TECHNICAL REPORT

Spectral Induced Polarization Response of Biofilm Formation in Hanford Vadose Zone Sediment

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INTRODUCTION

This technical report presents results of the initial column experiments relating to the Spectral Induced Polarization response of biofilm formation within vadose zone sediment during the Fall of 2016. These experiments help to advance the understanding of geophysical and geochemical processes that occur in the subsurface. Significant uranium contamination at the U.S. Department of Energy's Hanford Site exists within the vadose zone (up to 76 m). The mobility of uranium in the oxidizing, carbonate-rich Hanford subsurface at pH ~8.0 is relatively high, which is explained by the formation of highly soluble and stable uranyl-carbonato complexes ($UO_2CO_3^0$, $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$) dominating in groundwater and pore water compositions. Uranium-bearing solid phases are mostly present as uranophane and Na-boltwoodite. Remediation of this zone requires *in situ* sequestration of mobile uranyl-carbonate species (Szecsody et al., 2012) to control the mobility of uranium.

This research is focused on 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 forego 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. The second goal of this work is to measure and record changes in pore water characteristics after microbe injection in columns.

Column experiments at Florida International University (FIU) consisted of 1-D columns, which ran continuously for several months during Fall 2016 and were monitored using SIP and porewater chemical analyses. A continuation of this work is planned for Spring of 2017 and will feature higher sampling frequency in order to further analyze the effect of microorganisms.

Overview of the 200 Area Subsurface

The underlying bedrock beneath Hanford Site 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 the 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 Site 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).

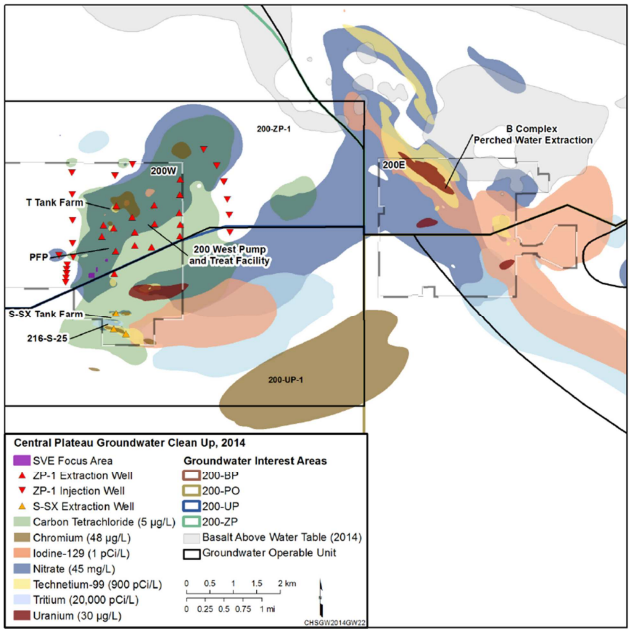


Figure 1. Contaminant plumes in the 200 Area subsurface (Source: DOE/RL-2015-07, 2015).

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 (Hao et al., 2015).

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 the individual cells excrete, and these polymers serve to strengthen their 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 which 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 electrical double layer (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

In June of 2016 researchers from Pacific Northwest National Laboratory (PNNL) arrived at FIU in order to build columns for SIP measurements. The general setup is pictured in Figure 5. There are six columns each with four potential electrodes and three sampling ports spaced equidistant on the sides. The potential electrodes are made from a silver wire encased within agar gel. This gel was prepared as a mix of agar and synthetic groundwater (Table 2) so that it would have a similar electrical conductivity to the adjacent pore water. The current electrodes are coiled Ag-AgCl and were placed on either end. These columns contain approximately ~700 grams of sediment from the Hanford 200 Area. In in the center of each column is a region composed of 100 mg of Ca-autunite particles that were mixed with sediment. Natural Ca-autunite, $Ca[(UO_2)(PO_4)]_2 \cdot 3H_2O$ obtained from Excalibur Mineral Corporation (Peekskill, New York), was previously characterized using ICP-OES, ICP-MS, X-ray diffraction and SEM/EDS methods to confirm the mineral composition, structure, and morphology as 98-99% pure autunite (Wellman et al., 2006). The autunite sample was powdered to have a size fraction of 75 to 150 μm or -100 to + 200 mesh with an average surface area of 0.88 $m^2\ g^{-1}$.

The body of each column is composed of clear PVC. Within the ends of the columns are filters designed to stop sediment from entering the inlet tubing. There is also a 3D printed plastic disk with holes (~5mm) at the ends to help support the filters.

Each column is fed solution from the bottom at a rate of 50 mL per day by an Ismatec peristaltic pump through a mix of flexible silicone and stiff Teflon tubing. The solution that is pumped through the columns is sparged with nitrogen gas for 10 minutes beforehand in order to remove dissolved gases. This is an effort to prevent gas bubbles from forming within the columns which can interfere with both geophysical measurements and pore water sampling.

Four different solutions are pumped through the columns (Table 1). These include: synthetic groundwater (Column 1), synthetic groundwater + 3 mM HCO_3 (Column 2), synthetic groundwater + 1 g/L glucose (Columns 3 and 5), and synthetic groundwater + 3 mM HCO_3 + 1 g/L glucose (columns 4 and 6). The synthetic groundwater base solution is made using only stock solutions A + B since the current setup only has HCO_3 in three of the six columns. Each container has enough solution to last ten days at which point new solution needs to be made.

Table 1. Contents of Each Column

Column Contents	
Column 1	0 mM HCO_3
Column 2	3 mM HCO_3
Column 3	0 mM HCO_3 + 1g/L glucose
Column 4	3 mM HCO_3 + 1g/L glucose
Column 5	0 mM HCO_3 + 1g/L glucose+ Inoculum
Column 6	3 mM HCO_3 + 1g/L glucose+ Inoculum

The medium in which the microorganisms have been cultured is synthetic groundwater (SGW1). Table 2 below shows the stock solutions (labeled A, B, and C) used to make SGW1 and the process used to make SGW1 follows.

Table 2. Stock Solutions for SGW1

SGW1 Stock Solutions	Concentration (g/L)
A	
$NaHCO_3$	12.1
$KHCO_3$	1.6
B	
$MgSO_4$	3.06
$CaSO_4$	0.82
C	
$Ca(NO_3)_2 \times 4H_2O$	5.43
$CaCl_2 \times 2H_2O$	9.56

To create 1 L SGW1: 10 mL each of solutions A and C and 20 mL of solution B were pipetted into 900 mL deionized water, then diluted to 1 L using deionized water. The SGW1 solution used also contained a concentration of yeast extract equal to 500 mg/L. The modified solution used did not contain bicarbonate and so was formed by pipetting 10 mL C and 20 mL B into 970 mL of deionized water. Pumping of the solution amended with glucose to Columns 3, 4, 5 and 6 began on day 33 of the experiment.

Microbial consortia were cultured at PNNL in 50 mL SGW1 (with 500 mg/L yeast extract added beforehand) with approximately 500 mg of Hanford sediment, 10 mg of autunite, and 50 mg of glucose. On a weekly basis, a 1-mL sample of each culture was taken and transferred to a fresh container. Microorganisms were originated from the sediment taken from a borehole and are naturally occurring in Hanford Site's vadose zone. Currently, the species of microbial consortia are unknown until molecular biology analyses can be conducted. These microorganisms were sent to FIU frozen and a new batch is being cultured at FIU in order to inject into Columns 5 and 6. Microorganisms were cultivated in the glucose with the tiny addition of Luria-Bertani (LB) media until the bacterial cell density (cells/mL) could be counted with the help of an INCYTO C-Chip disposable hemocytometer under a light microscope. Grown microbial consortia were injected via port 1 of Columns 5 and 6 in the amount of log 8.92 cell/mL on November 15, which was day 115 from the beginning of the experiment. The total number of cells injected to each column was log 9.62 cells/mL.

SIP measurements were taken once a week. These measurements were taken using a National Instruments data acquisition card which is inserted in the PCI slot of a PC. The measurement is controlled by the proprietary software Signal Express made by National Instruments. Current is injected at twenty-one different frequencies spaced logarithmically ranging from 0.1 Hz to 10,000 Hz with an amplitude and phase measured for each. Signal Express records data for amplitude, phase and frequency as ascii text files which are then analyzed using Python code written at FIU. In order to ensure reliability of measurements there is a reference resistor rated at

1800 Ohms placed in circuit with the columns which has amplitude and phase measured in order to correct for measured amplitude and phase in the columns.

The circuit through which SIP measurements are taken has a natural phase associated with it. This background phase was measured by running the sytem on a reference 120k Ohm resistor with a theoretical phase of 0. This background phase was then subtracted from the SIP results. During the experiment two different pci cards from NI were used, the first one was a loaner and was replaced by a new one. The data from the original card was corrected to match the results from the new card by finding the difference between old measurements and new measurements and adding it to the old measurements.

Pore water samples were taken once a week. These were taken by inserting a syringe into the sample ports and drawing water. Initially about 2 mL of sample was taken however in order to facilitate more chemical analyses this was increased to about 3 mL. Initial measurements included pH and conductivity (in mS/cm). Later on ORP was measured immediately after samples were taken and an extra 1.5 mL was taken for Fe^{2+} and total iron analyses.

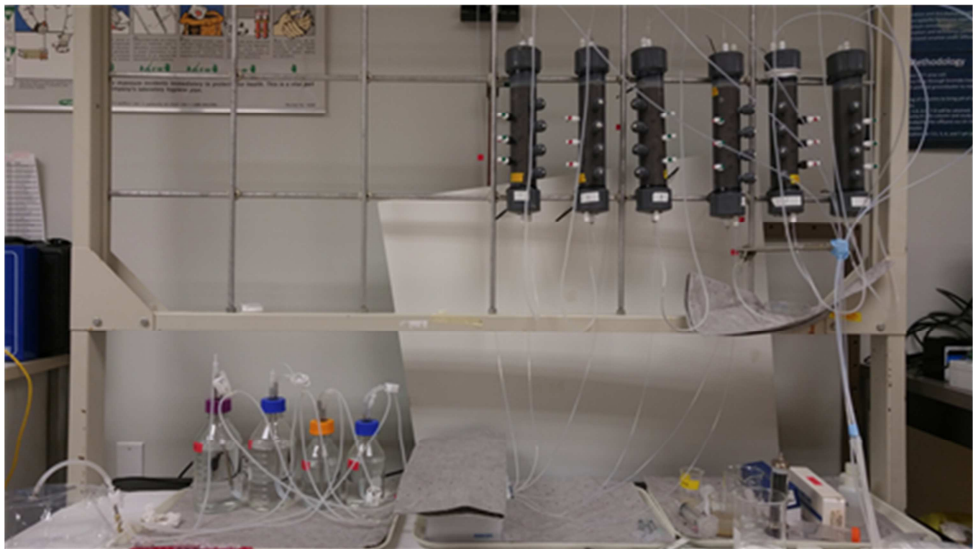


Figure 2. Current experimental setup at FIU.

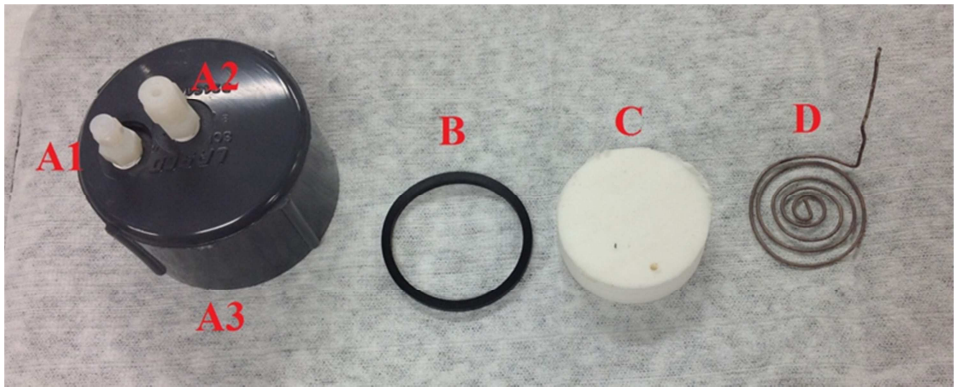


Figure 3. Various parts of end cap. A1 = current electrode port, A2 = influent/effluent port, A3 = end cap main body, B = rubber ring, C = porous plastic stopper (B and C were replaced by a 3D printed plastic stopper with mm scale holes in FIU's experimental column set-up), D = Coiled Ag-AgCl electrode.

Figure 4. Containers with solution, each connected to a nitrogen bag and to the pump.

RESULTS AND DISCUSSION

Initial SIP measurements as well as attempts at collecting porewater were commonly unsuccessful. This was due to various problems that were resolved over the course of the experiment, but nonetheless resulted in early data being very fragmented. These problems included the formation of air around potential electrodes as well as the clogging of sample ports. The presence of air around the sample ports also made sample collection unfeasible. In order to combat pore clogging FIU chose to leave syringes within the sample ports (Figure 5). This proved effective in preventing sediment from plugging the ports. This strategy, however, had the downside of allowing air into the columns through gaps in between the port and needle. In order to counteract this, FIU covered the connection between the needle and the port with Parafilm. FIU also moved the effluent container above the columns creating a hydraulic head gradient from the columns to the reservoirs preventing the siphoning of air at gaps.

Figure 5. Experimental columns with syringe and needles inserted in the sampling ports.

Spectral Induced Polarization Results

There was concern that the inclusion of metallic needles within the ports during SIP measurements would have a significant impact on the accuracy of the results. In order to test this, measurements were taken with and without needles and compiled into graphs showing the percentage difference (Figure 6). The difference was defined as:

$$\frac{(C_0 - C_n)}{C_0} * 100$$

Figure 7 shows that, in general, the difference between measurements conducted with and without needles inserted in the port is minimal (generally around 0). The first measurement at 0.1 Hz shows variation; however this is likely due to random error and limitations in the system being used rather than a systematic change due to the presence of the needle.

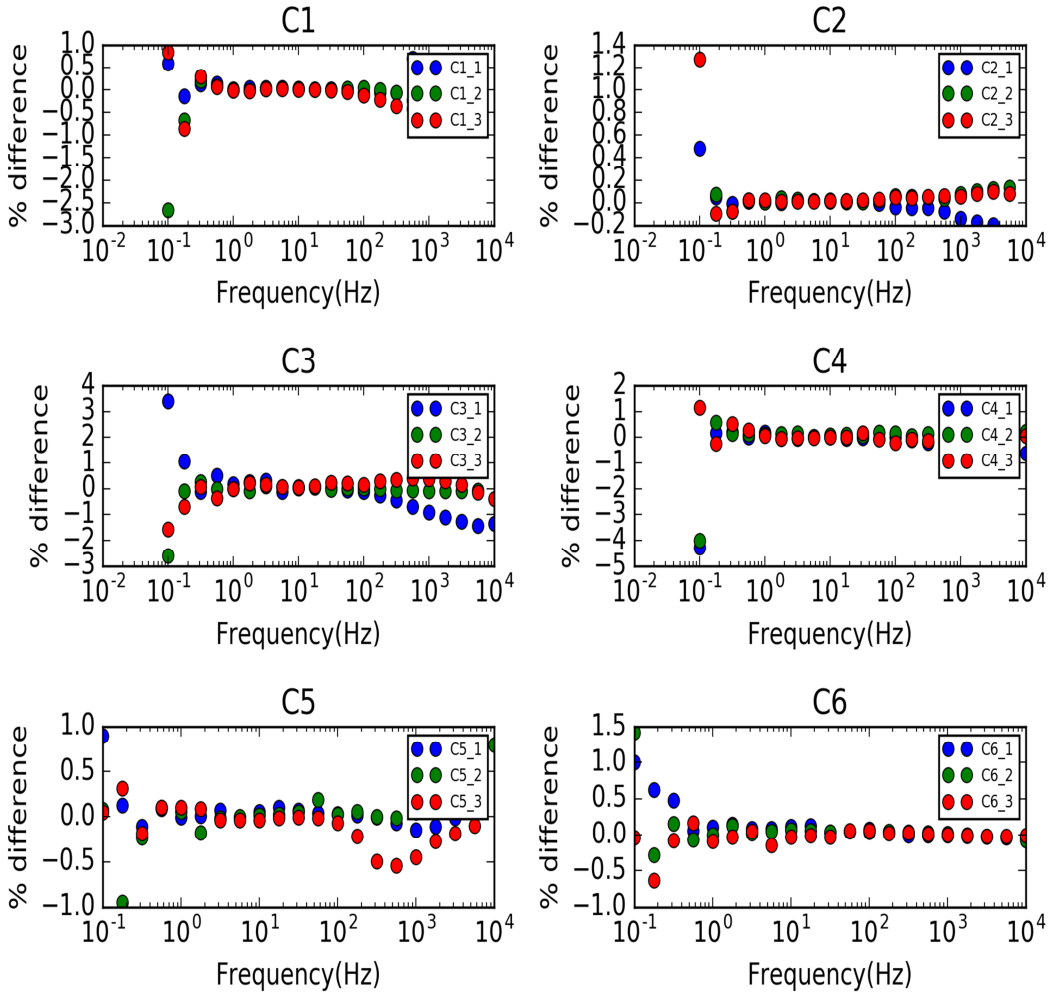


Figure 6. Comparison of SIP response with and without needles.

Based on the assessment, FIU contininued taking measurements with metallic needles inserted within the ports. The SIP measurements were taken from 08/03/2016 to 12/21/2016 once a week (although due to holiday closures some weeks were skipped or the day of sampling was changed). This data acquisition amounts to twenty-one measurements per column per port.

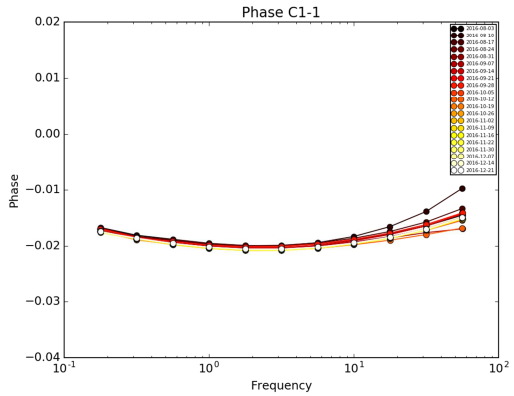
Figure 7. Bulk resistivity over time of Columns 1 – 6.

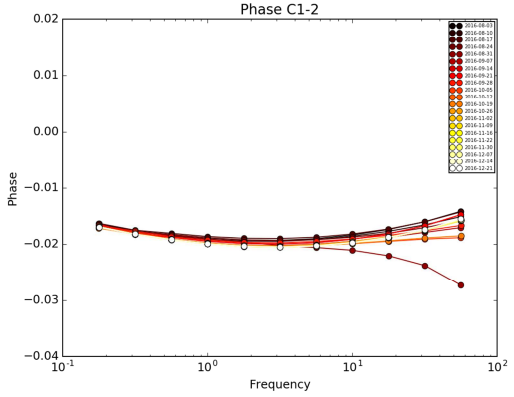
Figure 7 shows changes in the bulk resistivity over time in each of the columns. This was calculated by taking the Impedance magnitude and dividing it by a geometric factor based on the dimensions of the measured area. Column 1 shows a relatively constant bulk resistivity and illustrates changes based on height in the column. The blue line represents the bottom, the green line the middle, and the red line the top of the column. is the Column 1 graph shows a

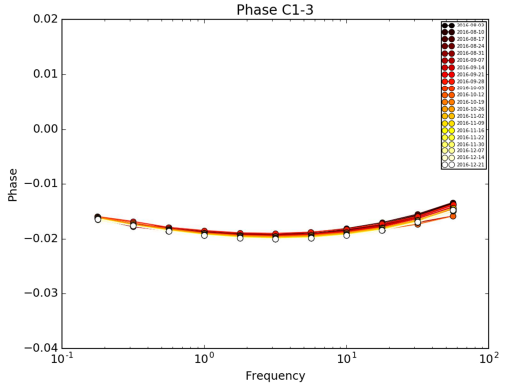
progressive drop in bulk resistivity from about 320 Ohm*m at the bottom to 270 Ohm*m near the top, which is likely due to an increase in dissolved ions in the pore water affecting the chemical reactions happening in the soil as the solutions slowly flow through the column. Column 2 shows a lower bulk resistivity at around 240 Ohm*m, which then drops to 225 Ohm*m by port 2 and remains relatively constant until port 3. Column 3 shows an erroneous high peak at the beginning (green) which is likely caused by a pocket of air on an electrode.

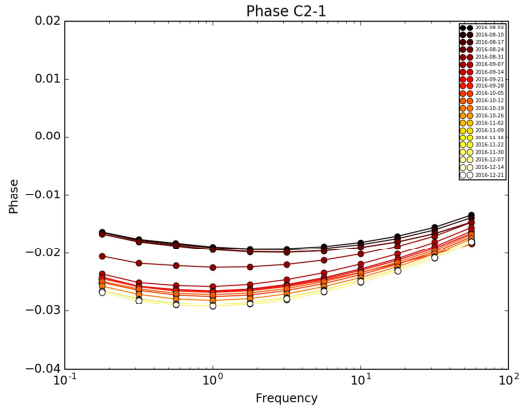
Columns that displayed microbial growth (whether from microorganisms naturally present in natural soil or injected from the cultivated microbial culture) all eventually reached resistivities of 100 Ohm*m. Column 4 showed the most gradual changes and went from 200 Ohm*m to 100 Ohm*m over the course of 4 months.

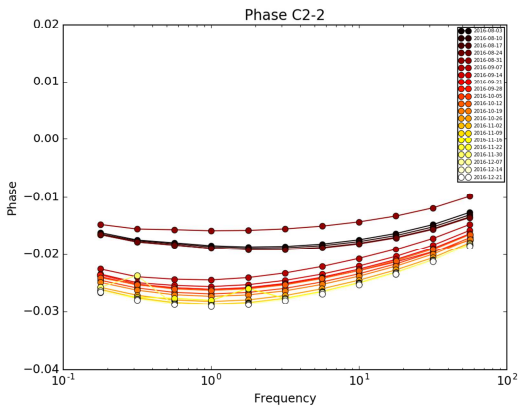
Figure 8 shows changes in all columns over time with Phase displayed in mrad and Frequency displayed in Hz. Darker lines are early measurements while later measurements are successively lighter ending with white. Column 1 (control) showed no difference in the phase response over several months (around -0.018 mrads), while Column 2, where the inlet solution contains bicarbonate, showed a steady increase in absolute phase until it reached a maximum at around -0.028 mrads. Columns 3 - 6 that displayed microbial growth, whether from natural soil microbes that developed due to the addition of glucose or injected as a microbial culture grown on glucose, had phase spectra which showed a decrease from the control. The phases for these columns appeared to stabilize around -0.06 mrads after a short period of time. Column 4 showed the most gradual changes in phase, which may be due to limited natural soil microbial growth.

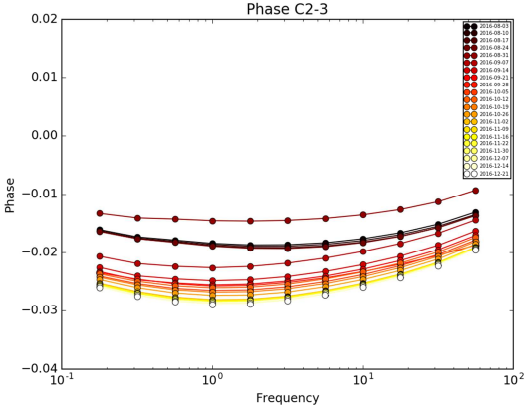

Phase C1-1
Phase
Frequency


Phase C1-2
Phase
Frequency

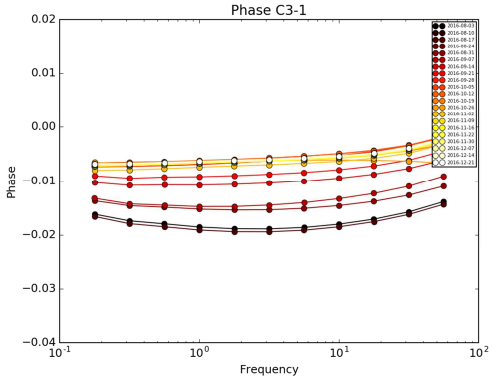

Phase C1-3
Phase
Frequency

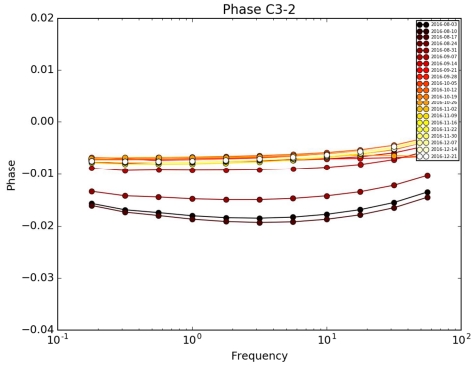

Phase C2-1
Phase
Frequency

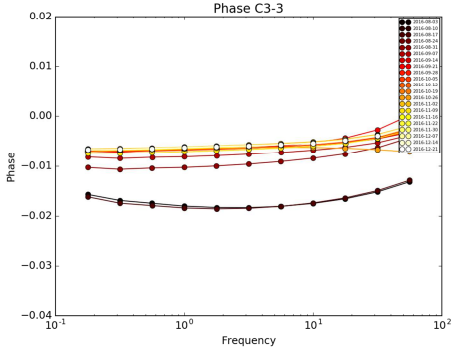

Phase C2-2
Phase
Frequency

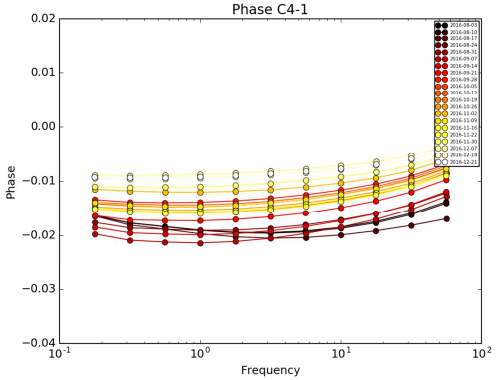

Phase C2-3
Phase
Frequency

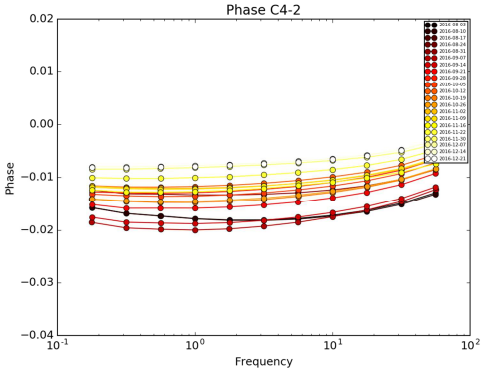
Spectral Induced Polarization Response

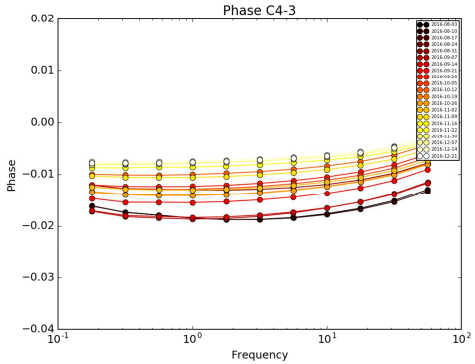












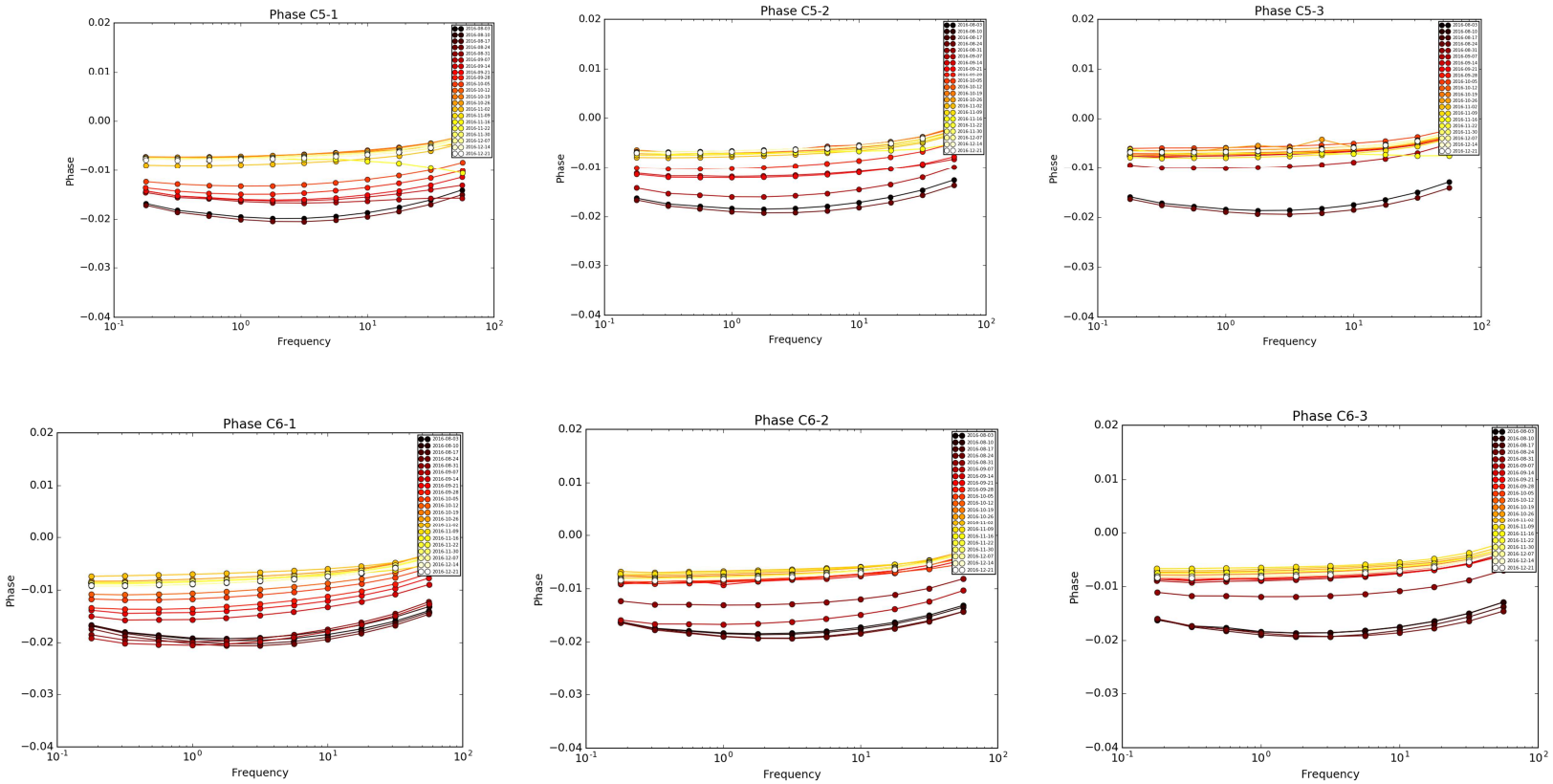


Figure 8. Phase spectra for Columns 1 - 6. Y axis shows phase in mrads while X axis shows frequency in Hz. Each line represents one measurement. Lines go from 8-03-2016 (Black) to 12-21-2016 (White) in a color gradient. Each line is separated by one week on average. Significant changes in phase correspond to vertical movements of the lines.

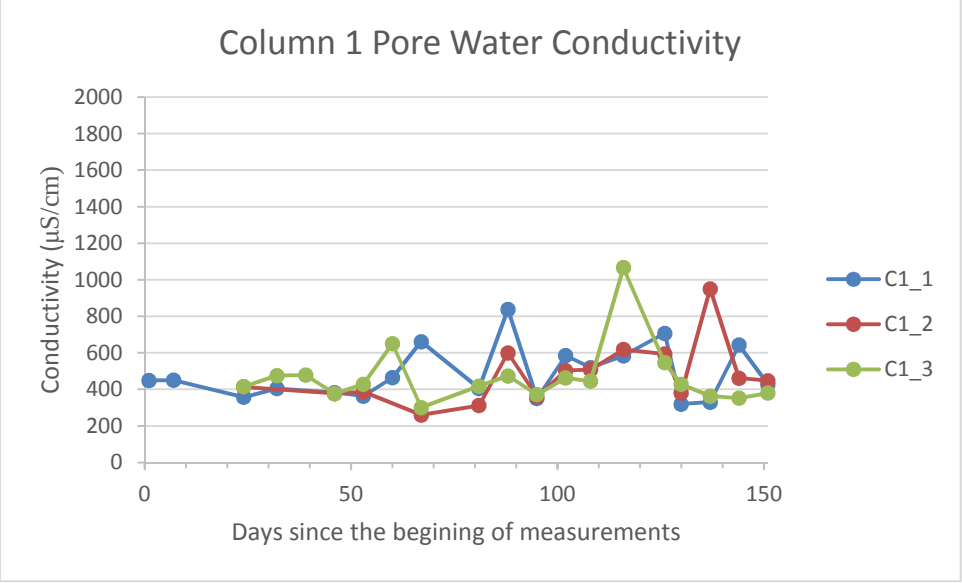
Basic Pore Water Analysis

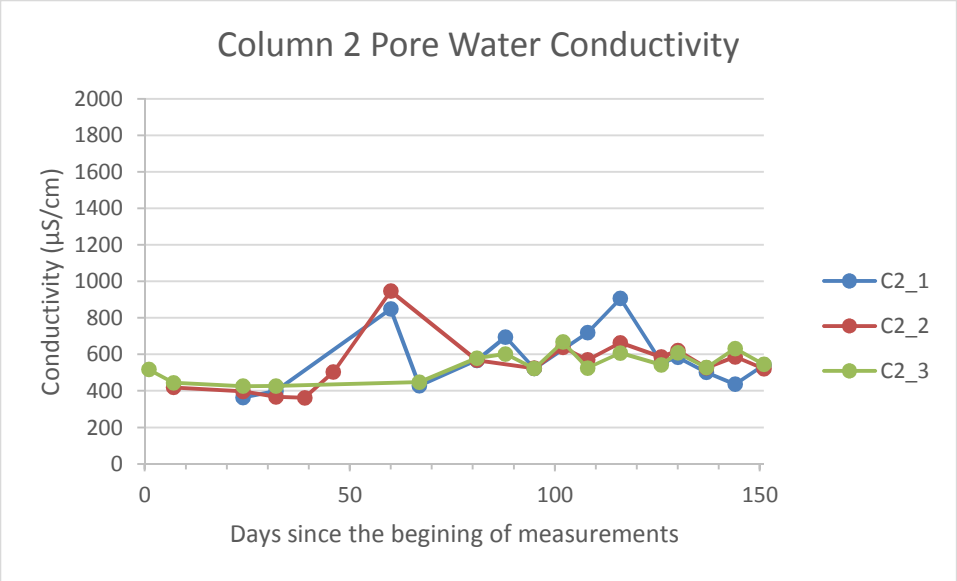
Pore water was collected once weekly and tested for conductivity and pH. Later, samples were tested for ORP in order to understand oxidation conditions within the columns. During the first half of the experimental run there was difficultly taking pore water samples. As such, there are many weeks with only partial data for the six columns and eighteen ports.

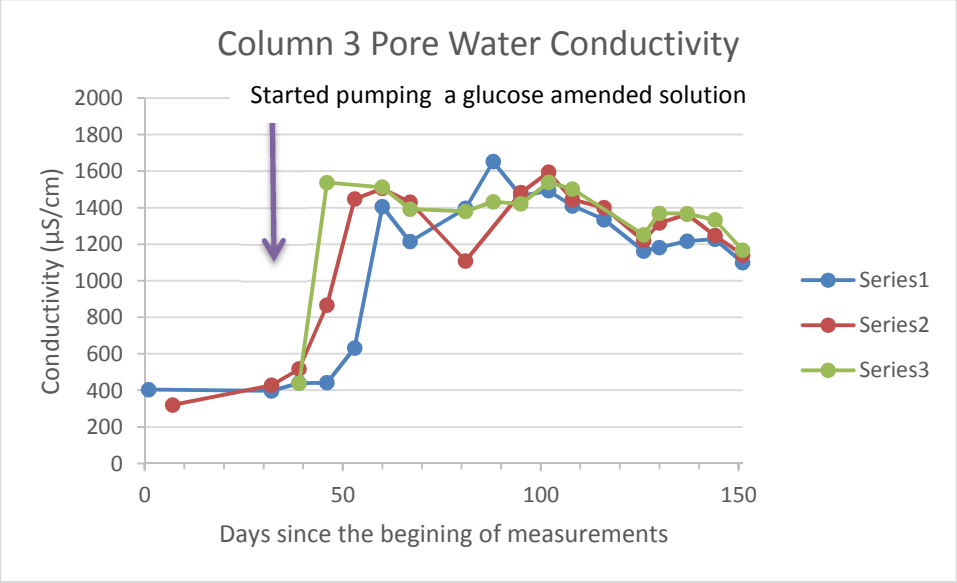
Figure 9 shows measurements of conductivity taken using a microprobe obtained from Microelectrode, Inc. These measurements are heavily dependent on the calibration of the microprobe and are not as accurate as the bulk resistivity readings from the SIP system. Column 1 and Column 2 hover between 400 and 600 µS/cm. Columns 3, 4, 5 and 6 show an eventual increase in conductivity to between 1,200 and 1,600 µS/cm. Column 4 shows the most gradual change in conductivity in the same way that it showed the most gradual SIP change.

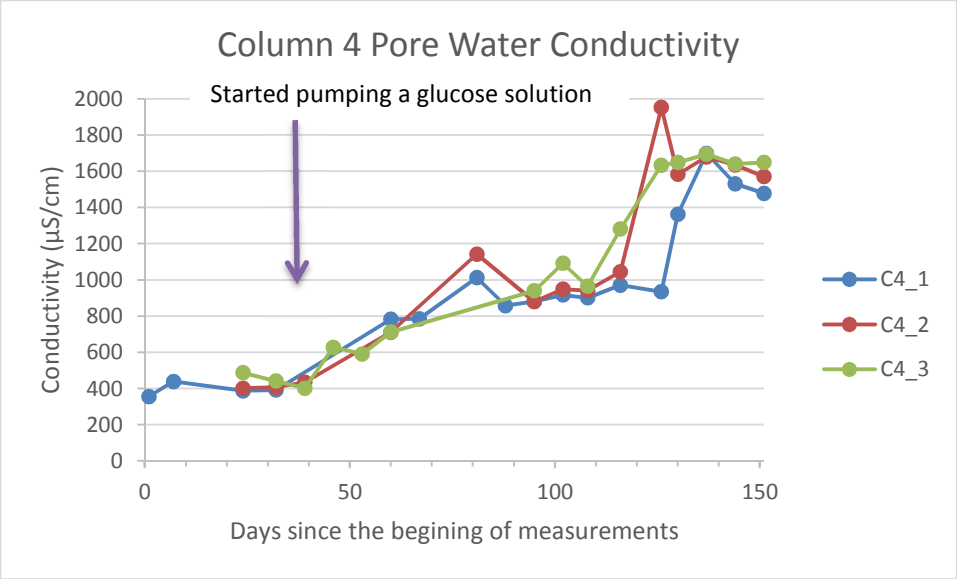
The pHs (Figure 10) of Columns 1, 3, 4, 5 and 6 seem to fluctuate between pH 7 and 8, and in Columns 3, 4, 5 and 6, pH values sometimes dropped as low as pH 6 during what may be a prolonged stop flow event or a calibration error. Column 2 remains consistent at approximately pH 8, which might be justified by the presence of bicarbonate in the effluent.

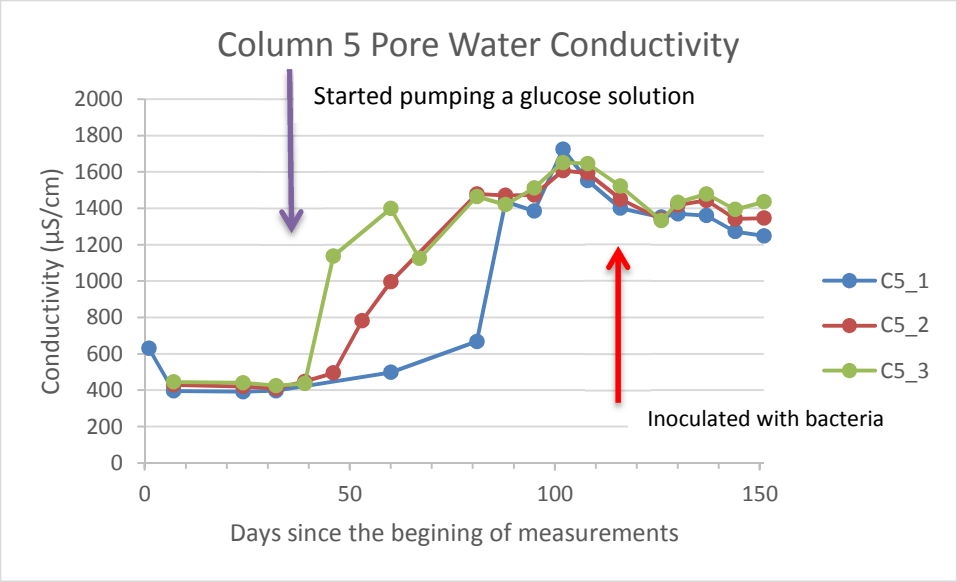
ORP measurements were taken late into the life of the experiment, as such conditions are likely close to equilibrium. Columns 3, 4, 5 and 6 all display reducing conditions likely due to a lack of oxygen in the system since the effluent solution contained glucose consumed by the microorganisms. Column 1 shows a high ORP measurement early on, which may represent oxiding conditions without the addition of organic substrate in the pumping solution. Neither Column 1 nor Column 2 ever display measurements less than 0 mV.




Column 2 Pore Water Conductivity
Conductivity (µS/cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
Days since the begining of measurements
C2_1
C2_2
C2_3


Column 3 Pore Water Conductivity
Started pumping a glucose amended solution
Conductivity (µS/cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
Days since the begining of measurements
Series1
Series2
Series3


Column 4 Pore Water Conductivity
Started pumping a glucose solution
Conductivity (µS/cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
Days since the begining of measurements
C4_1
C4_2
C4_3


Column 5 Pore Water Conductivity
Started pumping a glucose solution
Conductivity (µS/cm)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
Days since the begining of measurements
Inoculated with bacteria
C5_1
C5_2
C5_3

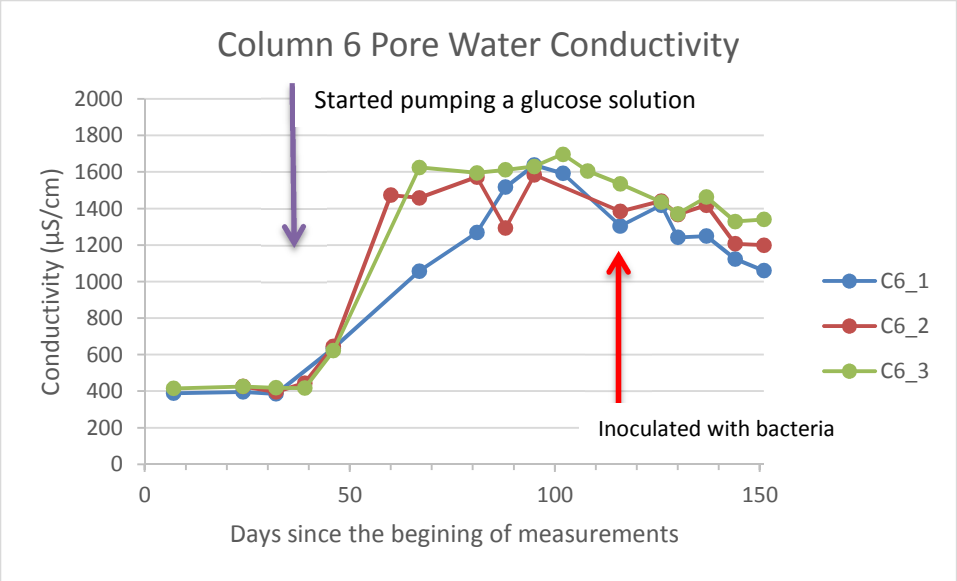
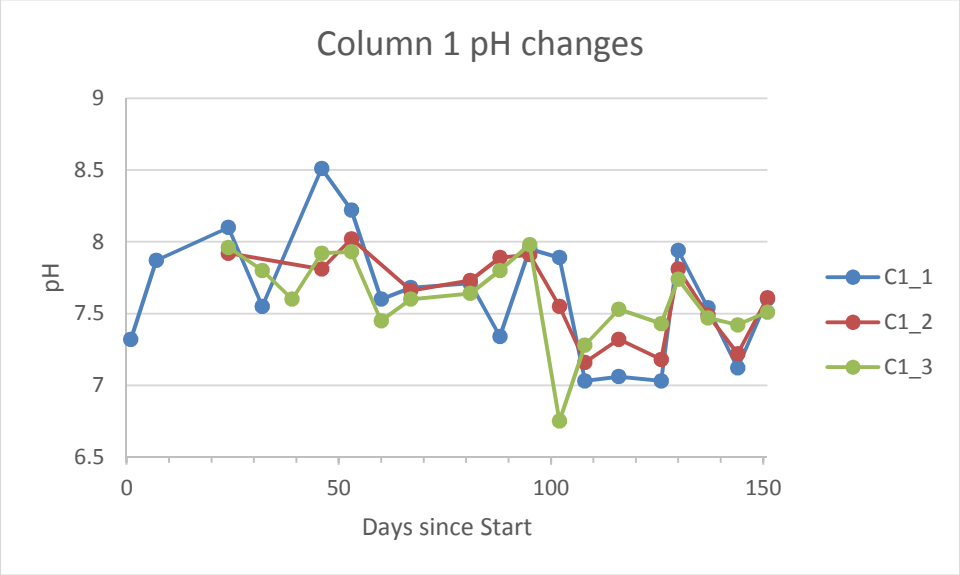
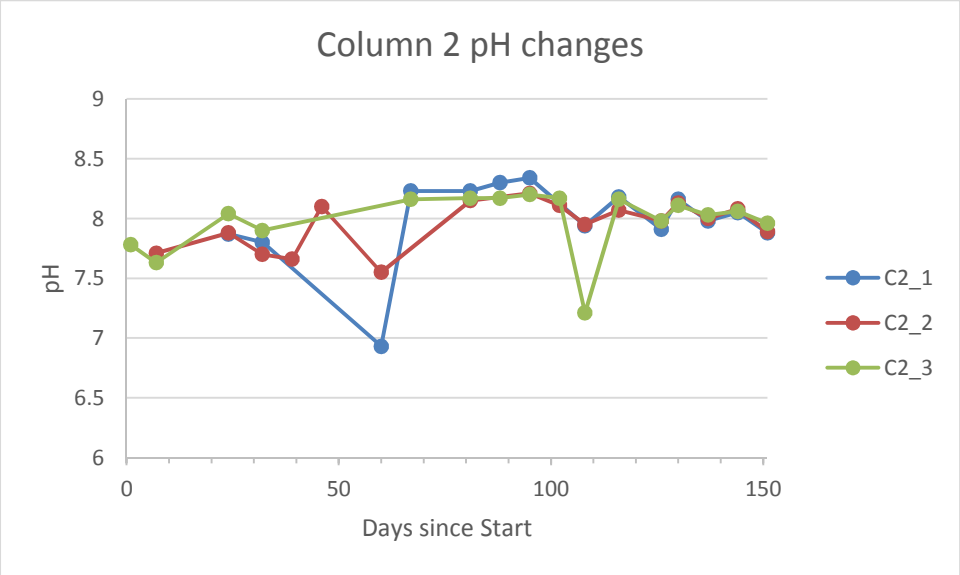
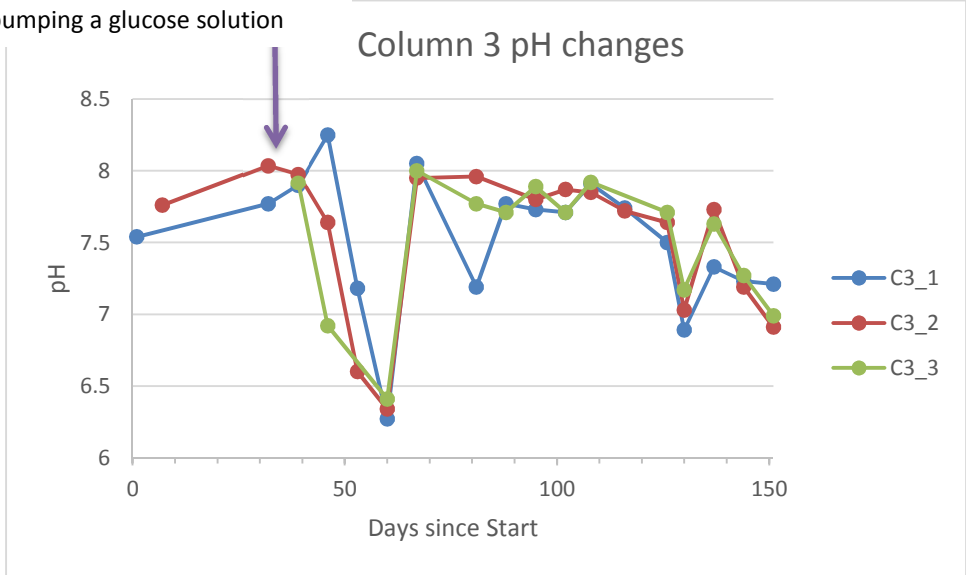


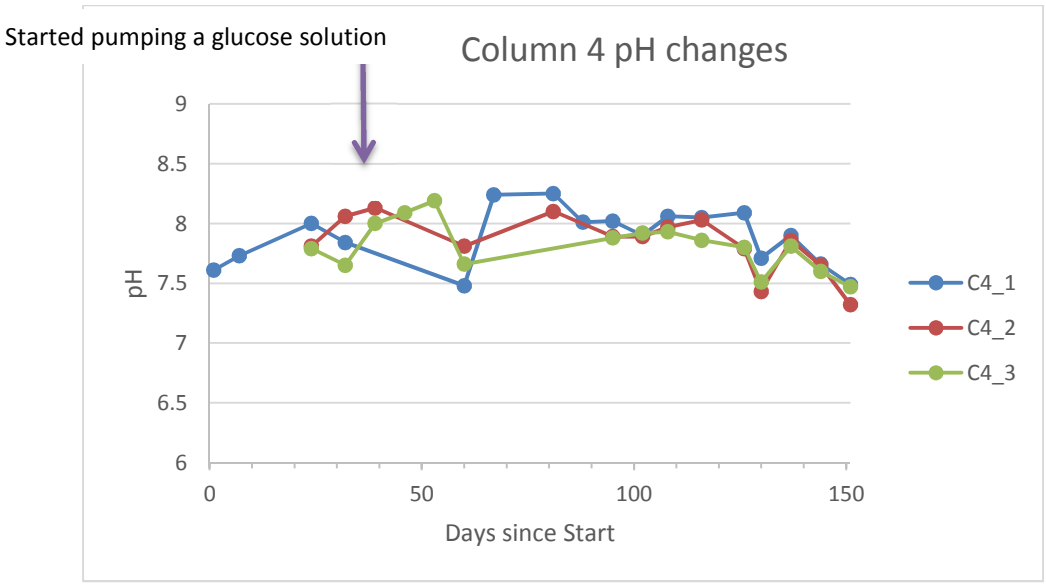
Figure 9. Pore water conductivity for Columns 1 - 6 in µS/cm.

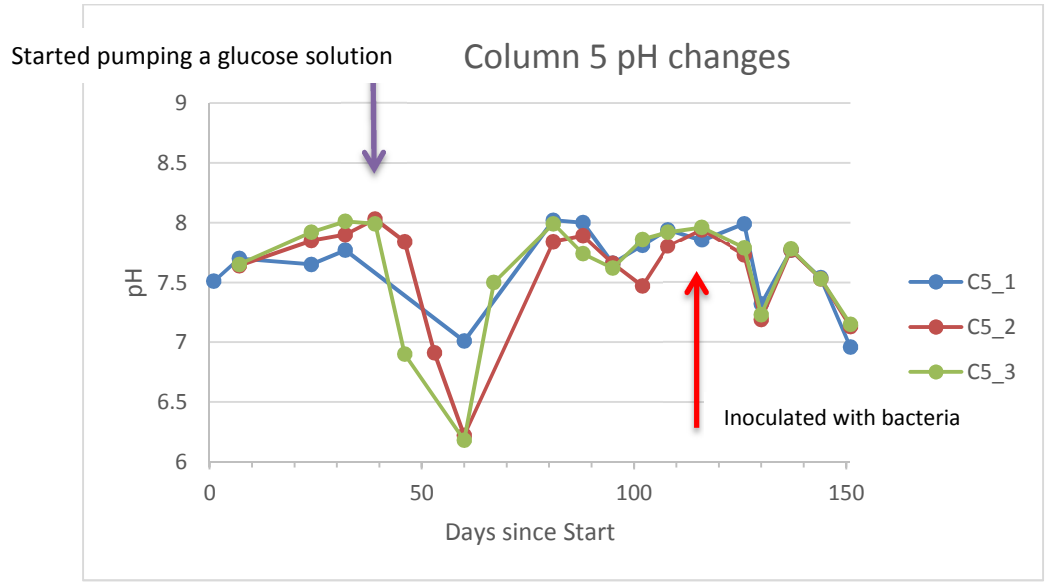




Started pumping a glucose solution




Started pumping a glucose solution
Column 4 pH changes
9
8.5
8
7.5
7
6.5
6
pH
0
50
100
150
Days since Start
C4_1
C4_2
C4_3


Started pumping a glucose solution
Column 5 pH changes
9
8.5
8
7.5
7
6.5
6
pH
0
50
100
150
Days since Start
C5_1
C5_2
C5_3
Inoculated with bacteria

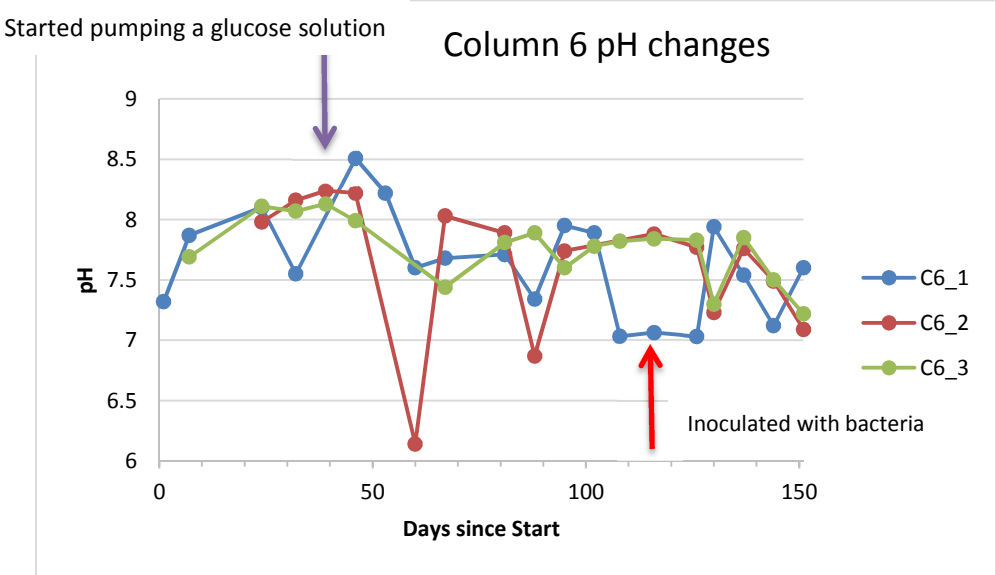
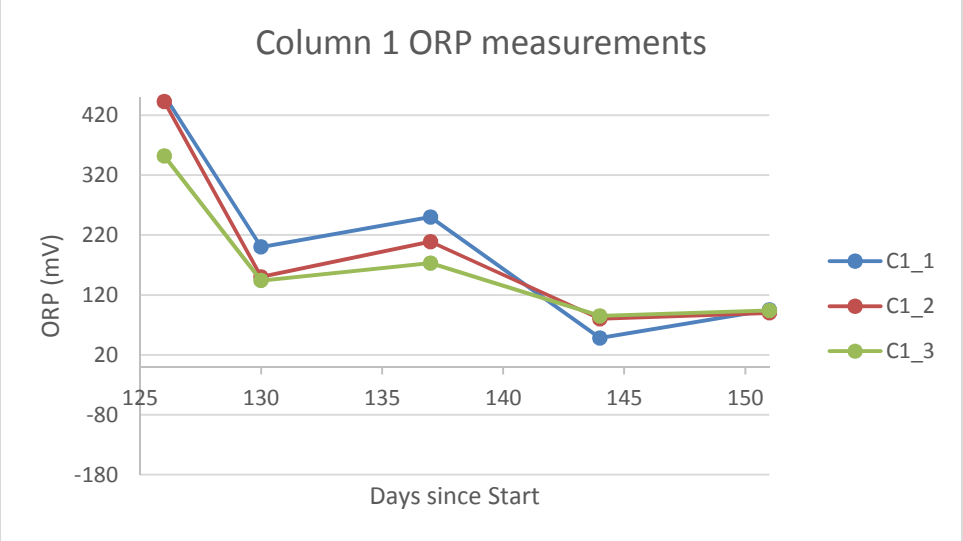
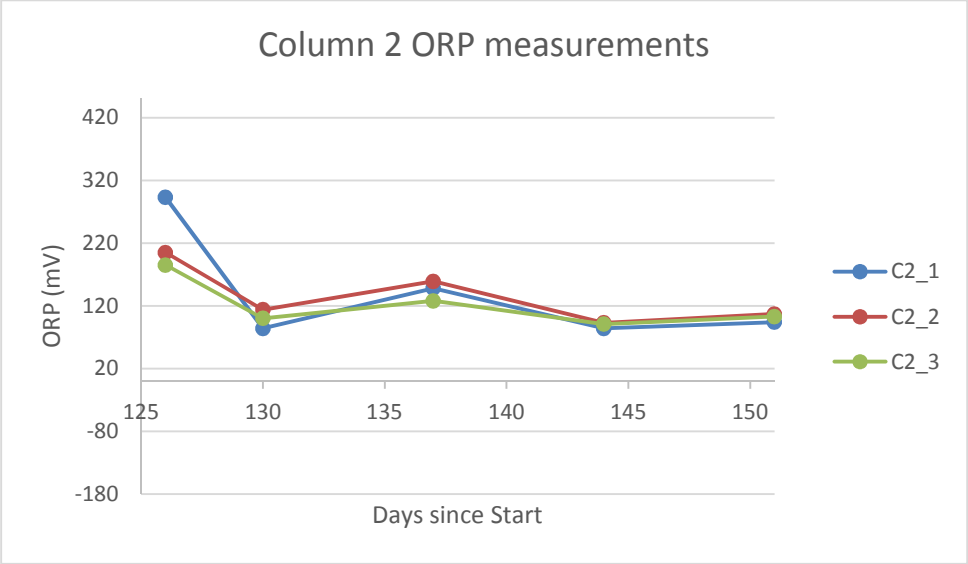
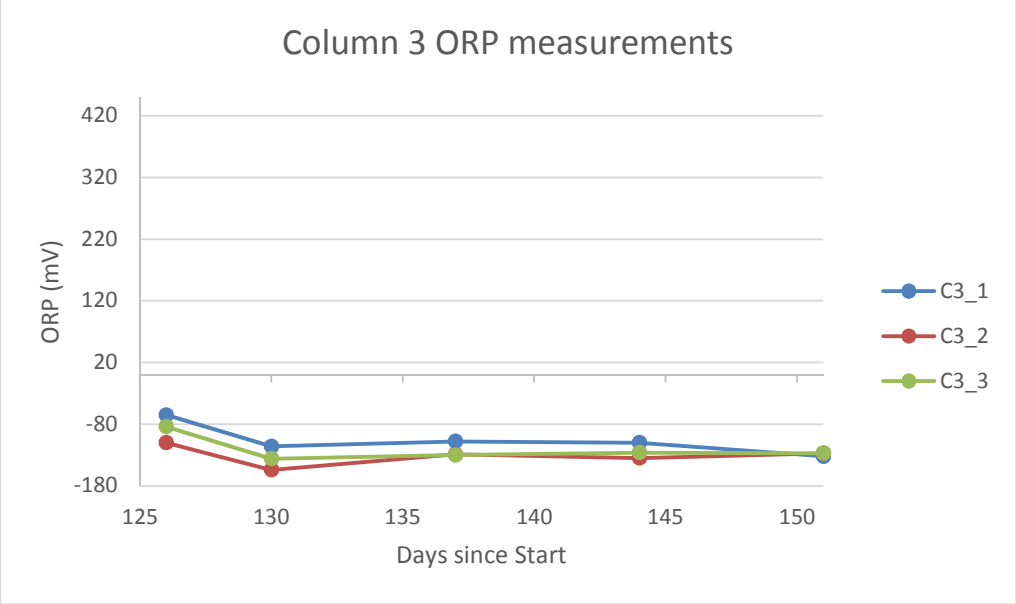
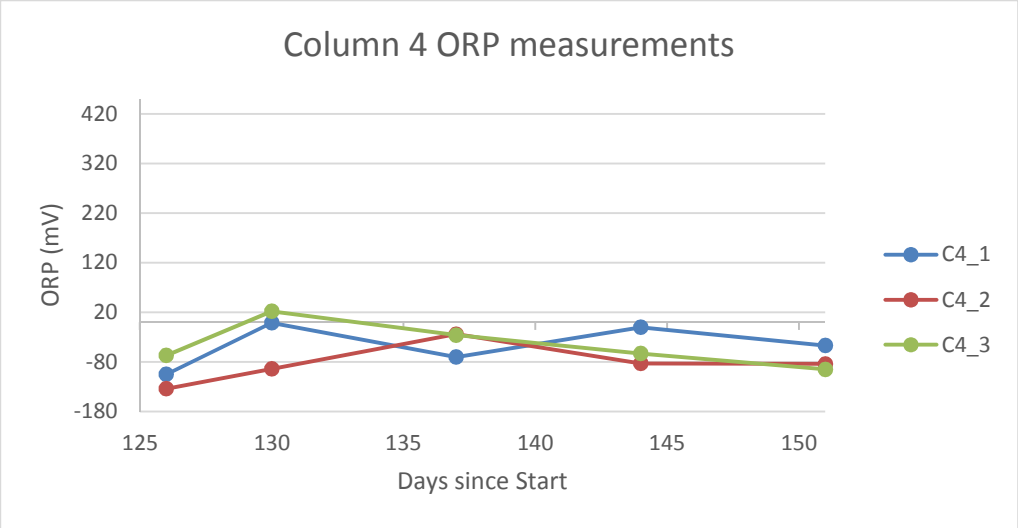


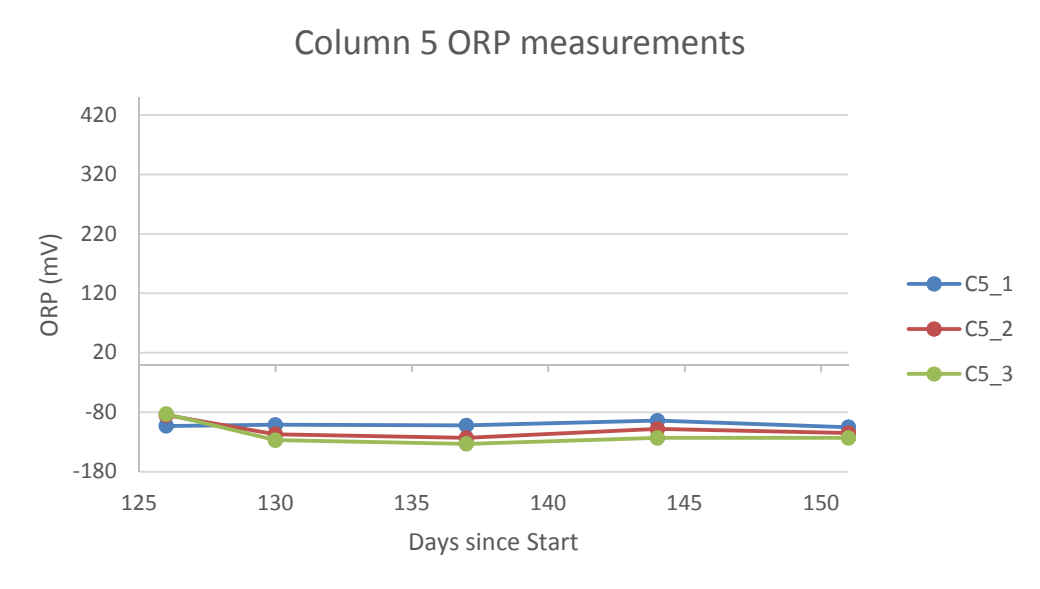
Figure 10. Pore water pH for Columns 1 - 6.




Column 2 ORP measurements
420
320
220
120
20
-80
-180
ORP (mV)
125
130
135
140
145
150
Days since Start
C2_1
C2_2
C2_3


Column 3 ORP measurements
420
320
220
120
20
-80
-180
ORP (mV)
125
130
135
140
145
150
Days since Start
C3_1
C3_2
C3_3


Column 4 ORP measurements
420
320
220
120
20
-80
-180
ORP (mV)
125
130
135
140
145
150
Days since Start
C4_1
C4_2
C4_3


Column 5 ORP measurements
420
320
220
120
20
-80
-180
ORP (mV)
125
130
135
140
145
150
Days since Start
C5_1
C5_2
C5_3

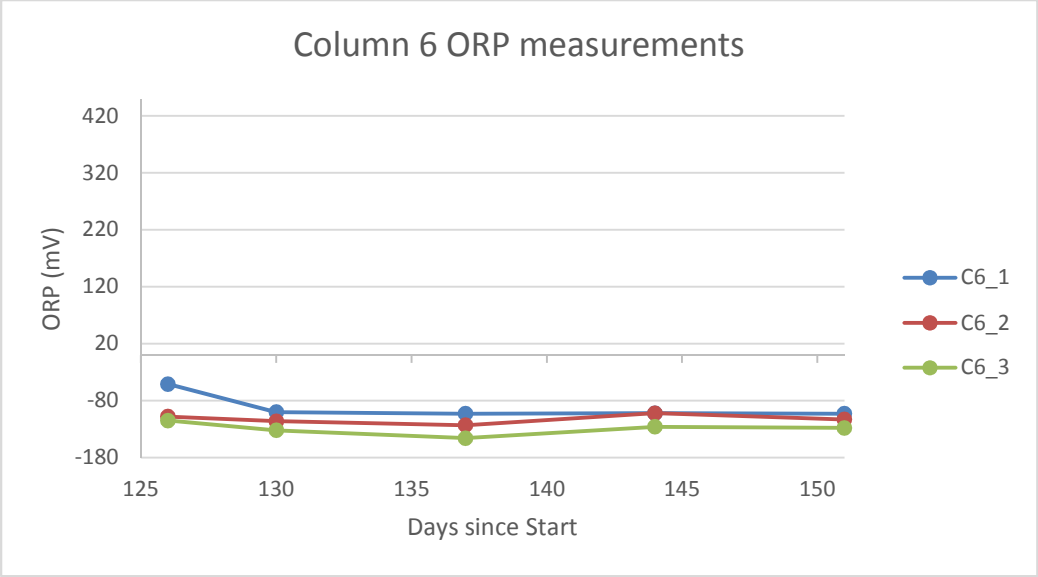


Figure 11. Oxidation reduction potential (ORP) for Columns 1 - 6.

FUTURE WORK

FIU has initiated the conversion of Column 1 (control) and Column 2 (SWG + bicarbonate) into columns inoculated with microorganisms. A consortia culture diluted to the amount of log 7.39 cells/ mL was injected to the port 1 of Columns 1 and 2. The purpose of this change is to allow a higher sampling rate for the columns as it is apparent from work during Fall 2016 that the biochemical changes of interest within the columns can occur very quickly. Difficulties were encountered when taking both SIP (due to bubbles positioned on electrodes) and pore water samples (due to clogging of ports with sediment) during the preliminary work conducted in the Fall of 2016 . In addition, samples for iron analysis were not taken until after the preliminary experiment had been running for two months.

During the Spring of 2017 SIP measurements will be taken daily from Monday through Friday and pore water samples will be collected three times a week. Samples will be measured for ORP, conductivity, and pH and will undergo Ferrozine analysis for the presence of Fe^{2+} and total Fe (Fe^{2+} + Fe^{3+}). In addition, FIU will initiate analysis for uranium via a KPA instrument and ICP-OES measurements for Si, Al, Ca and P for the past and newly collected samples. This higher frequency will improve the resolution of changes on a daily scale rather than weekly to gradually monitor changes that occur within the soil columns that contain autunite under continuous flow of glucose-amended solutions with and without the presence of carbonate.

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