STUDENT SUMMER INTERNSHIP TECHNICAL REPORT

The Role of *Chromohalobacter* on Transport of Lanthanides and Cesium in the Dolomite Mineral System

DOE-FIU SCIENCE & TECHNOLOGY
WORKFORCE DEVELOPMENT PROGRAM

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Principal Investigators:

Frances Zengotita, DOE Fellow Student
Hilary P. Emerson, Postdoctoral Fellow
Florida International University

Timothy M. Dittrich, Research Scientist
Julie Swanson, Research Scientist
Donald T. Reed, Research Scientist
Los Alamos National Laboratory

Florida International University Program Director:

Leonel Lagos Ph.D., PMP®

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FIU | Applied Research Center
Florida International University

ABSTRACT

The chemical behavior of actinide series elements and fission products is a concern for the Waste Isolation Pilot Plant repository due to their uncertain mobility in the subsurface salt formation. In this work, we are observing the behavior of the halophilic bacterium, *Chromohalobacter*, and its effect on the mobility of lanthanides and cesium in the presence of dolomite. Batch and mini-column experiments were conducted with Cs^{+} and lanthanides (Nd^{3+}, Eu^{3+}) to quantify potential transport with bacteria. Preliminary results show that Cs does not interact strongly with dolomite or *Chromohalobacter*, while the lanthanides can interact strongly with both minerals and bacteria depending on which the Ln contacts first.

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1. INTRODUCTION

Large amounts of radioactive waste began to accumulate in the 1940s as a byproduct from WWII and then the Cold War. A deep geologic repository was ultimately recommended as the best option for long-term disposal by the National Academy of Sciences. Deep geologic disposal in salt beds was suggested since they are relatively impermeable, stable rock formations which can seal themselves off with time and dissipate decay heat. During the 1960s, the Chihuahuan desert of southeastern New Mexico was chosen to be an appropriate site for transuranic waste disposal. This site is located in the Delaware Basin which was formed during the Permian period. The salt bed is over 250 million years old with a depth from approximately 1000 to 3000 feet below the ground surface.

During the 1990s, the Waste Isolation Pilot Plant (WIPP) became the first active deep geologic repository in the world for the permanent disposal of transuranic radioactive waste (US DOE-EM, 2017). The WIPP is located within a 42-km^2 area which amounts to around one tenth of the salt-bed having been mined (Figure 1 and Figure 2). To control actinide solubility and mobility by sequestering carbon dioxide, magnesium oxide (MgO) was placed into the repository (US DOE, 1996). To date, the facility has disposed of a total of 220,000 m^3 of transuranic (TRU) waste or >170,000 containers. TRU waste is defined as radioactive waste containing greater than 100 nCi/g of alpha from elements greater than or equal to atomic number 92 (uranium). The WIPP has disposed of over 36 million m^3 of waste with over one billion Curies of radioactivity from 22 DOE sites to date.

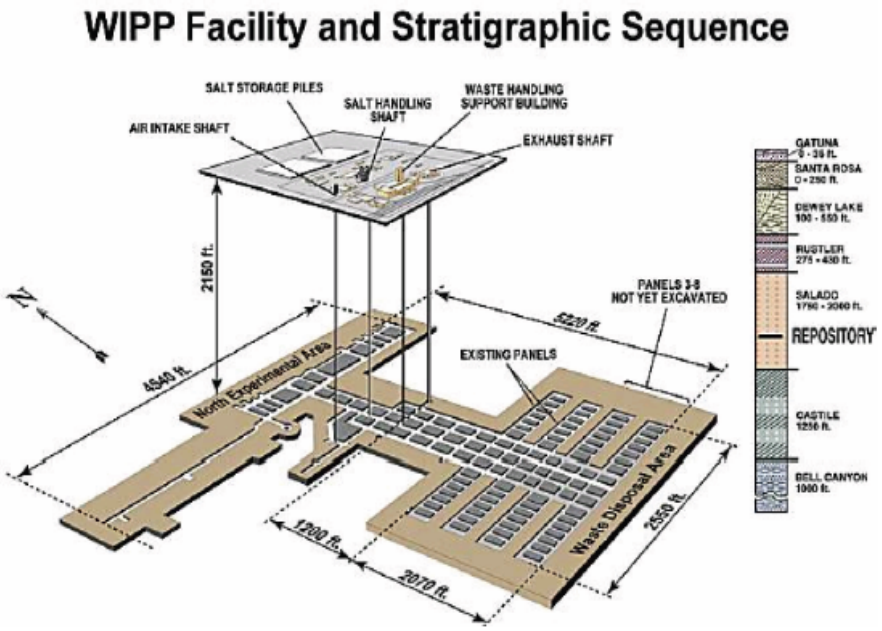

Figure 1. WIPP facility schematic (US DOE, 1997).

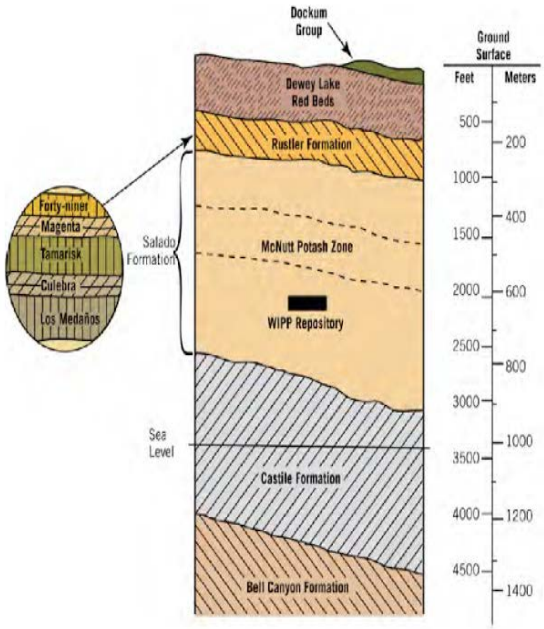

Figure 2. Depiction of the Waste Isolation Pilot Plant deep geologic repository and surrounding geology with respect to depth, located near Carlsbad, NM (U.S. EPA, 2006).

The chemical behavior of actinide series elements (the most long-lived byproduct of the waste created from development of nuclear weapons) is a major concern for the WIPP due to their long half-lives, significant radiotoxicity, and unknown mobility in the WIPP environment. In the most likely WIPP release scenario, human intrusion (cuttings, cavings, spallings) can lead to direct and/or long-term brine release (US DOE, 1995, US DOE 1996, Perkins *et al*., 1999). Once the brine is released, it may proceed through the Rustler formation (the most transmissive layer) and pose a potential threat to the environment and the public (Perkins *et al*., 1999). Within the Rustler formation, the 7-8 meter thick Culebra dolomite ($CaMg(CO_3)_2$) layer is the most transmissive member (Figure 2) (Domski *et al*., 2011).

It is important to understand the impact of microbial activity on the fate and transport of the actinide elements in the deep geologic repository environment. Although these deep subsurface conditions are challenging for bacteria to survive, previous work has identified microbial activity (Vreeland et al., 1998; Swanson *et al*., 2016). Moreover bacteria may affect actinide fate due to sorption and uptake of these elements, complexation by secreted ligands, and/or their impact on redox conditions.

A halophilic bacterium, *Chromohalobacte*r sp. strain PZ13, was isolated from near the Waste Isolation Pilot Plant (WIPP) site. *Chromohalobacter* is a member of the "*γ-Proteobacteria*" which is a phylum of gram-negative bacteria. The genus was established by Ventosa in 1989 (Ventosa, 1989). Halophilic bacteria have distinct metabolic patterns and a possibility of rapid adjustment to changes in external salt concentrations (Shivanand, 2011). They are also able to survive significant changes in pressure that may be associated with a deep geologic repository (Swanson *et al*., 2016). Therefore, they may be present in the WIPP repository and be able to affect the mobility of actinides.

Previous work has also shown that microorganisms can interact with contaminants and also affect their chemical form by changing their oxidation state (Tabak *et al*., 2005). Bacteria were able to remove heavy metals and radionuclides by the association with biomass in previous work which implies that metals can be sorbed onto the exterior of bacterial cells. Metabolism-independent metal uptake into bacteria can also occur. Several studies have documented the uptake of Cs^+ into various microbes, which is a radioactive contaminant present at the WIPP. Different microorganisms had different capacities to accumulate Cs^+, but Cs^+ was also found to potentially adsorb onto the exterior surface of some microorganisms (Avery, 1995). Cs^+ has been reported previously to associate strongly with organisms including *Bacillus* and *Cladosporium* and uptake into the organism alongside K^+ has been reported for all major microbial groups (Avery, 1995a&b; Johnson *et al.*, 1991).

Colloid-facilitated transport of cesium has been observed in saturated sediments, which can be compared to microbial transport experiments found in this report. In the colloid-facilitated transport of cesium, it was found that the cesium attached to the colloids via ion-exchange but could also adsorb onto colloidal particles in contaminated subsurface regions (Chen *et al.*, 2005). High sorption affinity for Cs was observed on native colloids and slight sorption occurred. (Chen *et al.,* 2005). In comparison to the results of this experiment, the cesium did not interact with the dolomite mineral, it did not sorb onto the surface and acted as a tracer.

The objective of these experiments is quantify the mobility of risk-driving contaminants in presence of bacteria under conditions relevant to the Waste Isolation Pilot Plant. In this work, we are observing the mobility of *Chromohalobacter*, the trivalent lanthanides (e.g. Nd^{3+}, Eu^{3+}) and cesium in the presence of Culebra dolomite with miniature column experiments. The lanthanide elements are used as non-radioactive, stable oxidation state analogs for the actinides (Am and Pu). These data will enhance the body of knowledge on microbial effects on actinide transport at the WIPP and decrease uncertainty in the probabilistic performance assessment.

Figure 3. TEM thin-section image of *Chromohalobacter* (left) and *Chromohalobacter* stock suspension (right).

2. EXECUTIVE SUMMARY

This research work has been supported by the DOE-FIU Science & Technology Workforce Initiative, an innovative program developed by the US Department of Energy's Environmental Management (DOE-EM) and Florida International University's Applied Research Center (FIU-ARC). During the summer of 2017, DOE Fellow intern Frances Zengotita spent 10 weeks at the Carlsbad Environmental Monitoring and Research Center working with the Los Alamos National Laboratory Actinide Chemistry and Repository Science (LANL ACRSP) team in Carlsbad, NM under the supervision and guidance of Dr. Timothy M. Dittrich and Dr. Donald T. Reed. The intern's project was initiated on June 2, 2017 and continued through August 12, 2017 with the objective of quantifying the mobility of contaminants in the presence of bacteria under conditions relevant to the Waste Isolation Pilot Plant.

3. RESEARCH DESCRIPTION

Dolomite was collected from the Culebra layer near the WIPP by Matthew Tomas (Sandia National Laboratory) and Timothy Dittrich. The rock samples were crushed, cleaned and characterized prior to experiments. First, dolomite rock samples were crushed in an impact mortar and pestle, washed with Milli-Q (>18 MΩ*cm) H_2O, and sieved through No. 45, 100 and 200 sizes (Figure 4). After repeated washing and sieving, all solids were dried for 24 hours at 40°C and re-sieved. The 355 – 500 µm (fraction between No. 100 and 200 sieves) size fraction was utilized for all batch and column experiments. If the finer fractions are used for columns, it will likely clog. Therefore, the 355 – 500 µm size fraction was used for all experiments for consistency. Bulk surface area was measured via the Brunauer-Emmett-Teller (BET) method (Micromeritics TriStar II 3020) at 1.70 m^2/g. XRD and SEM-EDS confirmed nearly 100% dolomite (Figure 5).

Figure 4. Images of Frances Zengotita and another LANL intern crushing and sieving dolomite rocks.

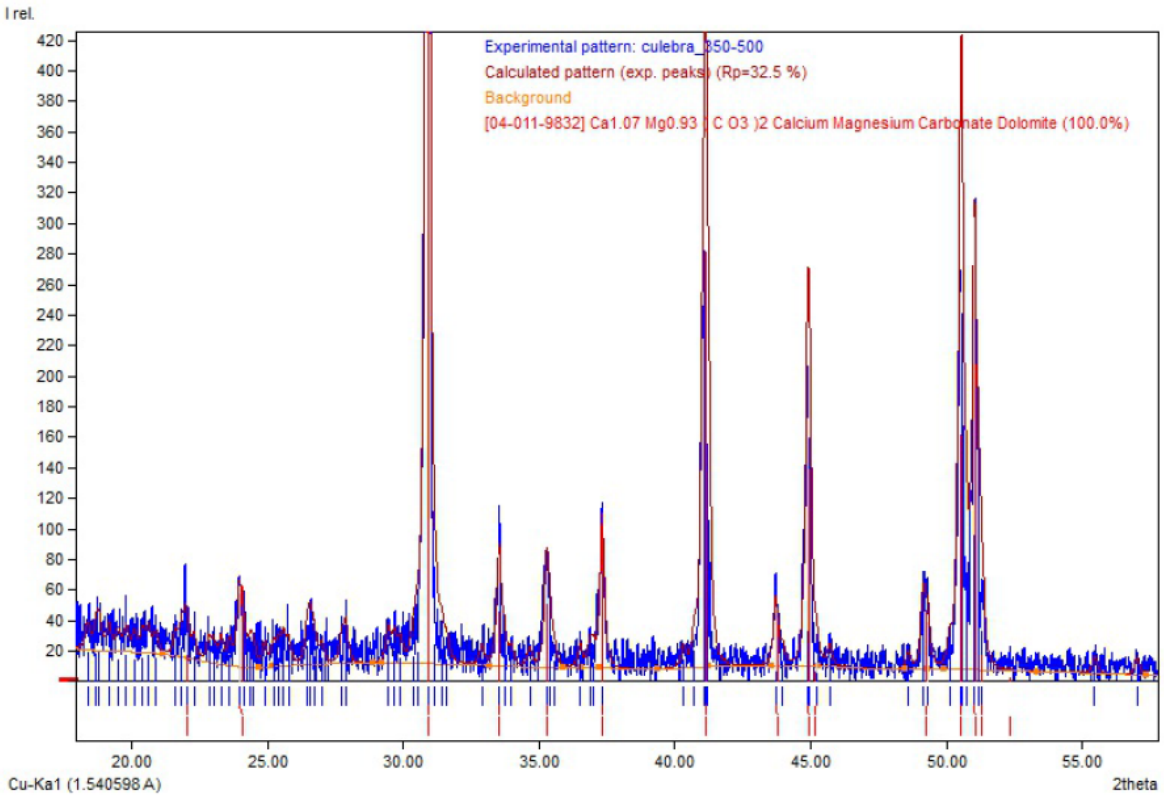

Figure 5. XRD spectra with reference spectra from Culebra formation, 355-500 µm size fraction for dolomite (Match! Software).

The design of miniature column experiments was based on previous work by Dittrich (Dittrich, *et al*., 2016). Briefly, one inch of polytetrafluoroethylene (PTFE) tubing (3/8" inner diameter, International Polymer Solutions) was packed with one gram of Culebra Dolomite [355-500 µm] for columns (Figure 6). The tubings were threaded with a 1/8" 27 National Pipe Tapered (NPT) carbon pipe tap (Drillco cutting tools). The fittings were sealed with silicone and the inlet and outlet of the columns was also covered with 35 µm polyether ether ketone (PEEK) mesh (Spectrum labs) to reduce the potential for clogging within the tubings. Attached to each mini-column were 30 cm inlet and 60 cm outlet tubings (PTFE #20 Cole-Parmer, 0.032" inner diameter) that were connected to 60 mL polypropylene syringes. The syringes were attached to a syringe pump (Kd Scientific Model 100 series) operating at a 0.013 min/mL flow rate and effluent was collected into a fraction collector (Gilson) at variable collection times. Samples and fraction collector remained within a clear plastic container throughout experiments to reduce evaporation from tubes. All mini-columns had an average pore volume of 0.4 mL.

Figure 6. 1-inch mini-column with (1 gram of dolomite + fittings).

Mini-column experiments were conducted to observe the behavior of *Chromohalobacter* and its effect on the mobility of relevant contaminants to the WIPP in a dolomite mineral system. *Chromohalobacter* was previously isolated from near the WIPP and grown by Julie Swanson for experiments. Each column was injected with a 15% (w/v) NaCl + 3 mM $NaHCO_3$ brine after allowing for approximately one hour of equilibration time with the contaminant of concern. The synthetic brine was equilibrated with atmospheric carbonate and had an approximate pH of 8.2. The bacteria (*Chromohalobacter* - with injection concentrations of ~10^8 cells/mL) were added as received and/or spiked with different concentrations of contaminants (Nd^{3+}, Eu^{3+}, and Cs^{+}). In these experiments, *Chromohalobacter* is injected in the stationary phase (i.e., no growth is expected). Both lanthanides act as chemical analogues to Am^{3+} and Pu^{3+} which are disposed of in the WIPP. Radioactive ^{137}Cs has also been disposed of in the WIPP and is represented by the stable ^{133}Cs isotope utilized in these experiments. Tracer experiments also were conducted to investigate the transport and transfer of material in a mini-column and tracked both Br^{-} and Cl^{-} as a tracer with measurements via ion chromatograph (Dionex). The specifics for each of the experiments quantifying transport of Nd^{3+}, Eu^{3+}, and Cs^{+} are described in Table 1.

Table 1. Description of Each Mini-Column Experiment in Terms of Metal Added, Initial and Final Concentration Injected

Element	Step #1 stock	Step #2 stock
Neodymium (Nd^{3+})	20 ppb + Brine + Bacteria	0 ppb + Synthetic Brine
Neodymium (Nd^{3+})	20 ppb + Brine	0 ppb + Synthetic Brine + Bacteria
Europium (Eu^{3+})	200 ppb + Brine + Bacteria	0 ppb + Synthetic Brine
Europium (Eu^{3+})	200 ppb + Brine	0 ppb + Synthetic Brine
Cesium (Cs^{+})	200 ppb+ Viable Bacteria + Brine	0 ppb + Synthetic Brine
Cesium (Cs^{+})	200 ppb+ Stressed Bacteria + Brine	0 ppb + Synthetic Brine

3.1 Tracer Experiments

Two syringes were filled and equilibrated with a 15% NaCl by weight solution and then were filled with a Br^- tracer solution. Both two columns were sampled (these columns were saturated with the NaBr tracer solution). The other two columns were pumped with the 15% NaCl synthetic brine. Samples were collected for thirty minutes with a one minute collection time. After an hour, the fraction collector was set for a 3-minute collection time. Tracer sampling protocol: 0.02 mL of sample was collected from the tubes in the fraction collector and diluted with 0.98 mL of high purity water for Br analysis by ion chromatograph (1:50 dilution).

3.2 Neodymium Mini Column Experiments

Two different experiments were conducted in parallel with duplicate mini-columns for each (Table 1). They were connected to syringes containing the 15% NaCl synthetic brine and 20 ppb Nd while one set initially injected *Chromohalobacter* and one did not inject bacteria until the second step. Prior to injection of bacteria and/or Nd, all columns were equilibrated with 15% NaCl + 3 mM $NaHCO_3$ overnight. These experimental protocols were used to investigate: (1) co-transport of Nd and bacteria and (2) re-mobilization of Nd associated with the dolomite surface by bacteria. All mini-columns were pumped at a rate of 0.013 mL/min and collected via fraction collector every 45 minutes in the first step and 90 minutes for the second step.

Set #1: *Chromohalobacter* was initially injected into two mini-columns with 20 ppb Nd in brine 15% NaCl by wt + 3 mM $NaHCO_3$. Solutions were injected for approximately nine hours before the input solution was switched to 15% NaCl by wt + 3 mM $NaHCO_3$ (note the absence of Nd) which was injected for ~11 hours.

Set #2: Neodymium was initially injected in the absence of bacteria to then analyze the re-mobilization of Nd. Solutions were injected for approximately nine hours before the input solution was switched to 15% NaCl by wt. + 3 mM $NaHCO_3$ + bacteria (note the absence of Nd) for ~11 hours.

Figure 7. Experimental setup with syringe pump (0.013 mL/min) and fraction collector.

3.3 Europium - Bacteria Transport Experiment

Similar to the neodymium experiment, duplicate columns were connected to syringes for two experiments. For one set, two syringes were filled with 200 ppb of Eu + 15% NaCl by wt. + 3 mM $NaHCO_3$. The duplicate set of mini-columns were injected with the *Chromohalobacter* solution (200 ppb Eu+ 15% NaCl brine + bacteria). Solutions were injected for approximately 15 hours before the input solution was switched to 15% NaCl by wt. + 3 mM $NaHCO_3$ which was injected for ~9 hours for all columns. All mini-columns were pumped at a rate of 0.013 mL/min with sampling protocols every 60 minutes and with the release solution it was switched to a 90 minute collection time.

Due to power-outages and syringe pump malfunctions, the experiment was stopped and two mini-columns leaked at the inlet due to temperature issues at the lab from a power outage.

3.4 Cesium - Bacteria Transport Experiment

Two suspensions of bacteria were prepared under different conditions: (1) stressed and (2) normal, stationary phase conditions. 100 mL of live *Chromohalobacter* was heated in a 70° C oven to obtain stressed cells (approximately 60% live but stressed, 40% dead, as determined by Live/Dead staining and microscopy) and 100 mL of *Chromohalobacter* were left completely viable (in the stationary phase as described above). These suspensions were then spiked with 200 ppb Cs for injection into the dolomite columns. Due to the small mass of bacteria available for these experiments, they were not conducted in duplicate as previously performed.

Syringes were placed onto the syringe pump and injected at a rate of 0.013 mL/min with collection every 45 minutes. Initial bacteria suspensions were injected for approximately 17 hours before the input was switched to 15% NaCl by wt + 3 mM $NaHCO_3$ for both sets which was injected for about 8 hours with collection every 90 minutes.

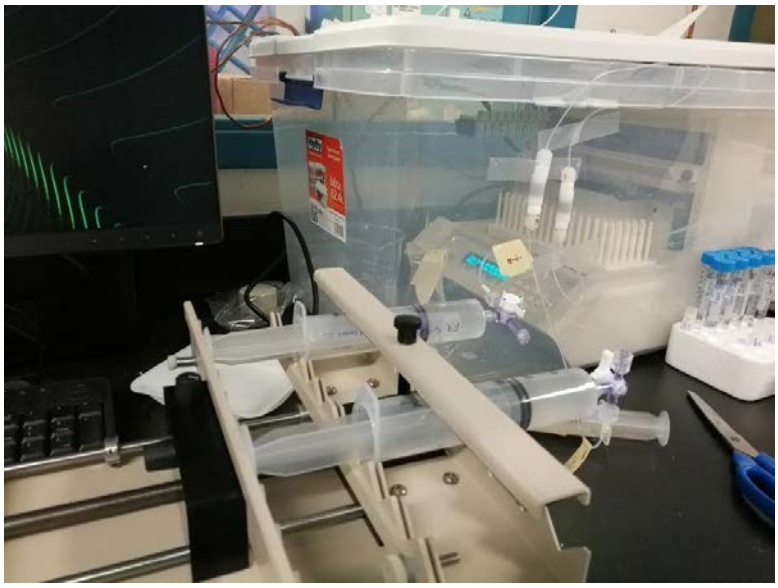

Figure 8. Image of the experimental setup for Cs experiments with two syringes (one for stressed and one for viable bacteria) connected to syringe pump and injected into columns with effluent collection in the fraction collector in a makeshift humidity chamber.

3.5 Sampling Protocol

The effluent was weighed to track total volume and flow rate of column by mass and samples were removed for ICP-MS (Agilent 7900) to be analyzed for metals (Nd^{3+}, Eu^{3+}, and Cs^{+}). The samples were analyzed in 2% HNO_3 with a 30 ppb In internal standard. In addition, 0.5 mL of sample was placed into the Genesys 20 spectrophotometer to measure absorbance of blank and sample solutions at 600 nm to correlate with the aqueous bacteria concentration. Then, 200 μL was filtered in 100k MWCO filters with centrifugation for 15 minutes at 13,500 rpm. After centrifugation in the filter tube, an aliquot was removed and prepared for ICP-MS in order to measure both total of the chemical analogues for the actinides and cesium in the aqueous phase and the fraction not associated with the bacteria (that which passes through the filter). The final step included analysis of pH for approximately 4 combined tubes to collect a sufficient volume for accurate analysis.

3.6 ICP-MS Limits of Detection

All samples in these experiments were analyzed through ICP-MS. The detection limit is the concentration that is obtained when the measured signal differs from the background. For each experiment, the limits of detection (LOD) were calculated through a linear regression on Excel. The specifics of the LOD values for Nd^{3+}, Eu^{3+} and Cs^{+} experiments are described in Table 2.

Table 2. Description of Each Element and its Limit of Detection (LOD) on the ICP-MS

Element	Limit of Detection (LOD) ppb
Neodymium-144	0.056 ppb
Europium-153	0.54 ppb
Cesium-133	0.081 ppb

3.7 Stock Solution Concentrations

As mentioned above, the concentrations of bacteria were correlated with the absorbance measured with a spectrophometer. The concentrations of each metal found in the sample was also supplied to visualize the concentration that associated with the bacterium. The specifics of these absorbances and ICP-MS concentrations are described in Table 1.

Table 3: Stock solution fraction associated with bacteria (based on filtration through a 100k MWCO filters

Element	Bacteria stock absorbance (nm)	Fraction of element associated with bacteria in stock
^{144}Nd	0.242	95.0%
^{133}Cs	0.262	5.6%

4. RESULTS AND ANALYSIS

The results and analysis are still ongoing for all results presented in this work. These results and conclusions are preliminary.

4.1 Neodymium – Bacteria Transport Experiment

The results from the first set of experiments shown in Figure 9 and Figure 10 are plotted in terms of the concentration normalized in terms of effluent recovery and cumulative pore volumes. A pore volume is the amount of space in a mini-column (the grains of dolomite) that is filled with liquid. In other terms, a pore volume is equivalent to one filling of the column and can be correlated with time based on the 0.013 mL/hr flow rate.

In Figure 9, the green points represent the bacteria recovery calculated based on absorbances and the blue represents the neodymium recovery in the effluent based on ICP-MS analysis. These data show that Nd is mobilized by the bacteria when it is reacted with *Chromohalobacter* prior to injection into the column. This is further observed as the majority of the Nd measured in the effluent is associated with the size fraction with the bacteria instead of the dissolved phase which passed through 100k MWCO filters and is depicted in Figure 10. This suggests that Nd is adsorbed to the bacteria surface.

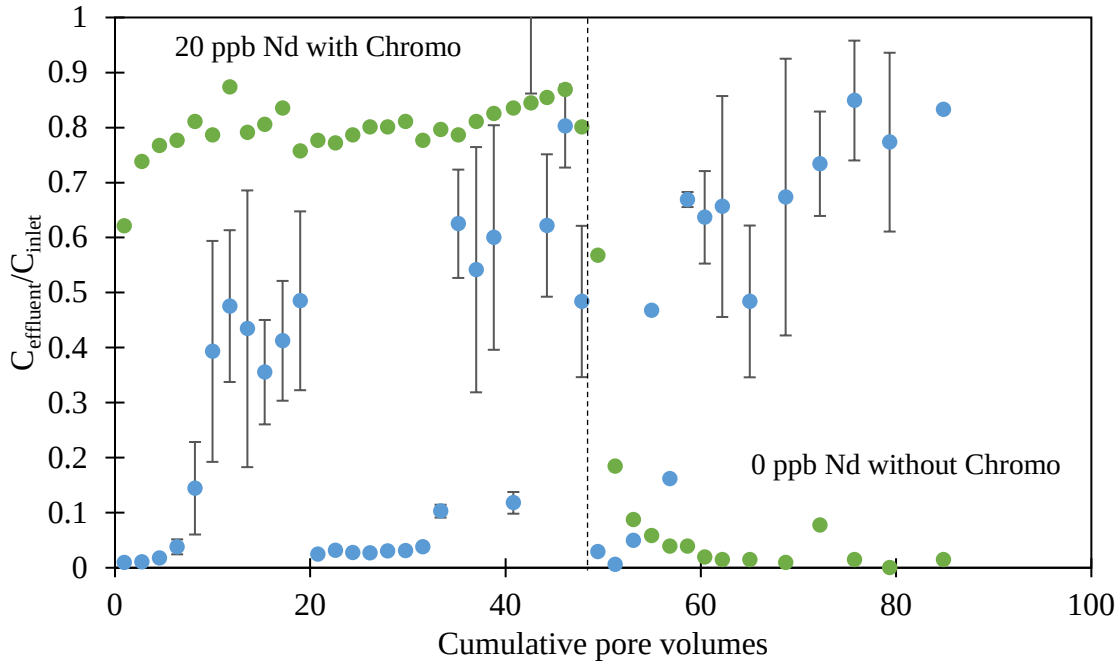

Figure 9. Results for total Nd (blue) and *Chromohalobacter* (green) recovery with initial injection of *Chromohalobacter* + Nd + brine followed by injection of brine only. Note: dotted line delineates between first and second injection steps as described in Methods. The drops in absorbance are a result of instrumental error.

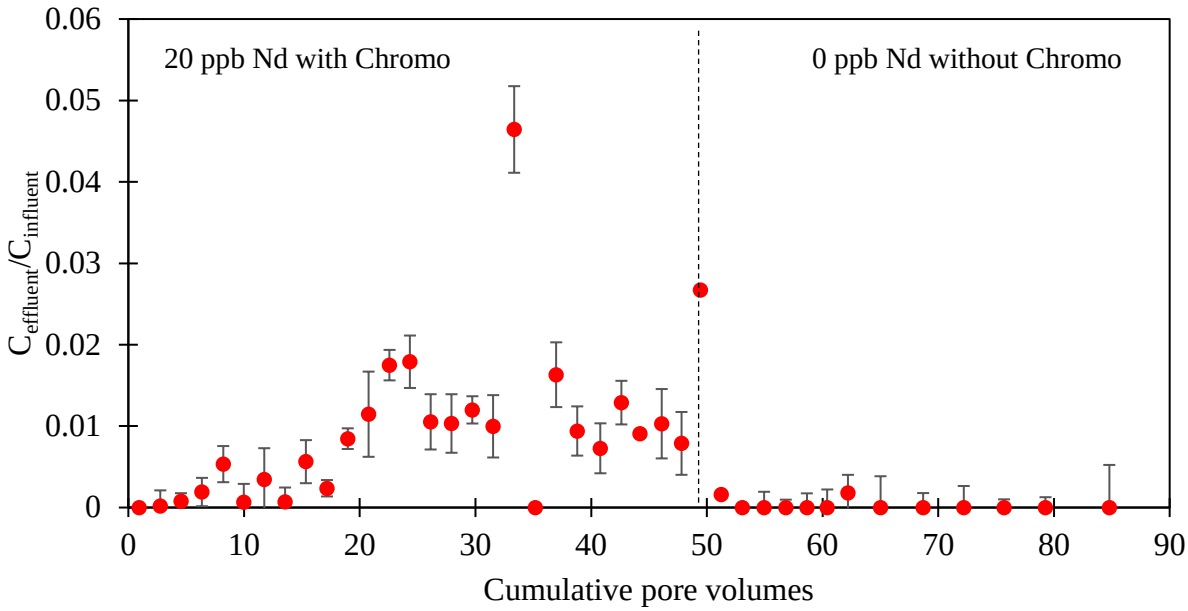

Figure 10. Results for Nd following filtration through a 100k MWCO filter for initial injection of *Chromohalobacter* + Nd + brine followed by injection of brine only. Note: dotted line delineates between first and second injection steps as described in Methods.

In Figure 11, the recovery is shown for Nd and *Chromohalobacter* with initial injection of Nd without bacteria followed by injection of bacteria without Nd. The graph below represents the unfiltered, total Nd recovery. Again, the green represents the bacteria and the blue represents the Nd concentrations. There is a neglibible amount of Nd recovered with the initial injection (20 ppb Nd + brine) and with the injection of the *Chromohalobacter* (Bacteria + brine, no Nd). Therefore, these data suggest that there is no remobilization of Nd by bacteria after Nd has contacted the dolomite, instead the Nd was nearly completely sorbed onto the dolomite and the bacteria were unable to pull the Nd off from the surface, i.e. sorption sites on bacteria cannot compete with available sites on dolomite.

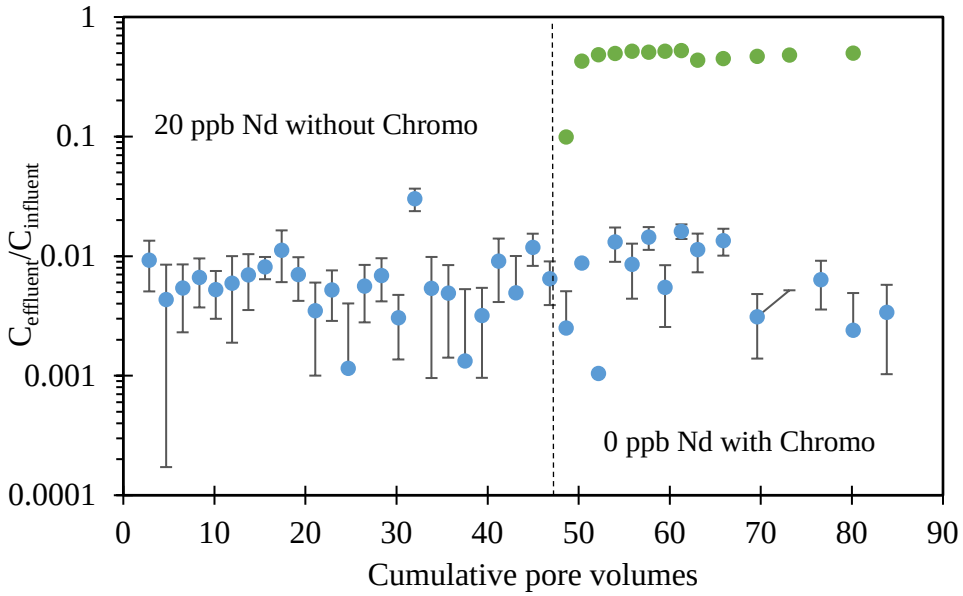

Figure 11. Results for recovery of total Nd and bacteria in column with initial injection of 20 ppb Nd + brine followed by injection of *Chromohalobacter* + brine only (only unfiltered data presented), , Note: error bars are not included for bacteria as only a single measurement was collected for each sample but error bars are smaller than the symbols for Nd.

4.2 Cesium - Bacteria Transport

Figure 12 represents the initial injection of viable *Chromohalobacter* with 200 ppb Cs + brine. Both the Cs unfiltered and filtered samples are plotted to show that there is very little difference between the two series. This result indicates that the bacteria did not have a significant effect on the mobility of Cs as they are not associated with the bacteria. Cesium also had no interaction with the dolomite based on the nearly complete recovery in the effluent. In comparison to the Nd experiment, the Nd was associated with the bacteria and was mobilized by them. However, cesium acted similarly to a tracer, it went through the column without interacting significantly with the dolomite or bacteria. This is significant as previous work observed strong interactions of Cs with other microbial species (Johnson *et al*., 1991, Avery, 1995). However, it is possible that uptake of Cs cannot occur in these experiments due to the short time frame and high concentration of competing ions (e.g., Na^+).

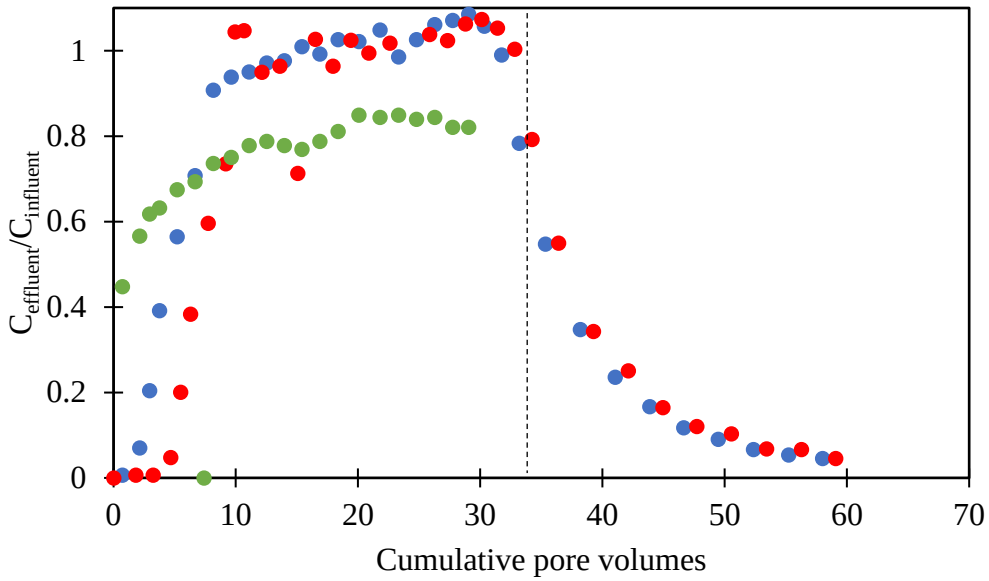

Figure 12. Results for column with initial injection of viable *Chromohalobacter* + 200 ppb Cs + brine followed by injection of brine only for viable bacteria. Recovery of unfiltered Cs (blue) and filtered Cs (red) shows minimal interaction of Cs with dolomite mineral and bacteria (green), respectively, Note: error bars are not included for bacteria as only a single measurement was collected for each sample but error bars are smaller than the symbols for Cs.

In the bottom of Figure 13 there are two green series: the filled green points represent stressed bacteria while the unfilled points represent viable bacteria. The top of the graph represents the concentration of cesium that was pulled in from both the stressed and viable bacteria suspensions. Since the graphs are very similar, this further indicates that the condition of the bacteria does not reflect on the cesium and that the bacteria do not have a strong impact on the mobilization of Cs. These data indicate that the bacteria have very little effect on cesium and cesium does not interact with the dolomite in terms of sorption. In comparison to Nd, cesium did not associate with the bacteria but was very mobile without their presence.

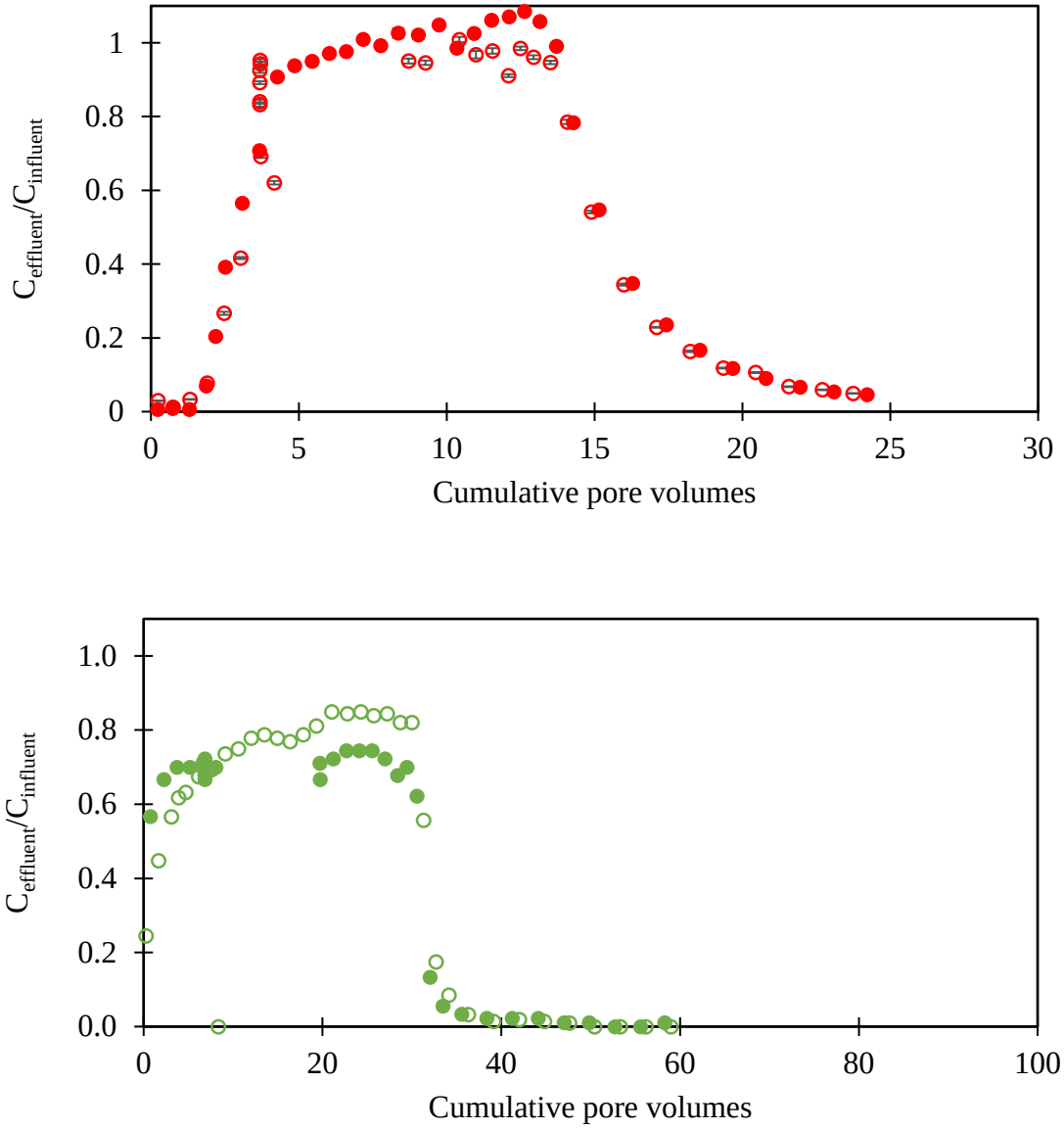

Figure 13. Results for columns with initial injection of viable (open) and stressed (filled) *Chromohalobacter* + 200 ppb Cs + brine followed by injection of brine only. Recoveries of Cs (red, top) and bacteria (green, bottom) shows no effect of stressed bacteria on Cs transport or bacteria recovery from the column, Note: error bars are not included for bacteria as only a single measurement was collected for each sample.

4.3 Europium - Bacteria Transport

Figure 14 represents the initial injection of europium with bacteria and brine. Both Eu unfiltered and filtered samples are plotted to demonstrate the mobilization of Eu and the association with bacteria. Similar to the results of the Nd experiment, there was a mobilization of Eu due to the bacteria which is indicated by the recoveries shown in unfiltered series. There is not a great recovery of Eu which suggests that: (1) Eu was less strongly associated with the bacteria than Nd or (2) the mass balance is incorrect. It must be noted that the initial stock solution concentration could not be checked via ICP-MS as all solutions were injected into the mini columns.

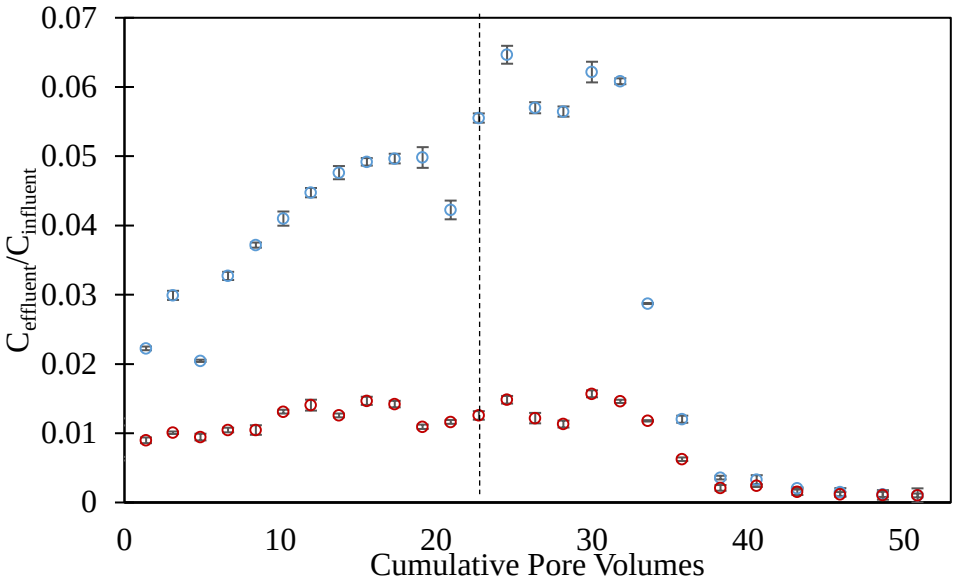

Figure 14. Results for the initial injection of *Chromohalobacter* + 200 pbb Eu + brine followed by injection of brine only. Note: Unfiltered results (open blue circles) suggest slight Eu mobilization in the mini-column and filtered (open red circles) suggests Eu association with bacteria, error bars are not included for bacteria as only a single measurement was collected for each sample.

As an extension of Figure 14, Figure 15 includes the absorbance. In Figure 14 and Figure 15, the amount of Eu that was mobilized by the bacteria was minimal. Figure 15 emphasizes that a significant fraction of Eu measured in the column effluent was adsorbed onto the bacteria and became associated with the bacteria. Unlike the Nd experiment, the effluent recovery was significantly lower than expected but still suggests some mobilization with bacteria in the aqueous phase.

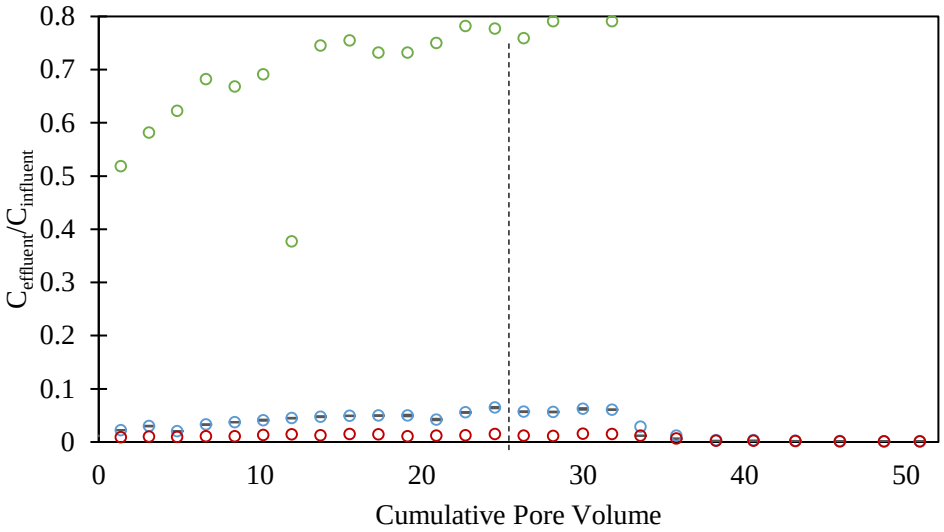

Figure 15. Results for the initial injection of *Chromohalobacter* + 200 ppb Eu + brine followed by injection of brine only. Note: Unfiltered recoveries (open blue circles) suggest slight mobilization and filtered (open red circles) recoveries suggest little Eu association with bacteria, error bars are not included for bacteria as only a single measurement was collected for each sample.

The top of Figure 16 represents total aqueous Eu recovery (unfiltered) for the initial injection of the Eu + brine. During the initial injection (Eu + Brine), low Eu recoveries were observed which indicates strong sorption onto the dolomite. There is a n increase in recovery of Eu in the second injection (brine only) which could imply that some Eu can be mobilized by the aqueous brine solution in the absence of bacteria. The bottom of Figure 16 represents the filtered results for the initial injection of Eu + brine. Like the unfiltered results, there was an increase of Eu recovery in the second injection which indicates a mobilization of dissolved Eu with the brine. In both graphs, Eu is mobilized at very low concentrations in the absence of bacteria. These results show that bacteria have a greater impact on the mobilization of Eu than the aqueous brine.

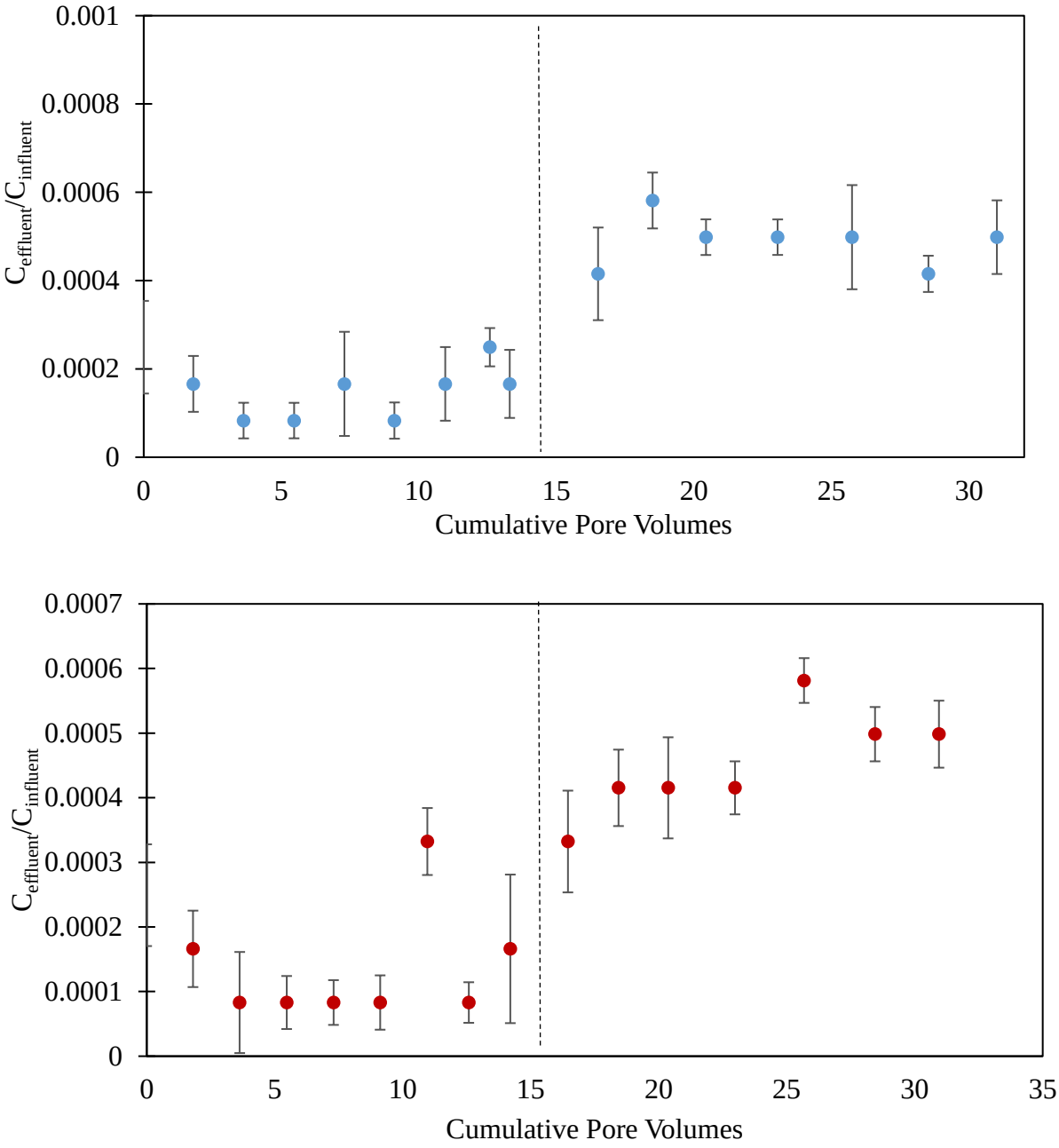

Figure 16. Unfiltered results for the initial injection of 200 ppb Eu + brine (top). Filtered results for the initial injection of 200 ppb Eu + brine, Note: Increase in mobility in the mini-column after the second injection at very low concentrations.

5. CONCLUSIONS

Bacteria can have a strong effect on mobilization and sorption processes of contaminants of concern. According to the results, *Chromohalobacter* can mobilize Nd when reacted with Nd before it contacts the dolomite surface but they cannot remove Nd from the surface of the dolomite. Further, the filtration results for Nd indicate that there is an association between the Nd and the bacteria, which means that without bacteria there is no mobility in the column due to strong sorption of Nd to the dolomite.

Cesium, on the other hand, did not associate with the bacteria and did not sorb onto the dolomite which means it went through the column. There is little effect on the sorption and mobilization of Cs in the mini-columns. However, it must be noted that uptake of Cs by *Chromohalobacter* is not expected under these conditions but could occur during the growth phase. Therefore, results show that there is little interaction of Cs with the surface of bacteria.

Eu data indicate that the bacteria can mobilize Eu in the aqueous phase through the mini-column. The filtration results for the Eu indicate an association between Eu and bacteria. Further, the second set of injection (200 ppb Eu+ brine) results indicate that Eu can also be mobilized by the aqueous brine but only at very low concentrations. The results for both Nd^{3+} and Eu^{3+} are very similar except that the fractions of Eu mobilized by the bacteria are significantly lower. Since Nd^{3+} and Eu^{3+} are both chemical analogues with the same oxidation state, their behavior should be similar in terms of co-transport by bacteria.

Although further experiments are required to fully understand these systems, preliminary conclusions can be drawn based on the data gathered during this internship. These data can be used to update risk assessment models for the Waste Isolation Pilot Plant because they show that bacteria could have an effect on the potential transport of actinides and cesium that are stored in the repository. Moreover, this work contributes to the body of knowledge of microbial interactions with cations.

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