored daily in the influent, effluent, and samples extracted from ports P1 to P9. A two-channel dynamic signal analyzer (National Instruments 4461) was employed to record SIP signatures over a frequency range from 0.1 Hz to 1 kHz at 100 logarithmic intervals on a daily basis. The measurement error is less than 1% for $|\sigma|$ and $\approx 0.1$ mrad for $\phi$ below 100 Hz. The current was injected through the coiled Ag-AgCl electrodes at both ends of the column and the resulting potential differences were recorded between Ag-AgCl electrode pairs spanning both sand zones (E1-E3, E3-E5, E13-E15, and E15-E17) and the silica gel zone (E6-E9 and E9-E12). The magnitude and $\phi$ were measured relative to a known high precision resistor on channel 1.

### 5.3.3 Spectral Induced Polarization data modeling

The SIP datasets from Experiment 2 were modeled with Equation 8 using a two-step procedure. First, a Bayesian model using a Markov-chain Monte Carlo (MCMC) based method was used to obtain the medians of unknown parameters by starting from an arbitrary set of initial values. This step provides marginal probability distributions but not explicitly optimal solutions [Chen et al., 2009]. The medians of the parameter estimates were then used as the initial values for an iterative least-squares deterministic method with Marquardt regularization [Kemna, 2000]. However, there were uncertainties and non-uniqueness in the parameter estimation. For example, the correlation matrix reveals that $m$ is highly correlated with $\tau$, the average correlation coefficient between these two parameters being 0.67 for measurements in silica gel. This problem is frequently experienced, especially for small measurement signals, and is not easily overcome.
5.4 Results and Discussion

5.4.1 Experiment 1: Urease mediated mineral precipitation

Scanning electron microscopy (SEM) images of solids excavated from another column with similar experimental conditions after the conclusion of the experiment (Figure 5.3) showed that crystals (CaCO₃ composition inferred by energy dispersive spectroscopy) were produced at grain-grain contacts with a length scale of 100 – 200 µm. The ICP-MS analyses showed that mineralized Ca²⁺ reached 0.15 mmol/g in the urease zones, and that precipitation continued downstream, with decreased mineralized Ca²⁺ concentrations at the end of experiment (Figure 5.4).

Figure 5.3 Scanning electron microscopy (SEM) images of CaCO₃ precipitation

Scanning electron microscopy (SEM) images of CaCO₃ precipitation showed that the calcium carbonate precipitation concentrated at the grain-grain interfaces, and the localized precipitation had insignificant impact on the porosity changes, which was confirmed by bromide tracer test. The images were from a similar experiment with the same design and configuration (a different flow rate was used).
The ICP-MS analyses of distribution of solid Ca and Sr in the column from destructive sampling of the solid media at the end of Experiment 1. The excavated samples were digested in 10% HNO$_3$.

5.4.1.1 Fluid conductivity ($\sigma_f$) and real conductivity ($\sigma'$)

The hydrolysis of urea, with accompanying changes in solution chemistry and the subsequent formation of carbonate precipitates, was reflected in the SIP measurements. The $\sigma'$ and the $\sigma_f$ data at different locations (Zones 1, 5, and 7) along the column are shown in Figure 5.5a, and the data for all zones (Zones 1 to 8) are plotted in Figure 5.6. Note that for the individual zones, the measured pH and $\sigma_f$ values at the two adjacent sampling ports were averaged to assist comparison with $\sigma^*(\omega)$.
Figure 5.5 Real and imaginary conductivity, fluid conductivity, and pH in Zones 1, 5, and 7

Experiment 1. (a) Real ($\sigma'$) and fluid conductivities ($\sigma_f$) and (b) imaginary conductivities ($\sigma''$) and pH changes in the pre-urease (Zone 1 as representative), urease (Zone 5 as representative), and post-urease zones (Zone 7 as representative) during ureolytically driven mineral precipitation experiment. The urea injection stage occurred from 0 to 544 minutes (Stage 1), followed by the co-injection of urea, Ca$^{2+}$, and Sr$^{2+}$ (Stage 2) began until the end of the experiment, and the two stages are separated by the vertical line. The $\sigma'_{\text{surf}}$ data are from 100 Hz.

In pre-urease zones (Zone 1 as representative), $\sigma_f$ was steady at 0.531 S/m during Stage 1 and $\sigma'$ also remained steady (≈ 0.33 S/m). During Stage 2, the $\sigma_f$ increased to 0.7 S/m due to the introduction of Ca$^{2+}$ and Sr$^{2+}$ into the column, and remained elevated through the experiment. Similarly, the $\sigma'$ also increased during Stage 2 to $\approx$ 0.42 S/m. The changes in $\sigma'$ and $\sigma_f$ in the pre-urease zone were directly proportional throughout the experiment, consistent with a dominant control of electrolytic conduction ($\sigma_{\text{ele}}$) on $\sigma'$, and it resulted in a formation factor of 1.64.
Figure 5.5 Real and imaginary conductivity, fluid conductivity, and pH in all zones

Real ($\sigma'$) and fluid conductivities ($\sigma_f$) (top row, (a) – (c)), imaginary conductivities ($\sigma''$) and pH changes (bottom row, (d) – (f)) in the pre-urease, urease, and post-urease zones during mediated ureolytically driven precipitation experiment. The urea injection stage occurred from 0 to 544 minutes (Stage 1), followed by the co-injection of urea, Ca$^{2+}$, and Sr$^{2+}$ (Stage 2) began until the end of the experiment, and the two stages are separated by the vertical line. The complex conductivity data are at 100 Hz.

In the urease zones ureolysis produces NH$_4^+$, HCO$_3^-$, and OH$^-$ (Equation 1), which will increase both $\sigma_f$ and $\sigma'$. In Zone 5 (as representative of the urease zone), $\sigma_f$ increased to 0.62 S/m but decreased later at the end of Stage 1 due to possible downstream migration of enzyme, which would have lowered the activity within the original urease zones. Urea arrival in Zone 5 during Stage 1 was evident in $\sigma'$ data as a consequence of the production of ionic species at the beginning of Stage 1. Afterward, the $\sigma'$ in Zone 5 increased significantly and reached a plateau at $\approx$ 0.52 S/m towards the end of Stage 1. With the onset of Stage 2, $\sigma_f$ initially increased then dropped thereafter. The increase in $\sigma'$
at the beginning of Stage 2 was associated with the arrival of Ca\(^{2+}\) and Sr\(^{2+}\). Although changes in pore space and \(\sigma_f\) in the urease zones during Stage 2 were very small, apparent formation factor calculated from the ratio of \(\sigma_f\) to \(\sigma'\) increased after the precipitation, consistent with a decrease in \(\sigma_{surf}^*\) as confirmed by \(\sigma''\) measurements. This was presumably due to lower surface charge and lower surface area of CaCO\(_3\) precipitation relative to silica gel [Eriksson et al., 2007].

In post-urease zones (using Zone 7 as representative), the \(\sigma_f\) changes were consistent with those observed in urease zones during Stage 1 except that the \(\sigma_f\) values were higher (\(\approx 0.64\) S/m) due to arrival of ions generated from upstream ureolysis. The correlation between \(\sigma_f\) and \(\sigma'\) is strong (\(R^2=0.96\)), and the \(\sigma'\) first increased when Stage 1 was initiated and remained relatively stable initially, although it started gradually increasing during the latter part of Stage 1. This may have been due to migration/spreading of the urease zone as a consequence of incomplete enzyme immobilization. With the onset of Stage 2, the \(\sigma'\) increase was much steeper before decreasing after 860 minutes. Qualitatively, the trends in \(\sigma_f\), \(\sigma'\), and pH were consistent with the migration of ionic species (background electrolyte and reaction products) coupled with ion depletion by CaCO\(_3\) precipitation.

### 5.4.1.2 The pH and imaginary conductivity (\(\sigma''\))

The \(\sigma''\) data compared with pH changes in Zones 1, 5, and 7 are plotted in Figure 5.5b, and the data in all zones are shown in Figure 5.6 (the \(\phi\) data are also plotted in the same manner in Figure 5.7). The pH in Zone 1 was steady around 7.5, and \(\sigma''\) was \(\approx 0.00072\)
S/m and remained steady during the urea injection. With Stage 2, the pH dropped in the pre-urease zones due to pH changes in the influent, and the $\sigma''$ increased slightly in Zone 1.

An important observation in the urease zones during Stage 1 is the correlation between pH and $\sigma''$ ($R^2 > 0.98$). The pH increases due to ureolysis were observed first in Zone 3, then 4 and 5, and the $\sigma''$ also increased in Zones 3 to 5 in that order. The polarization magnitude in urease zones was much higher than in the pre- and post-urease zones. The $\sigma''$ in Zone 5 increased to a peak of 0.003 S/m close to the end of Stage 1. Early in Stage 2, the pH decreased due to proton production from calcium carbonate precipitation. The $\sigma''$ dropped in all urease zones during Stage 2, coincident with the carbonate mineral precipitation. The variations of phase data (Figure 5.7) were similar to $\sigma''$ data, and also associated with pH changes in urease zones.

In post-urease zones, the pH was stable ($\approx 7.5$) during Stage 1, but increased during Stage
2. The pH in Zone 7 peaked at 950 minutes and then decreased by the end of the experiment. This can be explained by the migration of OH\(^-\) formed during Stage 1 that continued after the start of stage 2. During Stage 1, the \(\sigma''\) was much smaller (\(\approx 0.0004 \text{ S/m}\)) in the post-urease zones than in the urease zones. It increased slightly in Zone 7 near the end of Stage 1. After Stage 2 started, the \(\sigma''\) in Zones 7 first increased, but then fell again, although it was still higher than the initial Stage 1 value at the end of the experiment. The \(\sigma''\) changes coincided with changes in \(\sigma'\) in these three post-urease zones.

5.4.1.3 Polarization mechanisms

In this highly porous and high surface area medium, adsorption of ions on the surface of silica gel is significant. Ion or charge accumulation at the surface would presumably contribute to an increase in surface polarization, and therefore the \(\sigma''\). The OH\(^-\) adsorption, for example, would increase the negative surface charge, which would then attract counter-ions (i.e., H\(^+\), Na\(^+\), NH\(_4^+\), Ca\(^{2+}\), Sr\(^{2+}\)) into the Stern layer.

During Stage 1, \(\sigma^*_{\text{surf}}\) has risen as indicated by increases in \(\sigma''\). This could be attributed to an accumulation of surface charge resulting from adsorption of OH\(^-\) or other ions. Similarly, both \(\sigma'\) and \(\sigma''\) gradually reduced without significant changes in \(\sigma_f\) in the downstream direction during Stage 2, suggesting that \(\sigma^*_{\text{surf}}\) decreased as CaCO\(_3\) precipitation occurred and pH decreased. These observations indicate that \(\sigma^*_{\text{surf}}\) (not \(\sigma_{\text{ele}}\)) primarily controls the SIP responses to CaCO\(_3\) precipitation.
Mineral precipitation can result in changes in surface area, formation porosity, and pore throat diameter, with significant impact on SIP signals [Atekwana and Slater, 2009]. The electrical signals from precipitation of nonmetallic minerals such as calcite are small and associated with changes in pore volume/pore tortuosity and/or surface area/surface roughness [Atekwana and Slater, 2009]. However, both SEM image and tracer test suggests porosity change during Stage 2 was insignificant (≈1%) as the precipitation of CaCO$_3$ was localized at the grain-grain contacting points, indicating the contribution of pore space to decreased $\sigma^*_{surf}$ is negligible. Instead, the CaCO$_3$ precipitation has lower surface area relative to silica gel, and presumably reduced the total surface charge by forming a less negatively charged surface on the silica gel in the pH range 6-10 [Eriksson et al., 2007]. In addition, calcium adsorption on silica gel is strong and would also lower the total surface charge.

Our observations can be compared to [Wu et al., 2010], who conducted a column experiment with 3mm glass beads and observed increased polarization responses during calcite precipitation. They suggested that the increase of $\sigma''$ during a discrete precipitation phase was caused by an increase in total surface area, due to the accumulation of small, well distributed, discrete calcite particles (average size < 5 µm) relative to the glass beads. They specifically attributed the SIP response to polarization of the EDL surrounding calcite crystals. In our experiment, the porous matrix has much higher specific surface area than glass beads, and the surface area changes caused by CaCO$_3$ precipitation in silica gel is likely negligible. However, CaCO$_3$ should have lower surface charge than silica gel [Bolt, 1957; Eriksson et al., 2007], resulting in a decrease of $\sigma''$. In
a recent ureolytically-driven CaCO$_3$ precipitation study using natural sediment materials [Wu et al., 2011], an increase of $\sigma^\prime$ was not observed during the precipitation, but a positive correlation between $\sigma_f$ and $\sigma^\prime$ was observed during early urea hydrolysis, and was attributed to production of NH$_4^+$ and adsorption of hydroxyl ions on the mineral surface.

5.4.2 Experiment 2: Hydroxide ion adsorption experiment

5.4.2.1 The pH profile

![Figure 5.8 The pH profile in Experiment 2](image)

Experiment 2. The pH profile in the hydroxide adsorption experiment. The change of pH was monitored by extracting fluid samples at the 9 sampling ports as well as in the influent and effluent tubing.

Figure 5.8 shows the pH profile along the column in Experiment 2. As the influent pH increased from 7 to 10, the pH of samples from the bottom sand section (P1 to P3, Figure 5.2) rose to values close to the influent pH values. The slightly lower pH in P1 to P3
relative to the influent was likely due to buffering by the sand matrix. In the silica gel section (P4 to P6, Figure 5.2), the pH remained at values similar to the initial influent throughout the experiment, except for the pH at P6 on Day 5 and 6. There was no observable pH change in the upper sand section (P7 to P9, Figure 5.2) as the influent pH increased. The pH profile in the column suggests that OH$^-$ were buffered by (adsorbed by) the silica gel and the buffer capacity had not been exceeded by the end of the experiment.

5.4.2.2 The imaginary conductivity ($\sigma''$) dependence on pH

The spectral $\sigma''$ changes in silica gel as well as in the upstream sand section from each day are shown in Figure 5.9a. In the silica gel, the $\sigma''$ in the first two days were similar. When the influent pH increased on Day 2, $\sigma''$ also increased. The $\sigma''$ increased further when the influent pH increased to 10 on Day 3. By Day 4 the rate of increase had slowed and it reached a maximum near 1 Hz on Day 8. The characteristic critical frequencies (peak frequencies) for all of the SIP measurements were approximately 0.9 Hz.

The $\sigma''$ in the sand sections were much weaker (one magnitude lower compared to silica gel); only the signals from the upstream section are shown as the measurements in the downstream section were below the noise threshold ($\approx$0.1 mrad). With the influent pH increase, the $\sigma''$ in the sand first increased, but then decreased on Days 7 and 8. There were no consistent variations in the characteristic critical frequency. The spectral phase ($\varphi$) changes and pH dependence in both silica gel and sand are shown in Figure 5.10.
Experiment 2. (a) The imaginary conductivities (\( \sigma'' \)) from 0.1 Hz to 100 Hz in both silica gel and sand over the experimental duration. (b) The Cole-Cole model parameter normalized chargeability \( m_n \) for silica gel, the \( \sigma'' \) at critical frequency (0.9 Hz) for both silica gel and sand, and the pH of the influent. Data are from measurements between electrode pair 6 and 9 in the silica gel, and pair 1 and 3 in the sand.

Figure 5.9b shows the \( \sigma'' \) changes in silica gel and sand over time at the characteristic critical frequency (0.9 Hz), normalized chargeability \( m_n \) (\( m_n = m \times \sigma_0 \)) in silica gel over time and compared with influent pH. The \( \sigma'' \) was stable at 0.0009 S/m for the first two days, increasing by about 10% when the influent pH increased from 7 to 8. When the influent pH changed to 10, the \( \sigma'' \) increased and reached a plateau by Day 4, while the pH of the effluent stabilized around 6.8 throughout the rest of the experiment. The behavior of \( \sigma'' \) suggests that OH\(^-\) adsorbed onto silica gel after Day 4 did not contribute further to
Figure 5.10 Phase response of silica gel and sand

The spectral phase responses from 0.1 Hz to 100 Hz in both silica gel and sand over the experimental duration. Subplot (b) is a close-up look of phase responses in the sand. Data are from measurements between electrode pair 6 and 9 in the silica gel, and pair 1 and 3 in the sand.

The trend for $\sigma''$ (0.9 Hz) was very similar to the $m_n$ changes over time in silica gel, but the value of $m_n$ was 4 – 5 fold higher than $\sigma''$, since the $\sigma''$ here represents a single frequency whereas $m_n$ is the integrated measurement of charge storage over the investigated frequency range. The increased adsorption of OH$^-$ on the silica gel presumably caused the increase of $m_n$, which was apparent from Day 3 when the pH of the influent increased to 10. The $m_n$ decreased on Day 6 and stayed relatively stable thereafter. The $\sigma''$ responses in the sand were much smaller than in the silica gel, and differences between the $\sigma''$ values over time were insignificant.

We attribute the much weaker signals observed in the sand to the much smaller total surface area to pore volume ratio compared to silica gel. With similar particle size (300 - 212 µm) but no internal porous structure, the sands have surface area < 0.1 m$^2$/g. In
contrast, the surface area of the silica gel is approximately 480 m$^2$/g, which includes internal porosity. The $\sigma_{\text{surf}}^*$ is primarily controlled by the surface area, surface charge density, and surface ionic mobility [Lesmes and Frye, 2001; Slater and Lesmes, 2002a; Slater and Glaser, 2003]. In Experiment 2 with constant ionic strength and specific surface area, the surface charge density and effective mobility of ions in the Stern and diffuse layers of the EDL primarily determined the magnitude of the $\sigma_{\text{surf}}^*$ responses. [Lesmes and Frye, 2001] investigated the influence of pore fluid ionic strength and pH upon the SIP responses of sandstone. They reported that $\sigma''$ was dependent on pH, being reduced at pH 3, which is near the point of zero charge (pzc) of silica. In the case of silica minerals, the experimental data as well as model predictions of [Leroy et al., 2008] indicated that the surface charge density increased with increasing pH (pH > pzc) at salinity between $10^{-1}$ M and $10^{-3}$ M. [Skold et al., 2011] also showed increased $\sigma''$ as pH increased above the pzc in sand saturated by 0.01 M NaCl.

### 5.4.2.3 The Cole-Cole parameters $m$ and $\tau$

Figure 5.11a shows the change of the $m_n$ and $\tau$ over time in Experiment 2. The $m_n$ was well resolved from the model in both sand and silica gel. The $m_n$ for sand was significantly smaller than for silica gel, and it tracked the change in $\sigma''$ as expected (both being measures of the polarization magnitude). The $\tau$ in the silica gel had a similar pattern as $m_n$, slowly increasing to a maximum on Day 5, decreasing thereafter. It is reasonable to interpret changes in $\tau$ to changes in $\beta_s$ (Equation 9). However, this would imply an increase in $\beta_s$ after Day 5, which is unreasonable given continued accumulation of charge at the surface. Furthermore, the $\sigma''$ stayed stable after Day 5, consistent with a maximum
surface charge density on the surface sites. Therefore, the significance of changes in $\tau$ is uncertain, and possibly complicated by the non-uniqueness of this parameter. In the following section, we propose a conceptual model for OH$^-$ adsorption and polarization behavior in silica gel that could explain this plateau.

**Figure 5.11 Cole-Cole parameters**

(a) The Cole-Cole model parameters normalized chargeability $m_n$ for both silica gel and sand, and relaxation time $\tau$ for silica gel. (b) The $m_n$ and $\sigma''$ correlations in the silica gel.

### 5.4.3 Model for hydroxide adsorption on silica gel

Figure 5.12 is a conceptual representation for the formation of surface charge on silica gel resulting from reactions with OH$^-$. The silica gel matrix consists of silicon atoms joined by oxygen atoms in siloxane bonds. On contact with water, hydration of the silica surface
Figure 5.12 Conceptual model of hydroxide adsorption on silica gel

The conceptual representation for the formation of surface charge on silica gel resulting from hydroxide ion adsorption. The primary structure of silica gel is shown in subplot (a), and the hydration reaction and the reaction with OH\(^-\) in solution are shown in subplot (b) and (c) respectively. The electrical double layer (EDL) formed at the silica gel surface is also shown in the subplot (c).
initially produces multiple types of surface hydroxyl groups, and the surface of SiO$_2$ has a net negative change above pH 2.5-3. Addition of OH$^-$ drives the deprotonation reaction forward and increases the concentration of “fixed” negative surface (often described as OH$^-$ adsorption). Formation of these fixed charge results in the formation of the EDL on the surface of primary backbones of silica gel, which can be modeled as a consisting of a Stern layer of mobile counter ions that move tangentially along the surface and an outer diffuse layer. The arrangement of surface charges in the EDL with varying mobilities contributes to the SIP effect in the presence of an external electric field. We neglect the specific adsorption of Cl$^-$ anions at the surface since that usually occurs at pH < 3 \cite{Leroy et al., 2008} where the net charge is close to zero, but where positively charged (protonated) sites can be present.

The OH$^-$ ions are absorbed not only on the surface of the primary backbone, but possibly also in the numerous inner micro-pore structures. However, due to the small size of these pore structures and subsequent condensation reactions in silica gel, transport of Na$^+$ (a counterion of OH$^-$) may be restricted (Na$^+$ hydrated radius $0.79 \times 10^{-9}$ m) and will have less impact on the SIP signatures. We assume that the accumulation of OH$^-$ happened at a later stage (after Day 4) in our study, and this explains the plateau in $\sigma''$ since the excess negative charges are being stored in the internal structure. Because the chargeability $m_n$ relates to the polarizable interfacial charge storage, the behavior of $m_n$ (Figure 5.9b) is consistent with our conceptual model that the accumulation of OH$^-$ at the later stage did not contribute to the polarizability.
Similarly to the observations in Experiment 1, we attribute the polarization responses observed in Experiment 2 to electrochemical polarization occurring at the EDL. The EDL polarization involves charge movement in both the fixed and diffused layers, and pH has an important influence on the polarization. The size of the mineral particle is fixed, so the relaxation time is determined primarily by charge density and charge mobility in the EDL [Schwarz, 1962; Lesmes and Morgan, 2001]. We assert that the observed dependence of polarization on pH may reflect the relative contributions of charge density to the net surface conductivity response of the sample. Our conceptual model proposes that the adsorption of OH\(^-\) onto the primary silica gel surface contributes to the accumulation of negative surface charge, increasing the $\sigma^{*}_{\text{surf}}$. In reality, various surface conduction and competing physicochemical mechanisms likely combine to yield the net surface conductivity of the sample. To test the relative importance of the proposed OH\(^-\) adsorption behavior on the silica gel as illustrated in the conceptual model, further studies should analyze the inner structure of the material and characterize ion adsorption behavior.

We have shown that the SIP technique is sensitive enough to capture surface chemistry and associated interfacial properties of a solid-fluid surface. Our SIP measurements in the column experiments demonstrate the sensitivity of electrical geophysical methods to subtle and transient changes in porous media due to transport of ions and/or reactions such as precipitation and adsorption. The results suggest that pH controls the polarization response in silica gel. Although silica gel is a synthetic material, and the pH dependence of SIP is likely weaker for natural porous media, we believe our findings are still relevant.
to the assessment of methods for sensing and characterizing physical and chemical/biogeochemical events linked with subsurface remediation at larger spatial scales of natural environment. SIP can play an important role in improving the understanding of complex processes in porous media in both laboratory and field settings.
Chapter 6. Conclusions and future work

6.1 General conclusion

The results from laboratory experiments in these three topics provided information on the electrical geophysical responses of microbial cells, microbial processes and bio-mineral transformations, and evolution of physiochemical properties in mineral precipitation. The research examined the electrical geophysical signatures associated with remediation of metals in the subsurface and improved better understanding of biogeochemical processes at the mineral-fluid interfaces in the porous media.

For all the experiments, geochemical measurements were performed to facilitate the interpretation of collected geophysical data. In the first two research topics, microbiological analysis were also acquired in addition to geochemical data to support the geophysical observations and provide fundamental knowledge about SRBs. The geophysical detection of SRBs biomass and growth and SRB mediated sulfate reduction activities in natural river water suggested that electrical geophysical techniques could efficiently monitor the microbial processes and associated contaminant degradation processes. The urea hydrolysis mediated calcium carbonate precipitation experiment showed the potential of electrical geophysical methods (1) to track the position and chemical properties of reaction fronts involving mineral precipitation, and (2) to quantify geochemical processes at larger spatial scales in the natural environment.

Since common metal remediation efforts involve the microbial and chemical mediated
reduction of metals, this work could be applied in environmental remediation strategy and electrical geophysical methods could serve as a promising cost-effective monitoring tool.

### 6.2 Primary scientific findings and significance

In Chapter 3, multiple electrical measurements from microbial sulfate reduction in porous media revealed that large open-circuit potentials (>550 mV) were recorded at the reactive Ag/AgCl electrodes, and were generated from microbial sulfate reduction process in natural river water. The localized HS⁻ concentration at the measurement electrodes was estimated given EP and pH measurements based on Nernst equation and certain assumptions. However, SP signals were consistently only 1-8 mV in both biologically active column and control column, which probably represented the streaming potential due to the flow of the saturating fluid through the column. And it suggested no measureable geobattery (SP) effect associated with this microbial process. The SIP measurements showed increased imaginary conductivity in tandem with net microbial growth in our system. Thus, the electrical geophysical measurements, coupled with a reactive electrode measurement sensitive to pore fluid redox chemistry, could be a diagnostic tool for microbial drive sulfate reduction in the subsurface.

To further investigate (1) the inherent electrical properties of SRBs themselves and (2) how they might directly contribute to the electrical geophysical responses observed during microbial-mineral transformations, the study in Chapter 4 concentrated on the low frequency dielectric properties of *Desulfovibrio vulgaris* cell suspensions. In this topic,
two-electrode impedance spectroscopy was used to measure the complex dielectric response of *D. vulgaris* cell suspensions at varying concentrations (OD = 0.28 and 0.37) and at different growth stages (26, 49, and 73 hours of growth). At frequencies < 10 kHz, the relative dielectric permittivity, real and imaginary conductivity increased as bacteria concentration increased. As bacteria grew from mid-log phase to late stationary phase, the permittivity and complex conductivity were reduced. The subtle differences in permittivity and complex conductivity were resolved from electrode polarization error removal. This study is the first to explore the dielectric properties of SRB bacteria suspensions over a relatively broader range of frequencies.

In Chapter 5, the chemical reactions fronts associated with *in situ* urea hydrolysis and calcium carbonate mineral precipitation in porous media were studied using SIP in a flow-through column experiment. We observed distinct rise of imaginary conductivity associated with changes in hydroxyl ion concentration during urea hydrolysis in the enzyme zone, with both real and imaginary conductivity decreased when calcium carbonate mineral precipitation occurred. In a separate study focused on the reactions of the hydroxyl ions in Ottawa sand and silica gel, significant increases of imaginary conductivity were observed in the silica gel coinciding with an increase in the pH of the influent. Only small signals were found in sands. The diffusive relaxation time and chargeability increased, but subsequently decreased in the silica gel. This work opens the door to further investigate the correlation between complex conductivity and surface chemistry, and may ultimately provide valuable non-invasive information on carbonate precipitation reaction fronts.
6.3 Challenges

The sensitivity of electrical geophysical measurements to biogeochemical processes in the Earth has already encouraged efforts to apply various techniques for monitoring remediation processes at the field scale. However, there are many obstacles that still hinder the routine use of electrical geophysics for hydrogeological and biogeochemical characterization.

One major limitation is the incomplete knowledge of the linkages between biogeochemistry and geophysical signatures. Many of the relationships that link biogeochemical and hydrogeological parameters (such as ion concentration and mobility, type of pore fluid, mean grain size, porosity, and biological factors) within typical near-surface environments to electrical geophysical attributes are not well understood. Although a lot of research has been performed to investigate the relationships between lithology, texture and electrical geophysical signals for certain rock formations, very little research has been devoted to the connection between fluid chemistry, surface chemistry, quantified biological properties, and geophysical signatures.

The questions regarding the extent to which geophysical signatures can be reliably related to microbial activity as well as geochemical processes still remains. The research in this dissertation has recorded electrical geophysical signals that are only indirectly related to biogeochemical processes, e.g., due to changes in the fluid chemistry or physical alternations of surface area and pore spaces. Additionally, the responses measured are an integrated response to multiple yet complicated subsurface processes that happen
simultaneously. An improved interpretation of such signals may be possible as our understanding towards the relationships between different geophysical attributes and biogeochemical properties advances.

### 6.4 Future work

As an applied science, geophysics must balance between advancing our understanding of the fundamental principles while applying such principles toward subsurface characterizaton. Further fundamental research in better controlled laboratory conditions are required to improve understanding the some of the uncertainties in the source mechanisms generating electrical geophysical signals. Based on the research conducted in this dissertation, the following research is recommended in the future.

1. Investigate the fundamental relationships between the surface charge of mineral phases (generally a function of fixed charges and charges resulting from solute-solid partitioning) and complex conductivity signatures in realistic materials. Local and averaged reaction kinetics in porous media, ion partitioning, and mobility in electrical double layers of minerals should be studied to better interpret geophysical signals. High resolution and high accuracy electrical geophysical measurements should be applied to assess subtle changes in fluid-mineral interactions.

2. Intermediate scale (2D flow cell) laboratory experiment should be performed with and without microbial activities involved. The electrodes could be installed in both longitudinal and transverse directions. The biogeochemical reaction could introduce physical heterogeneity, and the changes in physiochemical properties of
reaction fronts should be monitored by multi-channel SIP system. The time-lapse
electrical data should be inverted using certain inversion algorithms.

(3) Investigate quantitative correlations between electrical signatures (complex
conductivity) and SRBs activities as well as mineral precipitation using
theoretical models. Physics-based quantitative model for electrical responses of
porous material could advance biogeophysics research, although microbial
activities and related processes within natural soil and water may be too complex
to model. The evolutions of electrical properties of the porous media should be
related to the pore-scale biogeochemical reactions through pore-scale reactive
transport and complex conductivity modeling.
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Appendices

Appendix A. Supporting information for Chapter 4

1. Microscopy image of *D.vulgaris* grown in LS4D medium in anaerobic chamber at 48 hrs.
2. SEM image of *D. vulgaris* grown in LS4D medium in anaerobic chamber at 48 hrs.
3. Fe K-edge X-ray near edge absorption spectra of *D. vulgaris* after 48 hours of growth in LS4D. Image courtesy of Prof. Treavor Tyson (New Jersey Institute of Technology). Left: Spectra reveal a local structure consistent with FeS$_2$. Right: A closer examination of the atomic structure shows no significant peaks for atomic shells above 3 Å indicating Fe in a nanostructured or amorphous matrix. Systematic studies as function of loading are being conducted. Also, total scattering x-ray measurements are planned to look at the structure over very long length scales.
4. Flow chart of measuring, analyzing, and removing electrode polarization procedure. Polarization impedance at the interface between the electrode and the sample (electrolyte), due to the polarization of the ionic double layer forming at the interface, and this effect is stronger at low frequencies.

Theoretical fitting for supernatant:

\[
Re(Z) = \frac{d\sigma}{\sigma A + \omega \varepsilon \varepsilon_0 A}
\]

\[
Im(Z) = \frac{\varepsilon \omega \cdot d / A}{\omega} + \frac{\omega \varepsilon \varepsilon_0}{\sigma}
\]
5. Example of electrode polarization correction strategy using different sizes of glass beads (0.1 mm, 1 mm, 3 mm, and 4 mm).
Real part and imaginary part of total impedance and corrected and uncorrected dielectric permittivities for 3 mm and 4 mm soda-lime glass beads saturated in water. The error bars in corrected dielectric permittivities of 3 mm and 4 mm glass beads were obtained by repeating three measurements.
Corrected and uncorrected dielectric permittivities for 0.1 mm and 1 mm glass beads saturated in water. The error bars in corrected dielectric permittivities of 0.1 mm and 1 mm glass beads were obtained by repeating three measurements.
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