Project Title: Solid State Electron Transfer via Bacterial Nanowires:
Contributions Toward a Mechanistic Understanding of Geophysical Response of Biostimulated Subsurface.

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Introduction

The degradation of organic matter by microorganisms provides a source of electrical potential or so-called "self potential" (SP) that can be measured by using a voltmeter. During this process electrons are being produced as a waste-product and bacterial cells have to dispose of these to allow for the complete biodegradation of organic matter. Especially in anaerobic microbial communities, exo-cellular electron transfer is the most important driving force behind this process and organisms have developed different, but also similar, ways to transfer electrons to other microorganisms. Recently, it has been postulated that direct electron transfer from cell-to-cell is actually done by "hard-wired" microorganisms (1, 2). This shuttling of electrons is most likely done by certain c-type cytochromes that form the functional part of electrically conductive nanowires (2). In this study we investigated if nanowires can explain the geoelectrical (self potential and spectral induced polarization) signals observed at some biostimulated environments such as DOE sites.

The objectives of our project are to: 1) investigate any temporal changes in the geophysical signatures (Self Potential (SP) and Induced Polarization (IP)) associated with nanowires of the bacterium Shewanella oneidensis MR-1, wild type and mtrc/ omcA deletion mutant, 2) demonstrate that mutant strains of bacteria that produce nonconductive nanowires do not contribute to geoelectrical responses.

We accomplished the following:

1) Provided training to students and a postdoctoral fellow that worked on the project. ,
2) Conducted several SP & IP measurements correlating the distribution of nanowires and SIP/SP signals in partial fulfillment of object # 1 and 2.

On the following we will report and discuss the results of our last experiment with some emphasis on the source mechanisms of both SP and IP associated with Shewanella oneidensis MR-1, wild type in sand columns.

Methods

Self potential and IP signals were simulated in the laboratory by suspending the bacterium Shewanella oneidensis MR-1, wild type in columns filled with saturated sands (Fig. 1).

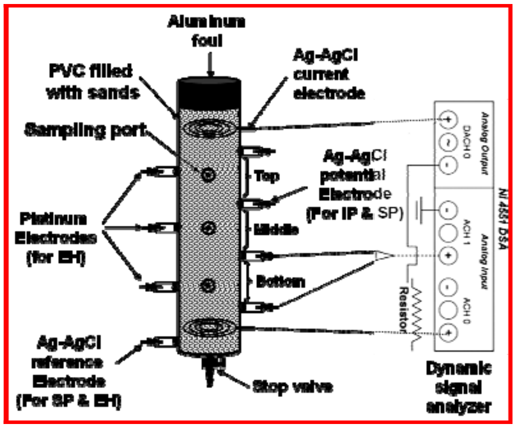

Figure 1. Schematic diagram showing the setup of the experimental columns.

PVC columns were filled with quartz sand and saturated with minimal medium containing 10 mM lactate. The wild type strain of S. oneidensis MR-1 was pre-grown in continuous cultures at low oxygen tension to provide the inoculum to the sand columns. The Keithley mutilmeter and data acquisition system were used to perform the self-potential (SP) and redox potential (EH) measurements at various electrode positions installed along the walls of the columns. Induced polarization (real and imaginary conductivity) was measured using NI Dynamic signal analyzer in the frequency range 0.1-1000 Hz at three different positions along the length of the columns (bottom, middle, top). Fluid samples were retrieved from the columns during the time span of the experiment (48 days) for biogeochemical analysis. By the end of the experiment, soil samples were retrieved for SEM analysis.

Results

The influence of microbial activity on biogeochemical parameters is presented in figure 2. The pH values decreased over time (Fig.2a) due to the production of low molecular weight organic acids (from the reduction of lactate) being higher in magnitude at the top of the column compared to the bottom. The organic acids will dissolve the sand grains and leach ions to the pore fluids and this in addition to some other metabolic byproducts will lead to increase in the fluid conductivity as shown in figure 2b. The redox potential (Eh) shows a gradient being higher at the top part of the experimental columns compared to the lower part which can be attributed to the gradient of oxygen concentration along the length of the column (Fig. 2c). The total

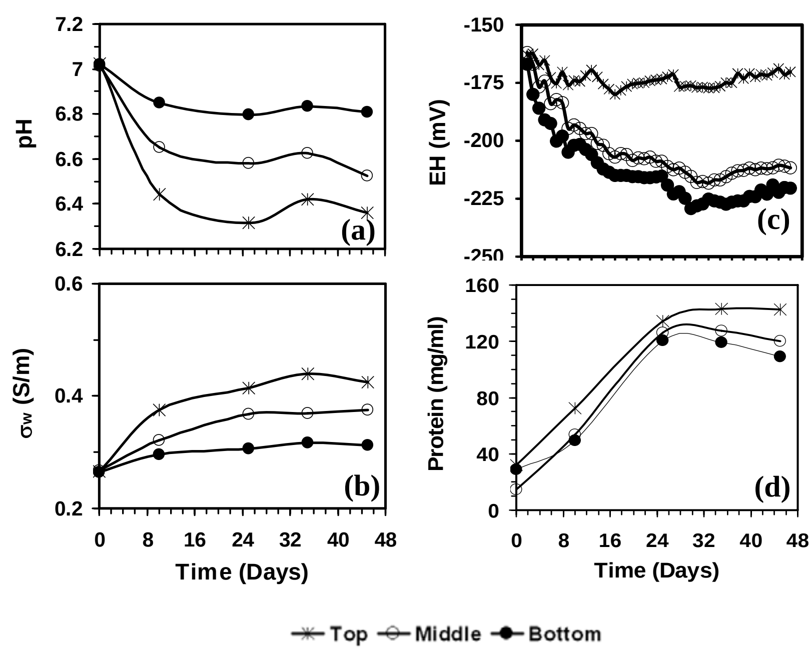

Figure 2. Temporal change in (a) pH, (b) fluid conductivity (σw), (c) redox potential (EH), and (d) protein concentration.

protein of Shewanella oneidensis MR-1 increases over time up to day 24 and remains steady thereafter (Fig. 2d). It is evident that the redox gradient developed along the length of the columns has impacted the microbial activity and hence the biogeochemical parameters.

Despite the fact that microbial growth along the column looks relatively similar in magnitude and pattern, the SEM images shows different magnitude in biofilm/EPS development and density of microbial nanowires as shown in figure 3. The top part of the column (more energy and oxygen) shows higher and healthy microbial cells, biofilm/EPS structures and nanowires compared to the bottom part of the column with less oxygen and energy.

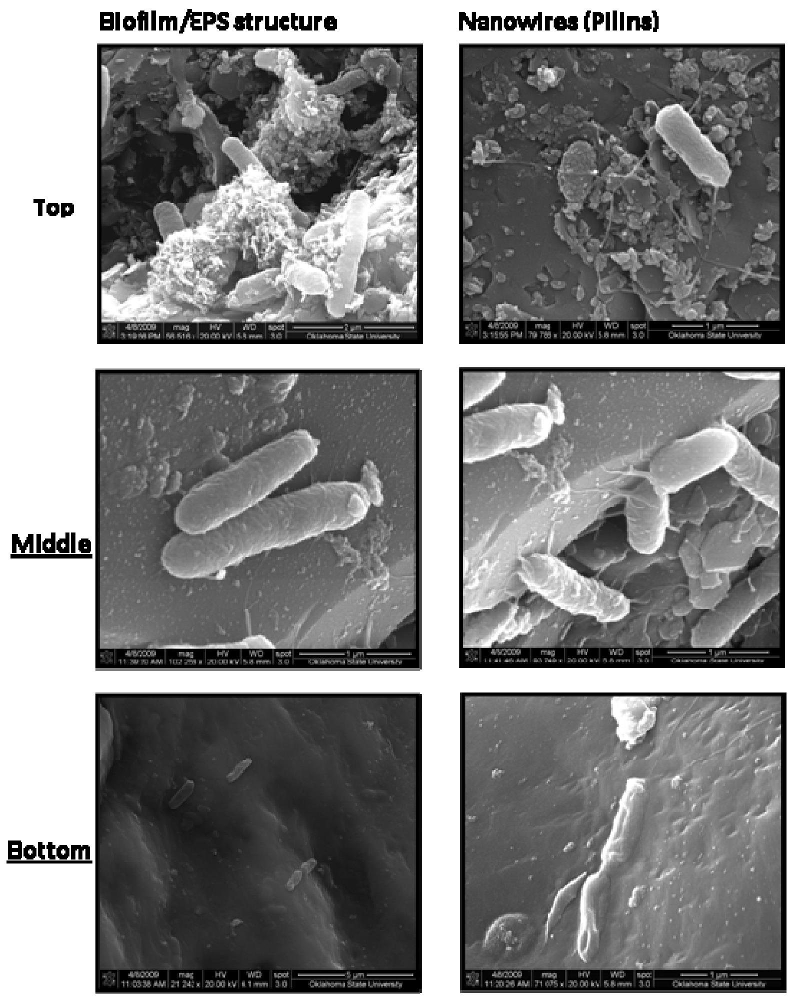

Figure 3. Representative scanning electron microscopy images of sand grains from different parts of the sand columns showing biofilm/EPS structure and nanowires (pilins) structure.

The experiment was conducted under limited terminal electron acceptor (oxygen) and the experimental procedure allow for more oxygen at the top part of the column compared to the bottom. This oxygen gradient impacts the microbial activity and the production of bioflm/EPS and nanowires structure. In addition, a redox gradient was developed along the length of the sand columns.

The results of the induced polarization (IP) and self-potential (SP) measurements are presented in figure 4. Both the imaginary (fig. 4a) and real (Fig. 4b) conductivity components show a gradual increase in the magnitude up to day 24 and remain steady to the end of the experiment. In addition, the top part of the column shows higher magnitude in the imaginary and real conductivity components compared to the bottom part which is coincident with the measured biogeochemical parameters. The real conductivity component is influenced by the increase in fluid conductivity over time as well as the enhancement of surface conduction due to biofilm development and microbial cell density. However, the imaginary conductivity component is influenced by the increase in surface area due to biofilm development as well as the density of microbial nanowires as shown in figure 3. The SP measurements show a gradual and relatively low increase during the first 24 days (~ 25 mV) as shown in figure 4c. During the time period between day 24 and 32, the SP values increase rapidly to almost 125 mV which can be attributed to the development of nanowire structures. After day 32 and to the end of the experiment the SP shows a gradual and relatively low increase up to 150 mV which can be attributed to the fully development of nanowires and steady state of electron transfer.

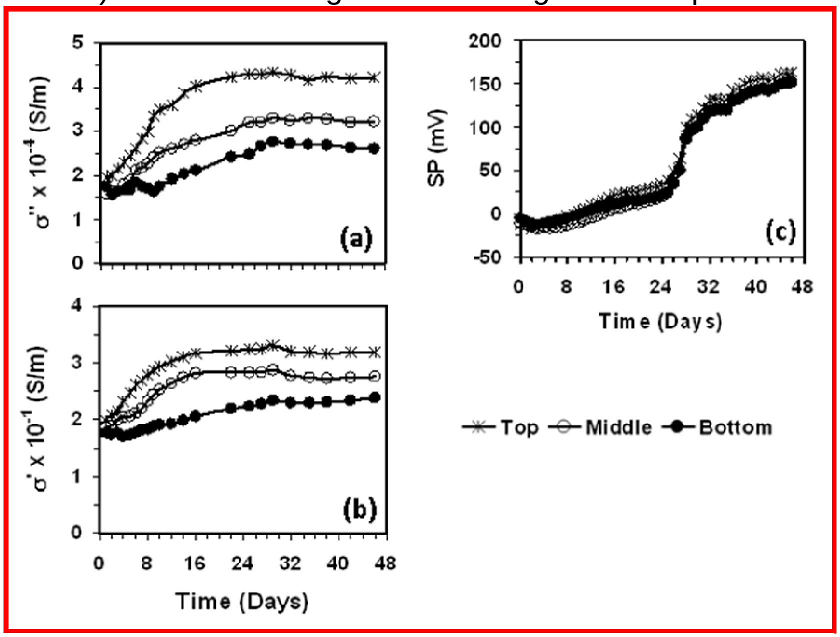

Figure 4. Temporal change in (a) imaginary conductivity (σ''), (b) real conductivity (σ'), and (c) Self potential (SP).

We infer that the IP response can be attributed to:

1- The increase in pore fluid conductivity
2- Biofilm development leading to bioclogging
3- The development of the conductive nanowires

The SP response can be attributed to the development of the conductive nanowires.

We hypothesize that both biofilm/EPS development and nanowire structures play important role in the measured geophysical signature associated with SP and IP measurements which in turn is related to the availability of oxygen and the redox gradient developed along the length of the columns.

Our results suggest that microbial activity and development of nanowires may impact the electrical properties of porous materials and may contribute to our understanding of the mechanisms that underlie geophysical methods for mapping microbial activity in near subsurface environments.

Partial finding from this project resulted in the following publications:

Abdel Aal, G. Z., E. A. Atekwana, S. Rossbach, and D. D. Werkema (2010), Sensitivity of geoelectrical measurements to the presence of bacteria in porous media, J. Geophys. Res., 115, G03017, doi:10.1029/2009JG001279.

Abdel Aal, G. Z., E. A. Atekwana, and E. A. Atekwana (2010), Effect of bioclogging in porous media on complex conductivity signatures, J. Geophys. Res., 115, G00G07, doi:10.1029/2009JG001159.

Abdel Aal,G., E. Atekwana, S. Radzikowski, and S. Rossbach (2009), Effect of bacterial adsorption on low frequency electrical properties of clean quartz sands and iron-oxide coated sands, Geophys. Res. Lett., 36, L04403, doi:10.1029/2008GL036196.

References Cited

1. Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., and Lovely, D.R. Extracellular electron transfer via microbial nanowires. Nature Letters 435(23), 1098-1101 (2005).
2. Gorby, Y.A, Yanina, S., Moyles, D., Marshall, M.J., McLean, J.S., Dohnalkova, A., Rosso, K.M., Korenevski, A., Beveridge, T.J., Beliaev, A.S., Chang, I., Kim, B.H., Kim, K.S., Culley, D., Reed, S., Romine, M., Saffarini, D., Shi, L, Elias, D., Kennedy, D.W., Pinchuk, G., Hill, E.A., Zachara, J.M., Nealson, K. & Fredrickson, J.K. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences, PNAS: 130:11358-63 (2006).
3. Davis, C. A., Atekwana, E. Atekwana, E., Slater, L.D., Rossbach, S., and Mormile, M.R. Microbial growth and biofilm formation in geologic media is detected with complex conductivity measurements. Geophysical Research Letters, L18403, doi:10.1029/2006GL027312 (2006).