TECHNICAL PROGRESS REPORT

Progress Report on Literature Review of Geophysical Resistivity Measurements and Microbial Communities (Subtask 1.3.3)

Date Submitted: March 18, 2016

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Prepared for:

U.S. Department of Energy Office of Environmental Management Under Cooperative Agreement No. DE-EM0000598



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Introduction

FIU performed a review of literature relating to the influence of microbial activity on the corresponding electrical response after ammonia injections in the vadose zone. Significant uranium contamination at the U.S. Department of Energy Hanford Site exists within the vadose zone (up to 76 m) in the form of mobile carbonate phases (rutherfordine, liebigite) and silicate phases (uranophane, Na-boltwoodite). Remediation of this zone requires *in situ* sequestration of mobile uranyl-carbonate species (Szecsody et al., 2012).

The first goal of this research is to investigate the influence and corresponding electrical geophysical response of microbial activity on vadose zone uranium sequestration using gas phase ammonia injections. The second goal of this work is to analyze the ability of geophysical electrical methods, particularly spectral induced polarization (SIP) and electrical resistance tomography (ERT), to detect subsurface microbial activity in a porous medium. Remote geophysical sensing of the subsurface allows scientists to forgo the drilling of expensive boreholes and rely instead on easily and cheaply deployed surface arrays in order to study processes occurring deep in the subsurface. Geophysical methods also allow the continuous collection of data autonomously, which can be remotely accessed and analyzed.

Initial experiments presented in this review will utilize 1-D laboratory columns.

Background

Overview of 200 Area Subsurface

The underlying bedrock beneath Hanford is the Columbia River Basalt Group; it is composed of hundreds of individual tholeiitic basalt flows that formed during the Miocene (23.03 - 5.3 Ma). Above that lies the Ringold formation which is composed of fluvial sediments approximately 125 m thick and is divided into three principal stratigraphic units: unit A (fluvial gravels), the lower mud unit, and unit E (fluvial gravels). The main aquifer under the 200 West Area is located mostly within unit E; the lower mud unit forms a low hydraulic conductivity base to this aquifer and confines groundwater stored in unit A. Between the Ringold Formation and the Hanford formation lies the Cold Creek Unit (formerly Plio-Pleistocene unit) which has a thickness up to 13.1 meters and is divided into two subunits: the upper CCUz (abundance of silt) and lower CCUc (abundance of pedogenic calcium-carbonate cement). Above the Plio-Pleistocene unit lies the Hanford formation which is composed of Pleistocene (2.58 – 0.0117 Ma) age deposits from cataclysmic floods during the Ice Age. The main constituents of the Hanford Formation are three distinct facies: a gravel dominated facie, a sand dominated facie, and a silt dominated facie (Serne et al., 2002; Xie et al., 2003).

The water within the principal unconfined aquifer under Hanford flows from recharge zones in the west towards the NE, E, and SE and eventually discharges into the Columbia River. Estimates of discharge from the Hanford aquifer into the river range from 1.1 to 2.5 m^3/s , which is considered to be relatively low. The hydraulic gradient of the water table is gentler under the 200 East Area compared to the 200 West Area due to the effects of a higher subsurface hydraulic

conductivity. This is on account of the fact that the top of the aquifer in the 200 East area lies within the Hanford formation which is more permeable than the Ringold formation (Hartman et al., 2007).

Subsurface contamination is split between the river corridor (wastes derived from the operation of the reactors, mainly strontium-90 and hexavalent chromium) and the Central Plateau (plutonium extraction activities, more varied waste streams). While most subsurface contamination at the 100 Area is strontium-90 and hexavalent chromium, there is a large plume of nitrate and a smaller plume of trichloroethene under the 100-F Area. In addition, all of the areas have nitrate concentrations greater than the maximum contaminant level (MCL) of 45 mg/L. Contamination within the central plateau includes carbon tetrachloride, nitrate, tritium, iodine-129, technetium-99, hexavalent chromium, and uranium. Downward migration of contaminants into the vadose zone and the groundwater was facilitated by the intentional and accidental addition of water from wastewater ditches and cribs, water pipe leaks, and meteoric water. Contamination within the vadose zone continues to supply the underlying groundwater with contaminants. Figure 1 shows a map of major contaminant plumes under the 200 East and 200 West Areas (DOE/RL-2015-07, 2015).



Figure 1. Contaminant plumes under the 200 Area taken from (DOE/RL-2015-07, 2015).

Ammonia Injection

Contamination at the U.S. DOE Hanford Site has spread into the vadose zone and requires *in situ* remediation. The end goal of this remediation is the transformation of mobile uranium carbonate species into less mobile uranium silicates and phosphates. One proposed method is the injection

of ammonia (NH₃) gas, which would partition into the pore water and cause an increase in the fluid pH. Ammonia gas injections are the preferred method over soluble amendments, such as injection of tripolyphosphate, due to the fact that liquid injections may lead to additional downward migration of contamination (Szecsody et al., 2012).

Szecsody et al. (2012) conducted experiments with ammonia gas injections using sediment samples collected from the Hanford Site 200 Area where uranium solid phases were identified as Na-boltwoodite. Experimental results suggested that ammonia injections were most effective in reducing the mobility of aqueous and absorbed U species. This decrease is likely due to the incorporation of contaminants into precipitates formed during ammonia treatment. While the mobility of U(VI) in the Na-boltwoodite sample was lowered, XANES and EXAFS analysis conducted on the sample have not revealed any changes in the amount of Na-boltwoodite. This was interpreted as coating of some existing U-bearing solid phases by a low-solubility non-U precipitate, which would isolate them from the surrounding pore water. The sediment sample high in carbonates did not show a significant decrease in uranium mobility.

SIP Method

Electrical geophysical methods allow geophysicists to understand subsurface properties by measuring the voltage response to an electric current. Similar to standard DC resistivity methods, most induced polarization (IP) methods employ four electrodes in galvanic contact with the sediment. Two of the electrodes are current electrodes which act as source and sink for an electric current; the other two electrodes are potential electrodes which measure a voltage response. Spectral induced polarization (SIP) is a type of IP method that measures a phase shifted voltage at various injection frequencies. An impedance, in terms of magnitude and phase angle, is then obtained and used as a measure of charge transport and storage (Binley and Kemna, 2005).

The SIP method allows geophysicists to quantitatively study charge storage and transport in porous media through the electrical complex conductivity. SIP has been used in the past to locate metallic ore bodies as well as subsurface zones rich in clay; however, recent work has focused on its applications in studying contaminant fate and transport. The injected current used for SIP measurements normally has a frequency below 1 kHz. The electrical complex conductivity (σ^*), the inverse of the complex resistivity ($\rho^*(\omega)$), is a function of ω (the angular frequency of the applied current). This can then be expressed in terms of σ' (the real part which represents charge transport) and σ'' (the imaginary part that represents charge storage). In the following equation defining the complex conductivity: $i = \sqrt{-1}$ (Hao et al., 2015)

$$\sigma^*(\omega) = \sigma'(\omega) + i\sigma''(\omega) \tag{1}$$

The impedance can be obtained by measuring the difference between two voltage electrodes and is defined as:

$$Z^*(\omega) = \frac{U}{I} = |Z^*(\omega)|e^{i\varphi(\omega)}$$
(2)

Where $Z^*(\omega)$ is the impedance, *U* is the voltage difference between the measuring electrodes (in V), *I* is the injected current (in A), ω is the angular frequency (in rad S⁻¹), and φ is the phase angle (in rad). The impedance can be related to the complex resistivity using a geometric factor *K* (in m) as:

$$\rho^*(\omega) = KZ^*(\omega) \tag{3}$$

This geometric factor is based on the position of the electrodes, the size and shape of the samples, and boundary conditions on the grain surfaces. The complex conductivity is then just the inverse of the complex resistivity:

$$\sigma^*(\omega) = \frac{1}{\rho^*(\omega)} \tag{4}$$

The conductivity magnitude can be expressed in terms of the real and imaginary components:

$$|\sigma| = \sqrt{(\sigma')^2 + (\sigma'')^2} \tag{5}$$

The phase angle can be described in terms of the components of the complex conductivity and an approximation can be made when the phase magnitude is less than 100 mrad. The phase angle is based on the ratio between the polarization and conduction (Okay et al., 2014):

$$\varphi(\omega) = \tan^{-1} \left[\frac{\sigma''(\omega)}{\sigma'(\omega)} \right] \cong \frac{\sigma''(\omega)}{\sigma'(\omega)}$$
(6)

There are two proposed mechanisms governing the low frequency polarization response, both relying on a structure known as the electrical double layer (EDL) at the solid-fluid interface. The EDL is formed when an electrolyte solution comes into contact with a charged surface; counterions from the solution are then attracted to the surface, forming two parallel charge layers of opposite polarity. Modern interpretation of the EDL actually designates three layers: the solid phase, the Stern layer, and the diffuse layer. The first theory asserts that at pore constrictions, where a pore becomes drastically thinner, the EDL comes together to form an ion selective

membrane that would cause the charge to build up at those locations. The second theory argues that charge builds up when excess ions move at differing speeds in the Stern layer tangential to the solid surface (Scott, 2006).

Scott (2006) conducted SIP measurements on sandstone samples saturated with an agar gel solution in order to prevent the motion of the bulk fluid during current injection. The results show that the quadrature conductivity is mainly unaffected by the use of an agar gel solution. This indicates that polarization is mainly controlled by grain surfaces rather than an ion selective membrane since ionic motion was constrained.

Skold et al. (2011) argues that there may exist a proton hopping mechanism at silica surfaces which may also contribute to polarization. This charge movement would be based on a Grotthuss cooperation mechanism where the charge moves along a surface through the breaking and reforming of hydrogen covalent bonds.

SIP Responses to Inorganic Factors

The specific surface area (S_p) is the ratio between the pore surface area and the pore volume. Generally, as grain size increases, specific surface area decreases due to the decreasing ratio between surface area and pore volume. There exists a positive correlation between the specific surface area and the imaginary conductivity (Lesmes and Friedman, 2005).

Sediment clay content has been shown to have a positive correlation with both the in-phase and quadrature conductivities. Clays contribute greatly to the cation exchange capacity (CEC) of sediment, which is a measure of a sediments ability to absorb cations that in turn leads to higher quadrature conductivity. For kaolinite rich sediments, the quadrature conductivity increases in response to higher salinities; on the other hand, smectite rich sediments display a quadrature conductivity that is mostly independent of salinity (Okay et al., 2014).

Sediment grains generally have a negatively charged surface; however, if pH reaches the point of zero charge (PZC), the net surface charge becomes neutral, leading to reduced sorption of cationic groups. Reductions in pH below the PZC can lead to a positive surface charge as H^+ attaches to all negative sites on the grain surface. Low pH can reduce the effects of surface conductivity and polarization (Lesmes and Frye, 2001).

SIP Responses to Subsurface Biofilm Formation

Bacteria in the subsurface are seldom found as solitary mobile organisms; rather, most microorganisms form interconnected immobile colonies known as biofilms. These biofilms are supported by extracellular polymers, which are the individual cells excrete and these polymers serve to strengthen attachment to a solid surface as well as to provide structural integrity to the biofilm. Biofilm formation can produce various changes in the physical and electrical properties of a porous medium; these changes include: clogging of pores (changes to porosity, permeability, and hydraulic conductivity), changes to overall shear strength and elastic moduli of media, production of proteinaceous extracellular appendages that facilitate electron transport and

increase bulk electrical conductivity, alterations to pore fluid electrolyte concentrations, dissolution of minerals leading to increased surface roughness, and precipitation of magnetosomes (Atekwana and Slater, 2009).

Modern research towards the direct detection of bacteria in subsurface porous media has placed a significant focus on the SIP method. Most bacteria have higher concentrations of anionic groups, which lead to a negatively charged cell wall; this in turn, when in the presence of an electrolyte solution, causes the formation of an EDL by counterions. Due to this effect, the bacterial surface can store charge when in the presence of a time-oscillating electric field in a fashion similar to charged mineral grains. Only bacteria that are alive contribute to the SIP response (Atekwana and Slater, 2009).

Experiments using artificial biofilm consisting of alginate mixed with microbial cells in a silica bead packed column have shown significant low frequency (0.1 - 1 Hz) SIP responses to biofilm formation. By using artificial biofilm and silica gel beads with a very smooth surface area, this study isolated the SIP response to the actual presence of biofilm rather than grain roughness or changes in the chemical makeup of pore fluid (Ntarlagiannis and Ferguson, 2008).

Methodology of Column Experiments

Literature Research Overview

Johnson et al. (2013) used a column for the purpose of determining pore water saturation. The main focus of the geophysical measurements was to obtain bulk conductivity; however, a similar setup should be usable for SIP measurements. The column used plate electrodes at opposite ends as current source and sink; such a system allows for a homogeneous one dimensional current flow from source to sink along the length of the column. The potential gradient created by the current flow can then be measured using eleven Ag/AgCl electrodes placed flush along the column length. Since two potential electrodes are required for each measurement, this allowed for ten intervals for potential measurements. A standard resistor of known resistance was placed in the current loop in order to allow computation of resistance across electrodes using circuit analysis. The current used had a 1 Hz sinusoidal injection waveform.

Ntarlagiannis and Ferguson (2008) conducted column experiments on silica beads treated with artificial alginate biofilm. The experimental design used two Ag/AgCl coiled current electrodes at either end to supply current flow. The column had a length of 20 cm. Measurements were done using two pairs of potential electrodes, one pair 4 cm apart and a second pair 8 cm apart. In order to avoid polarization, the electrodes did not extend into the column. The artificial biofilm was a mix of alginate with a bacterial culture. The bacteria used were *Pseudomonas putida* 9816/11, which was cultured in 100 mL of nutrient broth at 37°C for 24 h. The general methodology used for the experiments was as follows: before the experiments with the alginate/bacteria mix were conducted, a baseline SIP measurement was taken on a tap water saturated column as well as fluid flow measurements. Artificial biofilm was rinsed out using

deionized water due to the fact that the presence of free Ca^{2+} and Cl^{-} ions would lead to an increase in the real conductivity. SIP measurements were then made on tap water saturated columns with the artificial biofilm present. The benefit of this approach is that it isolates the biological SIP response, although it may not properly represent the response in natural conditions.

Ntarlagiannis et al. (2005) studied the SIP response of iron and zinc sulfide biomineralization under anaerobic conditions in three columns. The three columns were as follows: one for electrical measurements (A), one for geochemical sampling (B), and a non-inoculated control (C). Both A and B were identical apart from the type of measurement being conducted. The geochemical sampling ports were extended into the center of the column and could affect current flow; so, the measurements were done on separate but identical columns. All three columns were 30.5 cm long with a diameter of 5.08 cm. The geochemical sampling column had seven ports positioned every 3.8 cm in order to coincide with the centers of each electrode pair on the electrical column; by extension, the electrical column had eight electrodes spaced 3.5 cm apart and flush against the surface, creating seven distinct measurement points. The electrodes were Ag-AgCl. The columns were filled with 20-30 mesh 99.8% quartz sand, which was packed by periodically tapping the column 15-20 times during filling. Since the experiment was designed to use anaerobic sulfate reducing bacteria, the columns were flushed with N₂ gas in order to evacuate any oxygen in the sand. The setup was contained within an anaerobic chamber.

Column Experiments at PNNL

PNNL column experiments to begin in spring 2016 will be based on existing, currently operational columns, which were designed and constructed by a team under Dr. Brady Lee. The current setup uses a total of 14 columns; future work at FIU will use only 6 columns. Using a large number of columns allows for testing of various variables simultaneously. Each column has one pair of potential electrodes for measurement at the middle of the column; there is also an injection point for the microbes under the lowermost electrode. The ends of each column contain two coiled Ag-AgCl current electrodes which provide homogeneous current flow parallel to the columns length. Figure 2 shows the basic setup of the electrodes on the column as well as an overview of the column construction. Figure 3 displays the makeup of the end caps that cover each end of the columns.



Figure 2. Structure of each column. C = Current Electrodes, P = Potential Electrodes, S = Microbe Injection Port.



Figure 3. Various parts of end cap. A1 = current electrode port, A2 = influent/effluent port, A3 = end cap main body, B = rubber ring, C = porous plastic stopper, D = Coiled Ag-AgCl electrode.

Lessons Learned

Kemna (2014) provided several suggestions on the application of laboratory SIP experiments. From his overview, great care needs to be taken when working with unconsolidated sediments due to the fact that small differences in packing and water saturation can lead to significant differences in the SIP response even between identical samples. The samples should be saturated with solutions of known composition and enough time must elapse for chemical equilibrium to be established. At low frequencies (<1 kHz), a four electrode measurement array should be used. Equipment should be calibrated against a reference resistor-capacitor circuit as well as water

samples and the calibration should be thoroughly documented. Potential electrodes should not be within the current path; rather, they should be placed parallel to the path and flush against the sediment in electrolytic contact with the pore fluid.

Flow through the columns should be from bottom to top in order to ensure homogeneity in sediment fluid saturation over time. If the direction of the flow is from top to bottom, then fluid could penetrate at varying rates depending on the permeability of the sediment.

Electrodes should be non-polarizing and not penetrate into the sediment. Current electrodes should be placed at the ends of the column and should be plate or coiled electrodes in order to create a homogeneous flow of current from source to sink. Potential electrodes should be flush with the sediment. Experiments at PNNL use coiled Ag-AgCl for both current and potential electrodes.

In Ntarlagiannis et al. (2005), sulfate reducing bacteria migrated towards the nutrient source (influent from the bottom to the top of the column) and remained there, causing almost all sulfide mineral precipitation in the bottom of the column. This meant that although there were eight potential electrodes spaced throughout the column, only those near the bottom measured a significant change in the imaginary conductivity; electrode pairs closer to the effluent saw little change. It has also been noted during column experiments at PNNL that microbes can swim up the influent and potentially reach the source if barriers are not prepared in advance. The solution that scientists at PNNL devised was to connect IV drip tube air chambers (Figure 4) to the influent so that the solution would drip down rather than flow continuously and present a path for microbes to travel. Both these situations highlight that the movement of microbes introduced into columns should be considered in the experimental design.

When conducting geophysical column experiments, it is important to properly label all electrical and fluid connections to the columns; doing so reduces human error in regards to collecting data as well as injecting the correct solutions into the corresponding columns. Future column work will use six columns with five potential electrodes per column, including the current electrodes; this adds up to forty two electrical cables and twelve fluid tubes.



Figure 4. Air chambers used to prevent microbes swimming into influent; lines show flow of water through tubing. (A) Marks the pocket of air that prevents microbe migration.

Something to note is that experiments at PNNL have experienced some difficulty due to sediment from the column entering into the thin effluent tubing as well as the sample ports, blocking fluid flow and potentially creating excess back pressure. For future work, it would be beneficial to use some sort of filter on these ports that would allow water to pass through but not sediment. The sediment can clog up tubing and create high undesirable back pressure. This excess pressure can lead to the air chambers being filled with water, which could allow bacteria to swim up into the source; it can also cause tubing to pop off, creating small spills and allowing air bubbles to enter the column.

The Design of Column Experiments at PNNL - Spring 2016

The current plan for collaborative geophysical experiments between FIU and PNNL involves the construction of six columns. These columns will use combinations of HCO_3 , a carbon source (likely glucose), and microbes in order to isolate the SIP response of each variable. Table 1 lists the proposed content of each column. Also, each column is planned to have a layer of Ca-autunite mixed with Hanford fine sand. The columns will have a very similar structure to existing columns displayed in Figure 2; however, the new columns will not use the porous plastic stopper shown in Figure 3. In order to prevent sediment from entering the tubing, the columns will use a fine plastic filter.

Proposed Column Contents					
Column 1	0 mM HCO ₃				
Column 2	3 mM HCO ₃				
Column 3	$0 \text{ mM HCO}_3 + \text{Carbon Source}$				
Column 4	$3 \text{ mM HCO}_3 + \text{Carbon Source}$				
Column 5	0 mM HCO ₃ + Carbon Source + Inoculum				
Column 6	$3 \text{ mM HCO}_3 + \text{Carbon Source} + \text{Inoculum}$				

Table 1. Planned Contents for Each Column

Each column is planned to have five potential electrodes along the length for a total of four distinct measurement points. Each column is also planned to have four sampling ports opposite

to the electrode sites. Similar to previous experiments, both the current and potential electrodes will be coiled Ag/AgCl. These electrodes are hand coiled from a long piece of pure silver wire. The bottom of the electrodes is then allowed to sit overnight in undiluted bleach which provides the chloride necessary to produce AgCl. The equipment necessary to conduct SIP measurements on the columns has been ordered; however, there have been delays on the side of the supplier.

The medium in which the microbes will be grown is synthetic groundwater (SGW1). The table below shows the stock solutions (titled A, B, and C) used to make SGW1 and the process used to make SGW1 follows.

SGW1 Stock Solutions	Concentration (g/L)
Α	
NaHCO ₃	12.1
KHCO ₃	1.6
В	
$MgSO_4$	3.06
CaSO ₄	0.82
С	
Ca(NO ₃) ₂ ×4H ₂ O	5.43
CaCl ₂ ×2H ₂ O	9.56

Table 2.	Stock	Solutions	for	SGW1

To create 1 L SGW1: Pipette 10 mL each of solutions A and C and 20 mL of solution B into 900 mL deionized water, then dilute to 1 L using deionized water. The SGW1 solution that we used also contained a concentration of yeast extract equal to 500 mg/L.

Microbes are being cultured in 50 mL SGW1 (with 500 mg/L yeast extract added beforehand) with approximately 500 mg Hanford sediment, 10 mg of autunite, and 50 mg glucose. On a weekly basis, a 1-mL sample of each culture is taken and transferred to a fresh container. Microbes originate from the sediment taken from a borehole and are naturally occurring in Hanford's vadose zone. Currently, the microbe species are unknown until DNA analysis can be conducted.

Due to the fact that the columns will contain autunite, they need to be treated with caution and work should only be conducted in areas capable of handling radioactive materials. The effluent that flows through the columns will also likely be considered radioactive waste.

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