12-2008

Insights Into Biogeophysical Signatures Using Polarization Force Microscopy

Elizabeth Bartosik
Clemson University, ebartos@clemson.edu

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses

Part of the Geology Commons

Recommended Citation
https://tigerprints.clemson.edu/all_theses/506

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.
INSIGHTS INTO BIOGEOPHYSICAL SIGNATURES USING POLARIZATION FORCE MICROSCOPY

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Hydrogeology

by
Elizabeth M. Bartosik
December 2008

Accepted by:
Dr. Treavor Kendall, Committee Chair
Dr. Harry Kurtz
Dr. Stephen Moysey
ABSTRACT

The success of bioremediation strategies is dependent upon effective monitoring of microorganisms in the subsurface. Induced polarization (IP) may represent a cost-effective, complementary technique to existing borehole-based microbe detection schemes. Recent studies show a significant, yet poorly understood IP effect associated with the presence of bacteria in aqueous and porous media. This effect is believed to be rooted in the physicochemical surface interactions between cells and minerals which we probe using polarization force microscopy. Polarization force experiments were conducted on a hydrated mica surface using the gram positive bacterium *Bacillus subtilis* and the gram negative bacterium *Escherichia coli*. On all surfaces, polarization force maximums ($F_{\text{max}}$) increase as relative humidity is increased and surface water content rises. The $F_{\text{max}}$ response exhibited by *E. coli* was higher than that of *B. subtilis* at relative humidities (RH>75%), which suggests a unique effect due to the gram negative membrane structure of *E. coli*. The additional fluidity of the outer and inner membrane and the additional mobile charge within the gram negative periplasm are possible sources of the enhanced polarizability. Based on similarities between modeled, frequency-dependent permittivity trends on a bacterium and our experimental polarization force measurements, we propose the polarization force as a proxy for local permittivity at the cell-mineral interface. In this framework, unique dielectric dispersions with increasing frequency are exhibited by all three surfaces. Moreover, decay constants of the time evolution of the polarization force at low frequency reveal similar, relatively slow mobile ion response associated with both bacteria, and an overall faster mobile ion response on
mica. This suggests either lower mobile ion density or higher intrinsic surface mobilities for the mica. Lower mobilities on the cells could be attributed to inhibited ion movement due to protein and lipopolysaccharide membrane structures. Overall, this work shows distinct differences in the mobile ion and polarization force response of bacteria and mica. The differences in the polarizability we observed for each surface provides nanoscale information on charge separation mechanisms that could potentially sum up to a bulk, i.e. column- or field- scale, biogeophysical IP response.
ACKNOWLEDGEMENTS

This work was accomplished with the help of many professors and students. I’d like to thank Dr. Treavor Kendall, Dr. Stephen Moysey, and Dr. Harry Kurtz for their professional guidance and help with the project as my committee and advisors and Dr. Yanru Yang for help and guidance with the growth and culture of the bacteria. I’d like to thank Dr. Arash Komeili for the live cultures of magnetotactic bacteria sent from his lab. I’d like to thank Charlie McDonald for his help in the electrical set up for this project. I’d also like to thank students Baishu Guo, Dylan Fowler, Tom Sicilia, Xiaoling Liu, James Henderson and Jaime Ryan for their intellectual discussions and ideas and aid while setting up the experiments and conducting data analysis. I’d also like to thank the biogeophysics community for their ideas and support at the American Geophysical Union conference in San Francisco, CA 2007 for their discussion and suggestions during the poster presentation of this work.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>CHAPTERS</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. BACKGROUND/MOTIVATION</td>
<td>3</td>
</tr>
<tr>
<td>3. METHODS</td>
<td>9</td>
</tr>
<tr>
<td>4. RESULTS</td>
<td>13</td>
</tr>
<tr>
<td>5. DISCUSSION/CONCLUSIONS</td>
<td>17</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>27</td>
</tr>
<tr>
<td>FIGURES</td>
<td>32</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>40</td>
</tr>
</tbody>
</table>
CHAPTER 1: INTRODUCTION

Biodegradation by microorganisms such as bacteria and fungi have been used to remediate various soil contaminants including creosote, chlorinated hydrocarbons, and other oil and petroleum related products that can cause adverse health effects\(^1,2\). The success of many bioremediation schemes hinges largely on our ability to locate and monitor microorganisms in the subsurface across spatial and temporal scales. Remote detection and monitoring of microorganisms in the subsurface could potentially reduce the time- and labor-intensive efforts of monitoring methods such as core drilling and water sampling. Remote detection also has the advantage of leaving the contaminant and bioremediating organisms intact with little to no disruption of the microorganisms and contaminants. Understanding the changes in geophysical signals associated with active biodegradation of environmental contaminants is critical to using geophysics as a remote biosensing technique.

Induced polarization (IP) has received much interest recently as a remote detection strategy to monitor microorganisms capable of remediating environmental contaminants at the lab and field scale\(^3-5\). Traditionally, IP has been used for ore exploration, characterization of geothermal fields, and mapping and detecting groundwater aquifers\(^6,7\). The IP geophysical signal is a measure of how well an earth material can maintain charge separation, or become polarized, in the presence of an applied electrical field. The IP signal therefore provides information on the dielectric properties of a rock or sediment. The dielectric properties of porous media have exhibited
distinct changes when field scale IP investigations are carried out in areas with known microbial communities. This impact has also been seen in lab scale studies\textsuperscript{8-10}. Active microbial populations in both aqueous and porous media impact these dielectric signatures; however, the mechanism behind the response is poorly understood\textsuperscript{8-10}.

IP responses in porous media may be attributed to pore throat constriction which causes charge to build up across the pore (Figure 1)\textsuperscript{6}. As such, bacteria could act to constrict pore flow, or could act as spheres that disrupt the mineral surface double-layer. Further, bacteria have cell structures such as phospholipid layers and teichoic acids that give cells a charge that can impact IP electrical measurements, potentially through the disruption of mobile charge migration. This work aims to understand the dielectric properties of bacterial cells at the cellular level in hopes to better understand the observed field and lab scale biological IP response.
Lab and field scale observations have paved the way for current investigations of IP as a remote sensing technique to detect microbes in the subsurface\textsuperscript{8-10}. A typical field scale IP survey is conducted by placing two electrodes into the subsurface and imputing a time varying current through those electrodes. The spacing of the measurement and current source electrodes control the depth of the measurements. The resulting time-dependant voltage response is then measured across two reference electrodes. These surveys can be carried out in the time-domain or the frequency-domain.

In time-domain IP, the applied current is cycled on and off over a tenth- to half-second time-scale (Figure 2). In the absence of charge storage (e.g., polarization or IP effects), the time-dependent voltage response and applied current traces are identical. Conversely, polarization phenomena appear as exponential lags or decays in the voltage response (Figure 2), from which parameters such as apparent chargeability may be extracted. Chargeability is defined by the following equation:

\textbf{Equation 2.1:} \[ M = \frac{V_p}{V_o} \]

where $M$ = chargeability, $V_p$ = overvoltage and $V_o$ = observed voltage.
In frequency domain IP, an ac sinusoidal current or voltage is applied through the electrodes and the phase between the input and the output current or voltage wave is determined. The phase lag between the two signals corresponds to the complex conductivity, which is dependent upon the charge transmission and storage capacity of a material. Dielectric and conductivity parameters can then be extracted\textsuperscript{9}.

Previous studies of lab scale IP measurements show an increase in complex conductivity measurements of samples undergoing active bioremediation\textsuperscript{8,9}. Complex conductivity, $\sigma^*$, consists of a real $\sigma'$ and imaginary $\sigma''$ component:

**Equation 2:** $\sigma^* = \sigma' + \sigma''$

The real part of the conductivity ($\sigma'$) typically increases in earth materials with increasing water content, solute concentrations, and porosity\textsuperscript{11}. The imaginary part of complex conductivity ($\sigma''$) can be seen in Equation 3 where $\varepsilon =$ dielectric permittivity, $\omega =$ frequency and $i = \sqrt{-1}$.

**Equation 3:**  
\[
\sigma'' = \frac{\varepsilon \omega}{4\pi i}
\]

The frequency dependence ($\omega$) and dielectric properties ($\varepsilon$) of the imaginary conductivity captures polarization phenomena associated with pore geometry, dissolved ion content, pore wall chemistry, and possibly microorganisms\textsuperscript{11-13}. IP is sensitive, particularly in the frequency domain, to small changes in the dielectric properties\textsuperscript{9}. This
work addresses how microbial cells impact this bulk dielectric response by examining these properties at the cellular level. In particular this work measures polarization force equivalents to the imaginary part of the complex conductivity and characterizes ion density and mobility on cell surfaces, all with nanoscale spatial resolution.

Real and imaginary conductivities have been shown to increase over time in a column of bacteria, nutrients, and diesel compared to a column without bacteria. In lab scale column measurements both the real and imaginary conductivities increased as microbial population numbers increased. At the field scale, the imaginary portion of the complex conductivity appeared to be more sensitive to microbial activity than the real part of the conductivity. Further experiments conducted with bacteria in sands attributed major changes in complex conductivity with increased cell density to pore throat constriction. Each of these experiments shows changes in complex conductivity, or dielectric properties, with the addition of microorganisms. A key component is hypothesized to be the behavior of mobile charge at the cell-mineral interface. The electrical characteristics of the cells and the cell-mineral interface need to be measured to ascertain the dominant contributing mechanisms.

*Electrical Properties of Microorganisms: Measured and Modeled*

The frequency dependent dielectric response of biological cells has been modeled and measured using various techniques. Dielectric dispersions in the $\alpha$ (sub kHz), $\beta$ (radio frequency range), and $\gamma$ (above MHz) domains have been identified for biological
material\textsuperscript{14}. Past bulk dielectric spectroscopy has focused on the $\beta$ dispersion range, in part because the $\alpha$ dispersion interpretation remains difficult due to electrode polarization that occurs at low frequencies\textsuperscript{15,16}. Yet, field scale IP measurements typically use frequencies in the $\alpha$ range between 0.1 Hz to 4 kHz\textsuperscript{17}. IP signal loss is observed at high frequencies\textsuperscript{18}. Therefore, to interpret the biological IP response, it is important to further investigate the sub kHz, $\alpha$ range response of microorganisms, which is a focus of this thesis.

Within the $\alpha$ range, the dielectric response of suspended cells in aqueous solution is thought to be dominated by mobile ion displacement on the external side of the cell membrane in response to the applied electric field\textsuperscript{21,22}. The exterior of the cell has a fixed charged “cell envelope” that attracts counterions to create a radial field around the cell\textsuperscript{21}. When an electric field is applied, the modeled electrical permittivity of cells within the $\alpha$ dispersion range strongly increases due to asymmetry in the electrical shell created by the applied field\textsuperscript{24}. It is believed that most low frequency range dispersions are caused by counterion displacement along the outer membrane\textsuperscript{19,20}. Further interpretation of cell models suggests that the charge on the interior of the cell is fixed due to the equal net charge on the inner side of the membrane yielding negligible ion mobilities\textsuperscript{21}. These models have been useful in addressing $\alpha$ range dispersion; however, little quantitative, molecular-scale experimental data exists.
Polarization Force Microscopy

Polarization Force Microscopy (PFM) allows measurements of mobile ion processes on surfaces at low frequency\textsuperscript{35-38}. Because electrodes are not inserted into the solution being measured electrode polarization is not a factor. In general, polarization on mineral surfaces is dominated by movement of mobile cations and anions in response to an applied electrical field\textsuperscript{32-38}. The electrical field causes surface cations and anions to move towards the opposing applied bias. After the applied charge is turned off, surface ions return to a state of equilibrium over a period of milliseconds to seconds. The degree of charge separation or polarization that occurs is manifested as an overvoltage in time-dependent IP or as a phase shift in spectral IP measurements\textsuperscript{6}. We hypothesize the bulk scale IP effect is a sum of surface IP phenomena at the scale of a single bacterium.

To investigate this scale, an ac electrical bias is applied to a conductive, micron-sized PFM tip. Ions of opposing charge on the surface accumulate beneath the tip and attract the tip to the surface in a non-linear fashion. When the bias is reversed, this results in an immediate repulsion force and the tip moves away from the surface (Figure 3). The time dependent tip response when using a low-frequency ac square wave for the tip bias produces characteristic saw tooth patterns that we term ion mobility traces (Figure 3). The maximum amplitude (F\textsubscript{max}) of the tip deflection and the time constant (\(\tau\)) of the attractive decay are two measurables that correspond to the density and mobility of surface ions and further to the chargeability as measured by IP (See Equation 3.2). Both ion mobility and density are a function of water content on a surface; therefore
measurements for these experiments were taken over a range of relative humidities. Here, direct comparisons are drawn to IP measurements made in the vadose zone field measurements where water contents may differ (Figure 3). Indirect comparisons are also made to bulk aqueous conditions and the evolution of the electrical double layer.

Research Objective

PFM has been previously used to measure polarization and ion mobility responses within the $\alpha$ frequency range on inorganic systems, including minerals surfaces such as mica and carbonates$^{32-38}$. While PFM has not been used on biological systems, traditional atomic force microscopy (AFM) has proven useful in investigating the physicochemical properties of a bacterium at a $\mu$m to nm scale. The objective of this work is to combine PFM and AFM to investigate mobile ion processes on a cellular surface$^{25-31}$ in an effort to mechanistically rationalize the biological IP response. Specifically, this work compares the ion mobility responses of mica (a common soil mineral) and two bacteria with different cell surface structures, which we hypothesize as an important variable in the polarization response. With this information, we supplement existing models of cells in an applied electrical field and ultimately address direct IP signatures putatively associated with the presence of microbial cells$^{8-11,13-24}$. Importantly, utilization of PFM to investigate dielectric properties on a bacteria surface can give insights at length scales that cannot be obtained from lab and field measurements.
CHAPTER 3: METHODS

A typical experimental protocol consisted of culturing and mounting microbes on a mica substrate for PFM use, followed by AFM imaging and PFM point measurements of surface ion density and mobility on a single bacterium. Our model systems included the gram positive bacteria *Bacillus subtilis* and the gram negative bacteria *Escherichia coli*. Each of these species exhibit significant differences in cell surface structure which we hypothesize as an important variable in the polarization response. These measurements were compared to ion mobility measurements made on the mica surface as a control and as a known mineral that occurs in soils.

*Bacterial Growth and Preparation*

Bacteria cultures of *Escherichia coli* and *Bacillus subtilis* were grown to stationary phase in Luria Broth (10 mg/L tryptone, 5mg/L yeast and 5mg/L NaCl). The growth medium was autoclaved prior to use for 20 minutes at 121°C. Cells were extracted from the medium via centrifugation at 12,000 rpm for two minutes then rinsed with singly distilled water to prevent cell lysis. The rinse step was repeated three times before the cells were resuspended in distilled water. Cells were pipetted onto freshly cleaved mica for use on the AFM. The mica substrate (~2mm thick) was attached to a glass slide using silver paste, which should have very little impact on PFM measurements, to prevent substrate drift as the relative humidity was increased. The cells
were allowed to dry on the mica surface for 10 minutes before AFM imaging or PFM measurements.

*Relative Humidity Control*

To address the impact of surface water content on ion mobility traces of mica and bacteria, the relative humidity was controlled and maintained using a chamber that encased the sample and the tip (Figure 4). Humidity was adjusted with controlled N₂ flow that was either added directly to the chamber or first passed through a bottle of deionized water. The humidity was measured using a HC-610 thermostet polymer capacitive humidity sensor from Ohmic Instruments (Easton, MD) positioned near the sample. Humidity was then recorded using the *Igor* software package (Wavemetrics, Lake Oswego, OR). For these experiments the relative humidity was adjusted between 5% and 95% and maintained within ±0.5% of the selected humidity value. Humidity was also held constant approximately 3 to 5 minutes prior to the start of each experiment to allow equilibration of the surface.

*Polarization Force Microscopy*

Experiments were conducted on an Asylum MFP-3D Atomic Force Microscope using a customized polarization force module developed by T. Kendall and K. Jones. Single bacterial cells suitable for ion mobility measurements were located using an ac
imaging mode on the AFM. Intact solitary cells not in contact with other cells and were chosen for the experimental analysis (Figure 5). Measurements were taken on the center of the cell using the image collected as a guide. To obtain ion mobility traces, a Pt coated, 12kHz, 0.2 N m\(^{-1}\) cantilever (Nanosensors, Inc.) was held \(\sim 1.5\) \(\mu\)m above the sample. Tip to sample distance was maintained and monitored using force curves which record the distance the tip has to travel to reach the surface. Force curves were taken throughout each experiment. A 10V peak-to-peak AC electrical bias was applied to the tip at frequencies between 1 and 100Hz. Frequencies were selected to obtain good coverage on a semi-log scale for the frequency range selected. The frequency range terminus was selected because at frequencies above 100Hz the saw tooth patterns begin exhibit a sine wave structure at our sampling rate of 50,000 samples per second (50 kHz). Tip deflection was recorded and converted to force using Hook’s law \(F = -kz\) where \(z\) = the tip displacement and \(k\) = cantilever spring constant. The spring constant was measured using the Thermal Method integrated in the MFP-3D interface. For each cell and the mica surface, 150 traces were taken and averaged at each frequency and RH value. \(F_{\text{max}}\) and decay constants were then extracted from the average IM traces. Measurements were taken over multiple cells that fit the criteria explained above. Thus, multiple cells of both \textit{B. subtilis} and \textit{E. coli} were used in separate experiments.
Polarization Force Analysis

The decay curves of the ion mobility traces are analyzed using the Igor software package to fit a double exponential function:

\[ F(t) = A e^{-\frac{t}{\tau_1}} + A e^{-\frac{t}{\tau_2}} \]

where \( A \) is a constant and \( \tau_1 \) and \( \tau_2 \) represent relaxation times for each trace. A double exponential function is used to account for the multiple relaxation times seen in the curve (Figure 6). In the double exponential function there is a fast response and a slow response that is accounted for by the \( \tau_1 \) and \( \tau_2 \) values, respectively. The greater the \( \tau \) value the slower the response. Using this double exponential fit, each response can be quantified.

To relate \( \tau_1 \) and \( \tau_2 \) to mobile ion behavior, the system can be modeled as a resistor/capacitor circuit between the tip and the sample.

\[ \tau = RC = \frac{1}{\sum \mu_i \sigma_i C} \]

In this equation \( C \) = overall capacitance and the resistance \( (R) \) can be broken down into ion mobility \( (\mu_i) \) and ion density \( (\sigma_i) \). Each \( \tau \) value calculated has an ion mobility and ion density component; however, differentiation of the two parameters can be difficult. Each of these components is influenced by surface water content, which is an important factor in field scale induced polarization measurements made in porous media\textsuperscript{8-10}. 
Amplitude values of each ion mobility trace are the result of the repulsion of the tip in response to the same charges on the surface accumulating under the tip on either the bacterium or mica surface. Figure 7 is an example of a raw ion mobility trace collected over mica, *E. coli* and *B. subtilis*. The more ions that accumulate under the tip, the greater the repulsion force when the applied voltage polarity is switched. The repulsion force is influenced by surface water content which increases ion hydration and ion mobilities therefore amplitude values were measured and averaged over the bacterium and mica as a function of relative humidity and frequency\textsuperscript{32}. Further information on the theory of the polarization force as measured with an AFM can be found elsewhere\textsuperscript{32-38}. 
CHAPTER 4: RESULTS

Maximum Force Amplitudes at Low RH < 50%

Mica exhibits the highest force maximums $F_{\text{max}}$ of all three surfaces at RH values below 50%. $F_{\text{max}}$ values for each of the surfaces tested reveal little change in the 5% to 50% RH range, therefore, all $F_{\text{max}}$ values collected on each surface at a RH below 50% were averaged (Figure 8). $F_{\text{max}}$ values on all three surfaces from 5%-50% RH decrease with increasing frequency. $F_{\text{max}}$ values are always greater on the mica surface than on both bacteria over every frequency tested for RH values below 50%. Within error, $F_{\text{max}}$ values on both bacteria surfaces are the same over the frequencies tested. Low RH $F_{\text{max}}$ values for mica exhibits a linear decay from 0.7 nN to 0.35 nN on the semi-log plot as frequency increases from 1 to 100 Hz. The mica surface begins to exhibit an exponential decay on the semi-log plot at RH values above 50% RH. Both bacterial surfaces exhibit a pseudo-exponential decay on the semi-log plot of $F_{\text{max}}$ values with values flattening to 0.1 nN from 10 Hz to 100 Hz. Differences in the shape of the $F_{\text{max}}$ decay as frequency increases indicate differing mobile charge density or behavior on the mica compared to the cell surface; however, little difference is observed between the two cell surfaces at low RH. These trends indicate that the mica surface has more mobile charge at RH values below 50% than both the $E. \text{coli}$ and $B. \text{subtilis}$ surfaces.
Maximum Force Amplitudes at High RH 50% to ~90%

The response of each surface as RH increases above 50% becomes more complex presumably due to the mobilization of charge at the higher water content. Each surface exhibits a unique $F_{\text{max}}$ decay pattern as a function of frequency at a given RH (Figure 9). At higher RH, the *E. coli* surface achieves larger $F_{\text{max}}$ values under drier conditions compared to mica or *B. subtilis*. For example, *E. coli* shows a $F_{\text{max}}$ at 1 Hz of 1.1 nN for 66% RH. The same value is not achieved for *B. subtilis* and mica surface until 79% and 78% RH respectively. Put another way, *E. coli* $F_{\text{max}}$ at 78% RH measured at 1Hz is twice as high as the *B. subtilis* at 79% RH and mica at 78% RH at the same frequency. This relationship remains true even as probe bias frequency is increased. At the highest RH ~88%, the spectral response of $F_{\text{max}}$ values are most distinct amongst the three surfaces (Figure 10). The *E. coli* $F_{\text{max}}$ values are higher than both the mica and *B. subtilis* values at each frequency tested. At 1Hz, all three surfaces have $F_{\text{max}}$ values within 0.4nN of each other. This difference increases to 0.9 nN at 10 Hz. At high RH, the mica $F_{\text{max}}$ values remain lower than both the *B. subtilis* and the *E. coli*.

In addition to the magnitudes, the shape and dispersions of the spectral responses differ between the three surfaces, particularly near 88% RH. As frequency increases, *E. coli* $F_{\text{max}}$ values exhibit one small dispersion at approximately 20Hz (Fig. 10). This dispersion is roughly bracketed by an $F_{\text{max}}$ decrease of 0.3 nN between 10 Hz and at 50Hz. *B. subtilis* $F_{\text{max}}$ values exhibit two dispersions; one between 1 and 2Hz and the other between 20 and 100Hz. The largest total $F_{\text{max}}$ dispersion (2 nN) over the frequency
spectra tested is observed for *B. subtilis*. The mica surface exhibits one dispersion that occurs at approximately 2 Hz at 88% RH. The total $F_{\text{max}}$ difference for the mica surface is approximately 0.8 nN at 88% RH. The single dispersion observed on mica shifts to higher frequencies as RH increases (e.g., from 2 Hz at 88% RH to 10 Hz at 95% RH). Furthermore, the maximum $F_{\text{max}}$ values on mica do not increase with RH once the RH moves above 85%. These observations agree with previous polarization force experiments conducted on mica by Xu et al\textsuperscript{38}. Overall at high RH, *E. coli* exhibits the smallest overall drop in $F_{\text{max}}$ values from 1 to 100 Hz and *E. coli* exhibits the highest $F_{\text{max}}$ values of all three surfaces.

Modeled dielectric permittivities on a bacterium show a single dispersion with increasing frequency. As ion mobility increases, the frequency at which the single dispersion occurs gets higher\textsuperscript{22}, roughly in the Hz to kHz range. A parallel is thus drawn between the modeled permittivities and the frequency dependent trends with $F_{\text{max}}$ (Figure 12). Our observed $F_{\text{max}}$ trends, particularly at RH values below 50%, exhibit one dispersion similar to modeled dielectric permittivities and the dispersions occur at higher frequencies as RH is increased. In addition, our data provides additional detail which suggests that multiple dispersions exist as water content is increased (viz. mobilities increase) on the cell.
Time Decay of the Polarization Force at the Cell-Mineral Interface

Analysis of exponential decay values ($\tau_1$ and $\tau_2$) values for all three surfaces over all RH values tested display differences that are most visible at frequency values below 10 Hz (Figure 11). Each PFM trace exhibits an initial fast response followed by a slower response which is why a double exponential fit is needed. In general, differences in $\tau_1$ and $\tau_2$ values point to a faster mobile ion response on the mica compared to the cells. Generally, $\tau_1$ values range from 0.5 ms to 3.5 ms and $\tau_2$ values range from 30 ms to 40 ms.

Figure 11 is a histogram of $\tau_1$ and $\tau_2$ values at 7 Hz. Both E. coli and B. subtilis exhibit similar $\tau_1$ (3.41 ms and 2.95 ms respectively) which are 2 ms slower than the mica $\tau_1$ value (1.51 ms). For $\tau_2$ values E. coli and B. subtilis exhibit similar (38.5 ms and 34.9 ms respectively) values which are approximately 15 ms slower than mica $\tau_2$ (22.8 ms) values. The similarities between decay values of both bacteria indicate comparable mobile ion response times on the bacteria. The $\tau_1$ and $\tau_2$ values on the mica surface remained unchanged as RH was increased. The cell surfaces showed a slight decrease in the mobile ion response time as RH is increased.

Although the data are variable, the findings for $F_{\text{max}}$ trends reported above were fairly consistent across multiple cell and mineral surface replicates. Averaged $F_{\text{max}}$ data within selected RH ranges for 5 to 6 different cells for each cell type and 6 different mica samples are shown in Figures 13 and 14. The highest $F_{\text{max}}$ response of all samples was E. coli at RH values >75%. The mica and B. subtilis surface at RH values >75% are similar within error. Between 50%-75% RH, mica has a larger $F_{\text{max}}$ response than E. coli and B.
*subtilis* with each cell having a similar response. The large variability in the data is a consequence of binning $F_{\text{max}}$ values from a large range of relative humidities as well as the inclusion of older, potentially less reliable data sets which were collected during protocol development.
CHAPTER 5: DISCUSSION AND CONCLUSIONS

Implications for Lab and Field Induced Polarization Measurements

Polarization force measurements act as a nano-scale analog for measurements made with column- and field-scale IP. Polarization measurements made using the AFM are attributed to the materials surface mobile ion characteristics. This relates to IP measurements made in the field where the applied current causes surface ions to migrate, separate, and presumably contribute to the spectral IP phase shifts in the frequency domain or apparent chargeability in the time domain. In particular, $F_{\text{max}}$ measurements on the AFM appear to capture the same phenomena that are observed as the imaginary ($\sigma'''$) part of complex conductivity measurements ($\sigma'$), i.e. changes in the dielectric properties. Further, decay time constants ($\tau$) yield information on both the ion mobility and charge density that exists on the cell surface, which is at the root of the complex conductivity measured in IP measurements of porous media. Therefore the trends we observed, if properly upscaled, could provide information on field IP measurements.

The higher observed $F_{\text{max}}$ values of *E. coli* and *B. subtilis* could suggest higher imaginary components to the complex conductivity in aqueous solution and hydrated porous media, although, as mentioned, upscaling is required to further investigate this effect. In general, the greater polarization forces measured on the cell indicate a change in dielectric properties compared to the mica surface when adequate water is available. Further, the lower $F_{\text{max}}$ values seen at low RH values (e.g., low water content in the
vadose zone) for the cells versus mica indicate that differentiation of the two cell types using IP may not be possible, but that encouraging cell-mineral differences still exist. Cell-mineral interactions could affect the bulk IP response of measurements in areas with low water contents. The addition of microbes to porous media may reduce the IP response below what would be exhibited by just porous media at low water contents.

The dispersions we observed in the spectral polarization force responses may also be useful in providing a framework for detecting microbial communities using spectral IP in the field. The single dielectric dispersion observed in our measurements for mica could indicate that a single dispersion would occur in IP measurements made in hydrated clay and porous media. Addition of a microbial dielectric signature would most likely yield multiple dispersions as observed in our spectral force data. This effect becomes more prominent as water content increases. Moreover, The $F_{\text{max}}$ values collected at high RH show distinct differences in the polarization forces on the gram positive and gram negative cell surface. $F_{\text{max}}$ values on the gram negative cell are greater than those on the gram positive cell. This could also yield differences in the complex conductivity depending upon the dominant cell membrane structure within a subsurface microbial community.

**Upscaling Polarization Force Data**

Polarization force analysis has some major differences from IP measurements that need to be considered for upscaling models. PFM measurements are taken over single
cells at a specific distance above the cell. Understanding the geometry of the electric field created by our PFM measurements and comparing it to the electrical field in an IP column is anticipated to be an important step, however column- or field-scale IP measurement sums across multiple cells, albeit at low cell densities. To connect the two, a parallel needs to be drawn between the mechanical response of the dip and the field generated by the electrodes in column scale IP.

Our data were collected using a single bacterium, while measurements in the lab and field will focus on microbial communities. Lab and field scale measurements might be hindered by low bacteria densities and variable water contents, particularly in the vadose zone, thus optimizing the signal response and frequency range will be paramount. Our data suggest that bacteria cannot be distinguished from the mica below 1 Hz, even at high water contents. Above 1 Hz, however, $F_{\text{max}}$ values for each surface are distinctly different. An intermediate step to further test this hypothesis might include coupling our force observations with traditional dielectric spectroscopy on equivalent systems. This would help further constrain the connection to column-scale IP.

The complex conductivities measured in lab scale experiments are likely impacted by ion mobilities associated with the bacteria surface. Our data suggests that as water content increases, the relative change in IP parameters associated with gram negative cells may be greater than those associate with the gram positive cells. And both are larger than mica alone. If these effects are applied over a whole cell, or a biofilm, these impacts can be even greater than those observed on just an area of the cell as in our PFM measurements. Moreover, the measured decay constant values ($\tau$) are faster on the mica.
surface than on both bacteria. This discrepancy in relaxation time for the cell versus mineral represents a potential pathway for charge separation or polarization that could be attributed to the presence of microbes. If the effect sums to a larger scale, the polarization could manifest as a phase shift in spectral IP.

*Cell Wall Structures: Impacts on Ion Mobility Measurements*

The complexity of a bacterial surface compared to the mica surface could explain differences observed in both $F_{\text{max}}$ and $\tau$ values. The mica response is caused by ions on the surface becoming mobile with the addition of the water layers as RH is increased$^{37,28}$. A similar process is anticipated for the cells, however, the bacterial surface is considerably more complicated with lipid membrane layers, proteins, polysaccharides and other cell wall structures that could affect charge migration (Figure 15). To understand the response of bacterium to an applied electrical field, and thus compare it to the response on the mica surface, all bacterial surface structures including phospholipid layers, membrane proteins and polysaccharides and transport processes that could impact these measurements need to be considered.

*Mica Ion Mobility Responses*

Ion mobility measurements on mica surfaces have been investigated as a function of RH and frequency previously$^{37,38}$. The mobile ion response on the mica surface is
believed to be dominated by hydrated surface ions on the surface that migrate though the
addition of water layers as RH is increased. Different types of surface ions such as Ca\textsuperscript{2+},
Mg\textsuperscript{2+}, K\textsuperscript{+}) exhibit different relaxation times ($\tau$) measured using polarization force
microscopy\textsuperscript{37}. This indicates that the different ions on the mica surface yield different
mobility rates, although, surface structure is also a contributing factor\textsuperscript{32,33}. Ions on a
cellular surface, may exhibit similar differences, depending upon which surface ions
dominate. Moreover relaxation times decrease with increasing surface ion density. One
explanation of the observed fast mobilities over mica versus the cells could be lower
mobile ion density and/or less heterogeneity in the mica surface structure.

*Cell Wall Structures and Ion Mobility Measurements*

The major differences in the cell wall structure of the gram positive and gram
negative cell wall envelope (Figure 15) likely play a major role in the observed
differences in $F_{\text{max}}$ trends as a function of RH. The addition of cell wall structures also
differentiates the polarization force measurements on the bacteria from measurements on
the mica surface. The general structure of most bacterial membranes consists of a
phospholipid bilayer. Generally, bacterial cells also have peptidoglycan which gives the
cell structure, but has no charge so this layer should have a little to no effect on the
polarization $F_{\text{max}}$ measurements. The phospholipid structure consists of a hydrophilic
(glycerol-phosphate) head group and hydrophobic (fatty acid) tail which naturally form
bilayers in both the gram positive and gram negative cell membrane. The phosphate
portion of the phospholipid tends to be in the $\text{HPO}_4^{2-}$ speciation at neutral pH giving the cell wall a net negative charge. The bacterial membrane also acts as a fluid when in water giving allowing it to be semi-mobile when water is available to the cell\textsuperscript{43}.

The gram positive cell wall consists of a single phospholipid bilayer and a peptidoglycan layer that gives the cell structural integrity. One possible reason for the low RH (below 50%) $F_{\text{max}}$ values that are below the values on the mica surface could be due to the phospholipid bilayer retains its structure and remains relatively immobile when there is not enough water to allow fluidity of the membrane. The membrane is rigid, and cannot respond to the applied charge of polarization force measurements, and thus is anticipated to have a small impact on polarization measurements at low water contents. As the water content surrounding the cell increases, the cell wall begins to hydrate and become fluid, allowing the charged phosphate portions of the cell membrane, in addition to mobile counterions, to respond to the applied electrical charge. Notably, because the gram positive cell wall has only one lipid bilayer, the cell may not be as responsive in the applied field as the double bilayer in the gram negative bacteria.

The gram negative cell wall consists of two phospholipid bilayers and a peptidoglycan layer in the periplasm between the two lipid layers. When the cell is not hydrated (e.g., below 50%RH) the gram positive cell wall could remain unresponsive within the applied electrical field. Again this would be due to the lack of water allowing fluidity of the membrane and due to impeded (dry) ion motion on the cell exterior. Once enough water is added, the cell membrane could once again act more fluid. The double phospholipid layer of gram negative bacteria has the capability of impacting the $F_{\text{max}}$
measurements with twice the effect than that of the single lipid layer seen in the gram positive cell wall. This could allow for two times the amount of phosphate to react to an applied electrical field, which can account for almost double the $F_{\text{max}}$ measurements observed on the gram negative cell observed at high RH. This double phospholipid bilayer is also the reason the gram negative cell wall is more electronegative than the gram positive cell wall\textsuperscript{43}. This means that, in general, there is a greater charge on the exterior of the gram negative cell wall. This increase in charge could yield the observed increased polarization and ion mobilities, particularly as RH is increased.

Periplasmic space is another component that exists between the two phospholipid bilayers in the gram negative cell that could have an influence on ion mobility measurements as RH is increased. This periplasm toward the cell exterior is composed of an aqueous solution of polysaccharides, amino acids, peptides, and biosynthetic components and is thought to be viscous and gel-like but with mobilities 0.1% of mobilities of ions in aqueous solution\textsuperscript{43}. The increase in water content on the exterior of the cell could allow for more water to be held within the periplasm allowing ions within this area to become more mobile and respond to the applied electrical field. These structural differences including both the addition of phospholipid layers and increased periplasmic space could be major contributing factors to the differences seen in the $F_{\text{max}}$ measurements of \textit{E. coli} and \textit{B. subtilis} at high RH.

Exterior cell components that extend above the phospholipid bilayer could also affect mobile ion movement on the cell surface, thus causing the slower polarization force time response observed on both \textit{B. subtilis} and \textit{E. coli} cell surfaces compared to
mica. The gram positive cell exterior has membrane structures that extend above the phospholipid bilayer such as teichoic acids and other cell wall polysaccharides and proteins. Gram negative cells contain similar exterior cell structures that include polysaccharides and proteins, but no teichoic acids. Because these structures extend above the cell, ion mobility could be impeded as ions respond to the applied electrical field. Specifically, electrostatic interactions between the charged moieties in the membrane structures and the mobile surface ions could occur. Fimbriae were also observed in images collected of *E. coli* cells used in the experiments. These extracellular structures which extend well beyond the phospholipid bilayer are thought to be used for cellular adhesion\(^43\), but also could impact the polarization force measurements. In general, extension of cell wall components above the phospholipid bilayer could obstruct mobile ion flow yielding slower mobile ion decay constants.

Trans-membrane ion transport by bacteria is also capable of impacting ion mobility measurements, particularly for the gram negative cell wall. Passive transport is an energy independent function that allows transfer of ions across the outer membrane of bacteria though porins. This process shuts down when there is not enough water to drive osmosis via the porins. Under low RH conditions porins are not expected to affect the polarization measurements, however, under wet conditions (e.g., RH > 50%) the porins could activate potentially giving the rise in polarization forces. Once the RH increases above 50% this could allow passive transport of ions including water across the membrane via aquaporins. Aquaporins are characteristic of the *E. coli* membrane. Therefore the increase in the movement of water molecules and ions across the
membrane could increase the \textit{E. coli} mobile ion response above values observed on \textit{B. subtilis}.

\textit{Significance of Work and Outlook}

Our work shows the polarization responses of gram positive and gram negative bacteria are distinct to that of mica. The data collected suggest an increase in ion mobility on the bacteria surface to levels above that of the mica, particularly when water contents are high. This could yield increased complex conductivities measured in field and column scale IP, at least for the imaginary component. These data also suggest that when the water content is low, a distinction between the cell and mineral responses still exists. The microbial signature may be observed due to the lower ion mobilities measured on the bacteria compared to the mica surface. Decay constants also indicate slower responses on the cell surface which may influence the frequency dependant IP response and influence the time ranges used to conduct time domain IP.

Field scale IP responses in areas where microbes could be detected will most likely be the result of a combination of the soil characteristics, the gram positive and gram negative bacteria and water content. This makes differentiation and interpretation difficult, but potentially feasible through scaling and inversion using the nanoscale measurements provided in this thesis. What we can hope for is the bacterial signal may increase the overall complex conductivity enough to determine if there are active microbial populations particularly in areas with high cell concentrations. Coupled with
aqueous chemistry changes microbes can have when metabolically active, IP as a remote sensing technique could have promise in the future. Additional studies need to determine what cell densities are needed to allow detection and how does the growth of microbial communities and changing aqueous chemistries in the subsurface couple to increase the IP response. Future research examining the mechanism of the biological IP response can be conducted utilizing the AFM and polarization force microscopy. This work focused on a gram positive and gram negative bacteria; however, additional studies can investigate the effects of additional cell structure variants (e.g., S-layers or EPS), and even intracellular and extracellular biominerals. While single cells were the focus of this work, cell-cell connectivity could be an important determinant in the polarization magnitudes. Also, changes in interior cell chemistry (i.e., osmolarity) may determine the polarization response of the cell and should be investigated.
APPENDIX

Preliminary Data for Future Work:

The research objectives behind this work aim to further increase our mechanistic understanding of the bioelectric IP effect. Further work can give insights into these mechanisms at a lab/column scale, including the variable of cell orientation with respect to the applied electrical field. This mechanism could be addressed using magnetotactic bacteria which can be oriented within a column. Using an established protocol, simple bar magnets or an electrical current passed through a coil wrapped around the column (e.g., a solenoid) can be used to generate the magnetic field. Orientation of bacteria will be achieved when intracellular magnetic particles called magnetosomes respond to the applied magnetic flux lines. Spectral IP data can be collected with bacteria in various orientations, with a particular focus on controlling the spatial relationship between the bacteria and pore walls.

Thus far, bacteria growth and optimization have been conducted in our laboratory as part of this study. The primary goal for the growth effort was to establish and propagate viable cultures containing magnetic particles of \textit{Magnetospirillum} sp. AMB-1 (American Type Culture Collection (ATCC)). Stock cultures of \textit{Magnetospirillum} sp. were grown using the revised magnetospirillum growth medium (MSGM) recipe provided by ATCC. This media was supplemented with polypeptone, yeast extract, L-Cysteine and tetracycline to enhance cell growth and bacterial magnetic particle production. Figure 16 is the growth curve calculated using absorbance measurements.
(600 nm) and cell counts obtained from micrographs of DAPI (4'-6-Diamidino-2-phenylindole) stained Magnetospirillum sp. slides. The exponential growth phase was reached at approximately 72 hours which is similar to optimal growth patterns previously reported for Magnetospirillum sp. AMB-1 \(^{24}\). In selected cultures, ferrous sulfate was also used as an iron source in place of ferric quinate to enhance bacterial magnetic particle production; however, little affect on cell concentrations and magnetism was observed (Figure 16). Magnetism was confirmed by subjecting the cells to a bar magnet and magnetic stir plate. Cells migrated toward the bar magnet and swirled when held above the magnetic stir plate.

IP measurements in a column can be made using a dynamic signal analyzer (DSA, National Instruments) applying and receiving signals from a PVC column \(^{41}\) (Figure 17\(^9\)). The DSA passes a sinusoidal current at a given frequency through two stainless steel disk electrode at the ends of the column. The voltage response is measured by two Ag-AgCl electrodes (A-M Systems, Inc\(^{©}\)) spaced across the column. An Analog Devices Operational Amplifier (AD620) is used to amplify the signal measured by the voltage electrodes which is fed back into the DSA for comparison to the reference sinusoid \(^{9,41}\).

Prior to making column measurements, a first step is to calibrate and determine the accuracy of IP measurements made with our electrical set up. This was done by comparing measurements made on an RC circuit that was designed to simulate a column of porous media \(^{14}\) with data generated by a circuit simulation program (Quite Universal Circuit Simulator (QUCS)). Figure 18 is the simulated data compared to our measured data. The calibration shows we can accurately measure >1 mrad phase changes between
0.5 Hz and 100 Hz. The greatest phase change is seen at approximately 1 Hz suggesting that this is the best frequency to conduct lab scale measurements. Additional efforts needed to reduce noise and improve accuracy include reducing column dimensions, shortening wire lengths, and optimizing the quality and value of the reference resistor.

Complex conductivity is the typical measurement calculated for field and column scale measurements. This is calculated using the measured phase shift ($\Phi$) and conductivity magnitude $|\sigma|$. Conductivity magnitude is calculated via two steps. First, current ($I$) is calculated using the measured voltage ($V_{\text{ref}}$) across the reference resistor, resistance ($R_{\text{ref}}$) of the reference resistor and Ohm’s Law (Equation 1).

**Equation A.1:**

$$V = IR$$

Second, the impedance magnitude across the column can be calculated as ($R_{\text{col}}$) using the current calculated in the first step and the amplitude of the voltage measured across the column ($V_{\text{col}}$). Phase and conductivity magnitude then determine the real ($\sigma'$) and imaginary ($\sigma''$) parts of the complex conductivity\(^8\).

**Equation A.2:**

$$\sigma' = |\sigma| \cos \phi$$

**Equation A.3:**

$$\sigma'' = |\sigma| \sin \phi$$
Reporting complex conductivity values facilitate comparison across samples and with data reported in the literature\textsuperscript{9}.

Preliminary IP measurements collected on a column filled with DI, tap water, and bacteria suspensions allowed further assessment of the accuracy, noise floor, and reproducibility of measurements made with our setup. Figure 19 shows the measured phase changes of water and an aqueous solution of water and bacteria. The phase change in bacterial suspensions is consistently higher than that of water suggesting an effect on the IP response influence by bacteria may be captured with our experimental set up. The variability in these data was high and absolute values were not reproducible; however, the relationship between bacteria and water remained unchanged (e.g., $\Phi_{\text{bacteria}} > \Phi_{\text{water}}$) throughout our measurements. The dielectric permittivity of cells in suspension is reported to be high at low frequencies suggesting an IP response should be observed\textsuperscript{22}. The magnitude of this response, however, is dependent upon both the cell suspension and the chemistry of the surrounding media\textsuperscript{42}.

This preliminary data can be a future step in understanding the IP response of bacteria in porous media approaching a field scale. As all scales are investigated, we can close the gap between measurements at all scales and better understand the field scale observations.
FIGURES

**Figure 1**: IP response mechanisms of bacteria in porous media.

**Figure 2**: Time domain IP measurements. The circle indicates the area of time domain IP that equates to AFM ion mobility measurements.
Figure 3: Applied tip bias and measured tip response. Amplitude is the response to the repulsion force of the tip. The attractive force is the tip responding to the accumulation of opposing charges on the surface.

Figure 4: Environmental control chamber schematic
**Figure 5:** AFM image of a single *E. coli* cell typically used for measurements.

**Figure 6:** Actual data fit to a single and double exponential function. Notice the double exponential fits the entirety of the data where the single fit does not.
Figure 7: Averaged ion mobility traces for mica, *E. coli* and *B. subtilis* at 5Hz, >50% RH.

Figure 8: \(F_{\text{max}}\) values for mica, *E. coli* and *B. subtilis* from 1-100 Hz on each surface over all RH values below 50%. Applied dc-bias = 10V peak to peak. Error bars represent the 95% confidence interval over all measurements on each surface. Mica data represents the average of 1,350 traces, *E. coli* is the average of 1,050 traces and the *B. subtilis* is averaged over 900 traces.
Figure 9: Frequency spectra for mica, *E.coli* and *B. subtilis* at increasing RH. Applied dc-bias had an amplitude of 10V peak to peak.

Figure 10: Frequency spectra for mica, *E.coli* and *B. subtilis* each at ~89% RH. Applied dc-bias had an amplitude of 10V peak to peak.
Figure 11: Histogram of $\tau_1$ and $\tau_2$ values for *E. coli*, *Bacillus subtilis* and mica. Mica exhibits faster responses than both bacteria.

Figure 12: Measured and modeled dielectric permittivities of bacteria cells and the mica surface.
Figure 13: $F_{\text{max}}$ values for mica, *B. subtilis*, and *E. coli* for RH values >75%. Error bars represent 95% confidence intervals.

Figure 14: Mica, *B. subtilis* and *E. coli* $F_{\text{max}}$ values from 50-75% for multiple specimens. Each point is the average of 5-6 different cells or mineral samples. Error bars represent 95% confidence intervals.
Figure 15: Schematic of a Gram Positive and Gram Negative Cell Wall

Figure 16: Magnetospirillum Growth Curve
Figure 17: Diagram of column scale lab set up

Figure 18: Measured phase changes made in the lab compared to the circuit simulated phase changes made in QUCS.
Figure 19: Measured phase changes for tap water and an aqueous suspension of bacteria in a column with 2 standard errors.
REFERENCES


