

# **STUDENT SUMMER INTERNSHIP TECHNICAL REPORT**

## **Potential for Transport of Cesium as Biocolloids in a High Ionic Strength System**

### **DOE-FIU SCIENCE & TECHNOLOGY WORKFORCE DEVELOPMENT PROGRAM**

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## ABSTRACT

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In a subsurface repository, there are several waste components that are chemically active which can directly influence the fate and transport of radionuclides. Deep geologic formations, such as the Waste Isolation Pilot Plant (WIPP), can have halophilic bacteria due to its location within a salt mine (Swanson *et al.*, 2016). The behavior of halophilic bacteria, such as *Chromohalobacter*, although previously studied for its adsorption to Np and known for its growth in WIPP relevant conditions (Ams *et al.*, 2013), its potential interaction with Cs remains unknown in the WIPP environment. As an extension of previous experiments conducted in LANL/CEMRC 2017, the primary focus of this research was on the effect of *Chromohalobacter* on the fate and transport of Cs. Column and batch experiments were conducted to monitor the potential for uptake of Cs by microbes, co-transport of Cs on microbe surfaces and remobilization of Cs via microbial interaction. These results imply that there is no association of Cs with microbes under high ionic strength conditions (2.78 M NaCl).

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## 1. INTRODUCTION

The production of nuclear weapons during World War II and the Cold War resulted in an exponential growth of defense-related radioactive waste. The monumental amount of radioactive waste that is a byproduct of nuclear weapons development is currently being disposed of by the Department of Energy at a deep geologic repository known as the Waste Isolation Pilot Plant (WIPP) located near Carlsbad, NM. Deep geologic repositories have been chosen as the most appropriate site to dispose of nuclear waste including, but not limited to, clay, granite, and salt formations. However, salt and clay formations have an additional advantage due their abundance in the environment. Clay repositories, have disadvantages such as the uncertainty of long-term behavioral effects of heat and radioactivity on barrier performances (Chegbeleh *et al.*, 2008). Salt formations (like that present in the WIPP) have advantages such as high strength, cavity stability, permeability and dissolution properties which counter-act some of radioactivity (NEA, OECD). The WIPP repository places intermediate to high level waste in a salt bed, also known as the Salado Formation, which is around 2,150 ft below the ground surface in a bedded halite formation (~600 m thick). Figure 1 is a thorough WIPP stratigraphic column (Thakur, 2015). Already, 90,600 m<sup>3</sup> of contact-handled transuranic waste (CHTRU) and 360 m<sup>3</sup> of remotely-handled transuranic waste have been disposed of at the WIPP (DOE Linking Legacies).

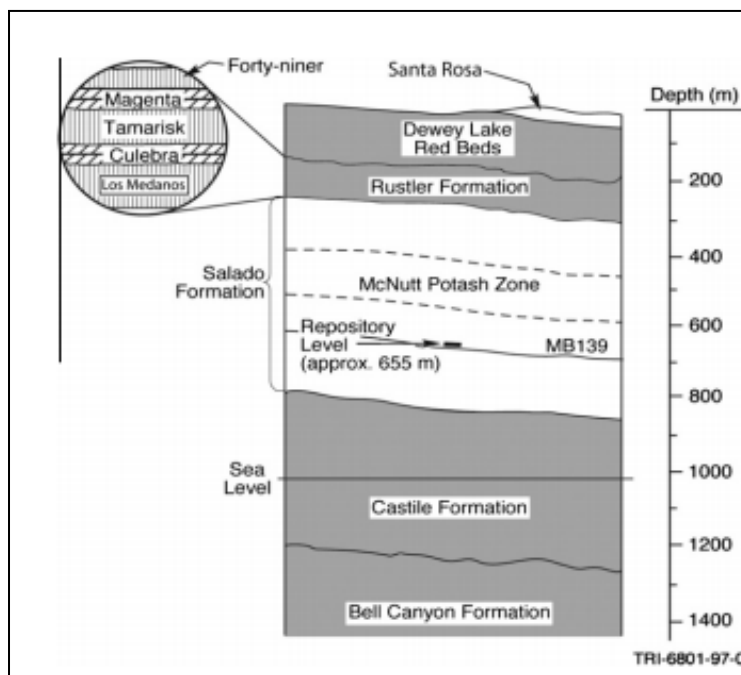


Figure 1. WIPP stratigraphic column (Thakur, 2015).

Cesium-137 is stored at the WIPP and also constitutes a major portion of the radioactive inventory at the U.S. Department of Energy's nuclear facilities such as Hanford (Chen *et al.*, 2005). The current WIPP inventory of <sup>137</sup>Cs based on radionuclide activity (Ci) has added up to 1.11\*10<sup>4</sup> Ci as of 2016 (DOE WIPP, 2016). However, nuclear weapons development was not the only contributor to radioactive waste generation and anthropogenic radionuclide releases. Another example is the Fukushima Daiichi Nuclear Power Plant (FDNPP) following the Great

East Japan Earthquake and tsunami on March 11, 2011, which caused the FDNPP's nuclear reactors to overheat and melt. The venting operation and hydrogen explosions that followed yielded significant releases of radioactive iodine ( $^{131}\text{I}$ ) and cesium ( $^{134}\text{Cs}$  and  $^{137}\text{Cs}$ ) to the atmosphere and the Pacific Ocean (Kubo *et al.*, 2018; Chino *et al.*, 2011) as shown in Figure 2. Additional releases from the development of nuclear weapons and nuclear reactor accidents, such as Fukushima in 2011 and Chernobyl in 1986, have led to significant releases of  $^{137}\text{Cs}$  into the environment (Burger *et al.*, 2018; Ashraf *et al.*, 2014a, b). Further, Cs may be highly mobile especially in high-ionic strength systems due to its dominant chemical form as a monovalent cation (Saiers *et al.*, 1996). Thus, there is a need to better understand the behavior of Cs under high ionic strength conditions in order to develop representative risk-assessment models.

The fate and transport of Cs in salt environments, with respect to sorption and mobility, have not yet been thoroughly understood. Further, there is uncertainty on the effects of microorganisms in high ionic strength environments on Cs transport (Swanson *et al.*, 2016). The Culbra dolomite layer, which lies around 450 m above the actual WIPP repository and within the Rustler formation is the most transmissive component and, therefore, the most likely formation for radionuclide transport in the event of brine release from the repository (US Department of Energy 2009; Thakur, 2015). Therefore, there is a need to better understand the partitioning of radionuclides in this layer in order to update performance assessment models for the WIPP.

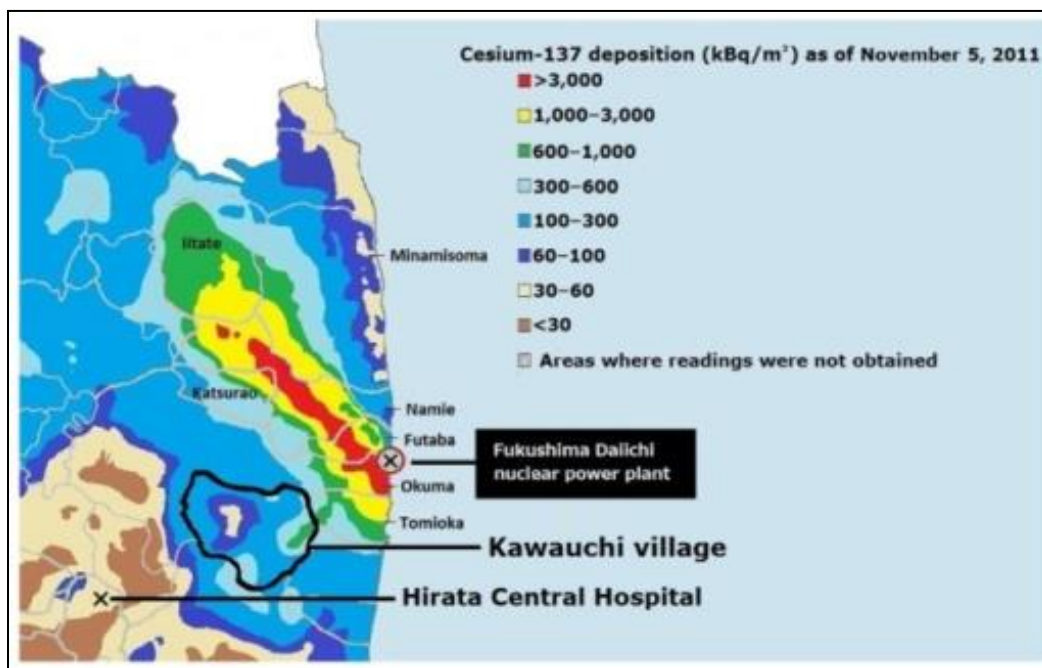
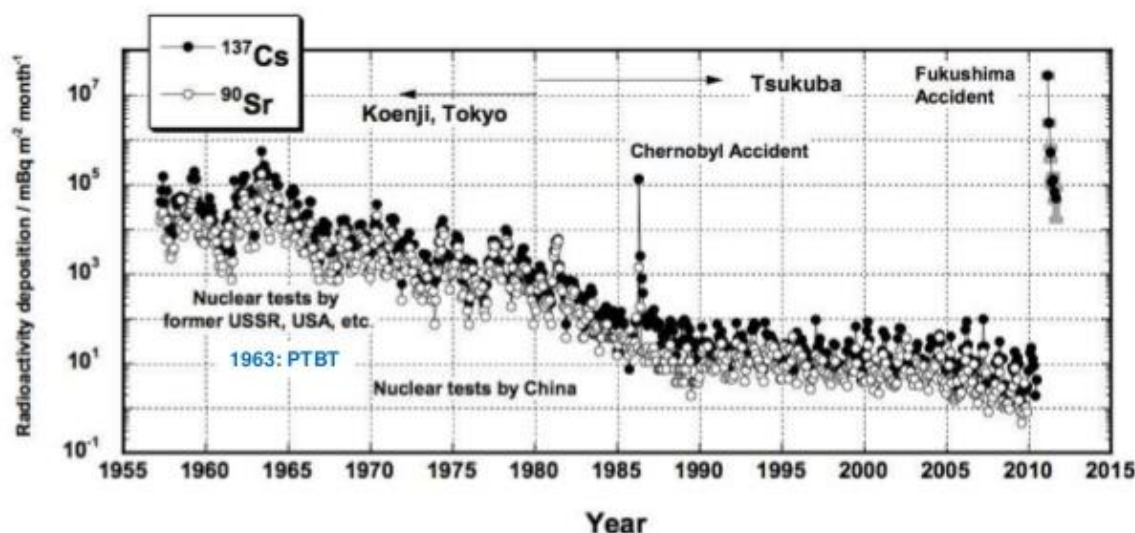


Figure 2. Radiation contour map underlying the Cs-137 deposition (Hayakawa, 2011).





**Figure 3.** Deposition of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  by nuclear testing (Meteorological Research Institute, 2011).

Porous media are an important factor in quantifying Cs and other contaminant transport in the subsurface, however, experiments are often conducted with simplified solid materials such as glass beads or pure minerals (Flury *et al.*, 2004). Some studies have investigated the interaction of  $^{137}\text{Cs}$  with minerals and sediments to understand the rate at which it moves through aquifers. Saiers and team previously utilized column experiments as they provide a more accurate representation of the subsurface because of the similar solid to solution ratios (Saiers *et al.*, 1996). In their research, columns were packed with quartz sand with pore water that consisted of variable ionic strengths from 0.0002 up to 0.102 M. The low ionic strength conditions in these experiments showed an inverse relationship for adsorption with respect to ionic strength (Saiers *et al.*, 1996).

The potential for colloid facilitated transport of Cs with inorganic minerals from natural sediments must also be considered. Chen *et al.* (2005) observed that inorganic colloids from Hanford sediments were capable of transporting Cs although significant desorption occurred depending on residence time. In columns packed with Hanford sediments and saturated with artificial groundwater (AGW) of 1.0 M NaCl and 1.67 mM  $\text{NaHCO}_3$ /1.67 mM  $\text{Na}_2\text{CO}_3$  at pH  $\sim 10$ , they reported that native colloids (flushed with the AGW) had a higher sorption capacity for Cs than modified colloids (flushed with [1.4 or 2.8 mol/kg NaOH, 0.125 or 0.25 mol/kg  $\text{NaAlO}_4$ , and 3.7 mol/kg  $\text{NaNO}_3$ ], Mashal *et al.*, 2004), which implied that Cs traveled in the presence of colloids. However, the potential for transport as a bio-colloid (with a microbe) has not yet been considered.

This research also observes the transport of Cs through natural porous media, Culebra dolomite under high-ionic strength conditions in the presence of microbes. The objective is to quantify the influence of microbes on the mobilization and transport of Cs through high ionic strength systems.  $^{133}\text{Cs}$  was utilized as a stable non-radioactive analogue and exhibits identical chemical behavior to the radioactive isotopes of Cs. There is potential for co-transport of Cs with microbes in deep geologic repositories due to contaminant adsorption to microbe surfaces or uptake into microbes. A halophilic bacterium, *Chromohalobacter* sp. strain PZ13, was originally isolated from the far-field groundwater of the WIPP and grown for these experiments. These

results are compared with previous Cs experiments conducted in 2017 where there was no interaction with the dolomite and no mobilization by microbes in the stationary phase.

## 2. EXECUTIVE SUMMARY

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This research work was 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) through cooperative agreement DE-EM000598. Additional funds for travel were provided by FIU's McNair program as well as the Los Alamos National Laboratory Actinide Chemistry and Repository Science team. During the summer of 2018, DOE Fellow Frances Zengotita spent 7 weeks working in a laboratory at the Carlsbad Environmental Monitoring and Research Center under the supervision and guidance of Drs. Juliet S. Swanson and Donald T. Reed. Her project was initiated on June 11<sup>th</sup>, 2018, and continued through July 27<sup>th</sup>, 2018 with the objective of quantifying the mobility of Cs in the presence of halophilic microbes under conditions relevant to the Waste Isolation Pilot Plant.

### 3. RESEARCH DESCRIPTION

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#### *Materials*

Dolomite utilized in these experiments ranged from 355 – 500  $\mu\text{m}$  in diameter to allow sufficient surface area for interaction and be large enough particles to reduce clogging. Rock samples were collected by Dr. Timothy Dittrich from the Culebra bluff outcrop located near the Pecos River parallel to the WIPP (Emerson *et al.*, 2018). The rocks were crushed with a stainless steel impact mortar and pestle (Chemplex, catalogue no. 850), sieved (No. 45, 100 and 200 sizes, Fisher, Stainless steel), cleansed with high purity water ( $>18\text{ M}\Omega\cdot\text{cm}$ ) and finally placed in the oven at  $40^\circ\text{C}$  for drying (Emerson *et al.*, 2018). This method was conducted to cleanse and remove any impurities from the dolomite and isolate an appropriate size fraction. The process was repeated 2-3 times before being collected.

All salt solutions utilized in these experiments were ACS reagent grade or better, including  $\text{NaCl}$ ,  $\text{NaHCO}_3$ , and growth broth components. The nitric acid employed for ICP-MS measurements was Optima<sup>TM</sup> concentrated nitric acid (67-69%) from Fisher Scientific (Waltham, MA; USA). The deionized water ( $18.2\text{ M}\Omega\cdot\text{cm}$ ) was produced in-house using a Barnstead<sup>TM</sup> GenPure<sup>TM</sup> water purification system from Thermo Scientific (Waltham, MA; USA). Indium, used as the ICP-MS internal standard, was obtained from High Purity Standards (Charleston, SC; USA) as a 1000  $\mu\text{g/mL}$  solution in 2% nitric acid. Likewise, cesium, used for ICP-MS calibration and all column experiments, was received from High Purity Standards as a 1000  $\mu\text{g/mL}$  solution in 1% nitric acid.



**Figure 4.** Steel mallet and impact mortar utilized to crush dolomite (*left*) and sieves (No. 45, 100 and 200 sieves) with a variety of different size fractions (*right*).

#### *Chromohalobacter*

The halophilic bacterium observed in this work was isolated from a groundwater environment (specifically, a monitoring well) that had an ionic strength of 4.5 M at the WIPP site in southeastern New Mexico, USA. The monitoring well was an open borehole at a depth of approximately 65 ft below the ground surface in the Dewey Lake Formation. This monitoring

well is assumed to be a combination of flow within the formations with seepage from nearby salt (NaCl) removed from the WIPP. This monitoring well was within the WIPP demarcation zone which suggests that this bacterium could potentially be a representative model for high-ionic strength experiments (Ams *et al.*, 2013). Figure 5 is an image taken under a transmission electron microscope, or TEM, which yields a thin-section image of the halophilic bacterium in question.

*Chromohalobacter* was grown in a generic halophile broth (GHB) containing 15% NaCl (w/v; equivalent to 2.6 M NaCl) and 3 mM bicarbonate and other micronutrients for growth. Cells were harvested during log-phase growth (~14-16 hours of growth) and concentrated by centrifugation. They were then washed three times with sterile 15% NaCl + 3 mM bicarbonate solution. After the final wash, they were re-suspended in the test solution to obtain an Optical Density (OD) of ~0.23-0.26. This corresponded to approximately  $10^8$  cells/ml.



**Figure 5. Thin-section image taken by Dr. Peter Cooke at New Mexico State University-Core University Research Resources Laboratory.**

#### *Miniature column experimental setup*

The design of the miniature column experiments was inspired from previous work (Dittrich *et al.*, 2016) and remained the same as experiments from summer 2017 to have the pore volume of the column to remain relatively constant (~0.45 mL). In brief, one inch of polytetrafluoroethylene (PTFE) tubing (3/8" inner diameter, International Polymer Solutions) was packed with one gram of the Culebra dolomite [355-500  $\mu$ m] for the columns. The one inch PTFE column was then threaded with a 1/8" 27 National Pipe Tapered (NPT) carbon pipe tap (Drillco cutting tools) to allow the fittings to be screwed and tightened into the column. To prevent clogging and particle breakthrough in the tubing, the fittings (MC-F-12, iPolymer) were covered with 35  $\mu$ m polyetheretherketone (PEEK) mesh (Spectrum labs). The PTFE fittings were then tightened and sealed with silicone. The mini-columns were attached to 0.032" of tubing (PTFE #20 Cole Palmer, 0.032" inner diameter) for the influent and effluent lines that

were then connected to polypropylene syringes (B-D, Luer-LOK) through a syringe pump (kd Scientific 100 series) with a flow rate of 0.013 mL/min.

Pore volume is the space in between the grains that is liquid in the mini-column. The pore volume was calculated based on the total volume of the column, volume of the tubing and volume of dolomite added to the column. The cumulative volume injected into the columns is converted to a cumulative number of pore volumes based on the pump flow rate and pore volume.



**Figure 6. Mini-column setup with fittings attached with inlet and outlet tubings.**



**Figure 7: Gilson Fraction Collector (left) and kdScientific 100 Series syringe pump.**

#### *Column transport of Cs with Biocolloids*

A synthetic brine that consisted of 15% (w/v, 2.78 M) NaCl + 3 mM NaHCO<sub>3</sub> was utilized to keep conditions relevant to repository conditions and to have a stable, high ionic strength environment for the bacteria. Bicarbonate, NaHCO<sub>3</sub>, was added to buffer to pH of ~8.2 and to represent equilibration of the solutions with dolomite and the atmosphere. Variations on the introduction of Cs to microbes were conducted to observe the impact of *Chromohalobacter* (~10<sup>8</sup> cells/mL) on the fate of Cs in the dolomite mineral system.

The following column experiments were run each in duplicate columns:

1. Cs injected in the absence of microbes (negative control).
2. Co-transport of Cs with *Chromohalobacter* – Following growth of *Chromohalobacter* in generic halophile broth (GHB), microbes ( $10^8$  cells/mL) were equilibrated for one hour with 5000 ppb Cs prior to injection into columns. After a steady-state was achieved in the columns, injection solutions were switched to brine only to monitor potential release of Cs and microbes.
3. Re-mobilization of Cs with *Chromohalobacter* – Columns were initially equilibrated with 5000 ppb Cs. Following growth of *Chromohalobacter* in GHB, microbes ( $10^8$  cells/mL) and brine were injected into the columns until a steady-state was achieved and then brine was injected to consider the potential for uptake of Cs by microbes within the columns.
4. Co-transport of Cs after growth of *Chromohalobacter* in the presence of Cs – Following growth of *Chromohalobacter* in GHB and 5000 ppb Cs, microbes ( $10^8$  cells/mL) were injected into columns until a steady-state was achieved. Injection solutions were then switched to brine only to monitor potential release of Cs and microbes.
5. Co-transport of Cs after growth of *Chromohalobacter* in the presence of Cs with additional Cs - Following growth of *Chromohalobacter* in GHB and 5000 ppb Cs, microbes ( $10^8$  cells/mL) were equilibrated for one hour with 5000 ppb Cs prior to injection into columns. After a steady-state was achieved in the columns, injection solutions were switched to brine only to monitor potential release of Cs and microbes.

#### *Batch experimental protocols*

Alongside bio-colloidal column experiments, growth batch experiments were also conducted. The purpose of growth experiments was to monitor the uptake of Cs through rapid sampling periods. The samples were grown in generic halophile broth (also regarded as GHB) in 15% NaCl (w/v) + 3mM NaHCO<sub>3</sub>. The growth samples were collected at variable time periods every few hours due to the exponential growth of the microorganisms. Polypropylene centrifuge tubes (50 mL, BD Falcon) were prepared with a 20 mL total volume into each individual sample. Samples were taken at t=0, 4, 8, 12, 16, 20, 24, 28, 32 and 49 hours. After each sampling period, the samples were placed into the incubator shaker (I2400 Incubator Shaker, New Brunswick Scientific) at a rate of 220 rpms and a constant temperature of 37° C. At 37° C. Figure 8 demonstrates the instrumental set-up and supplies an image of the batch samples based on turbidity.

The conditions are found below (in triplicate):

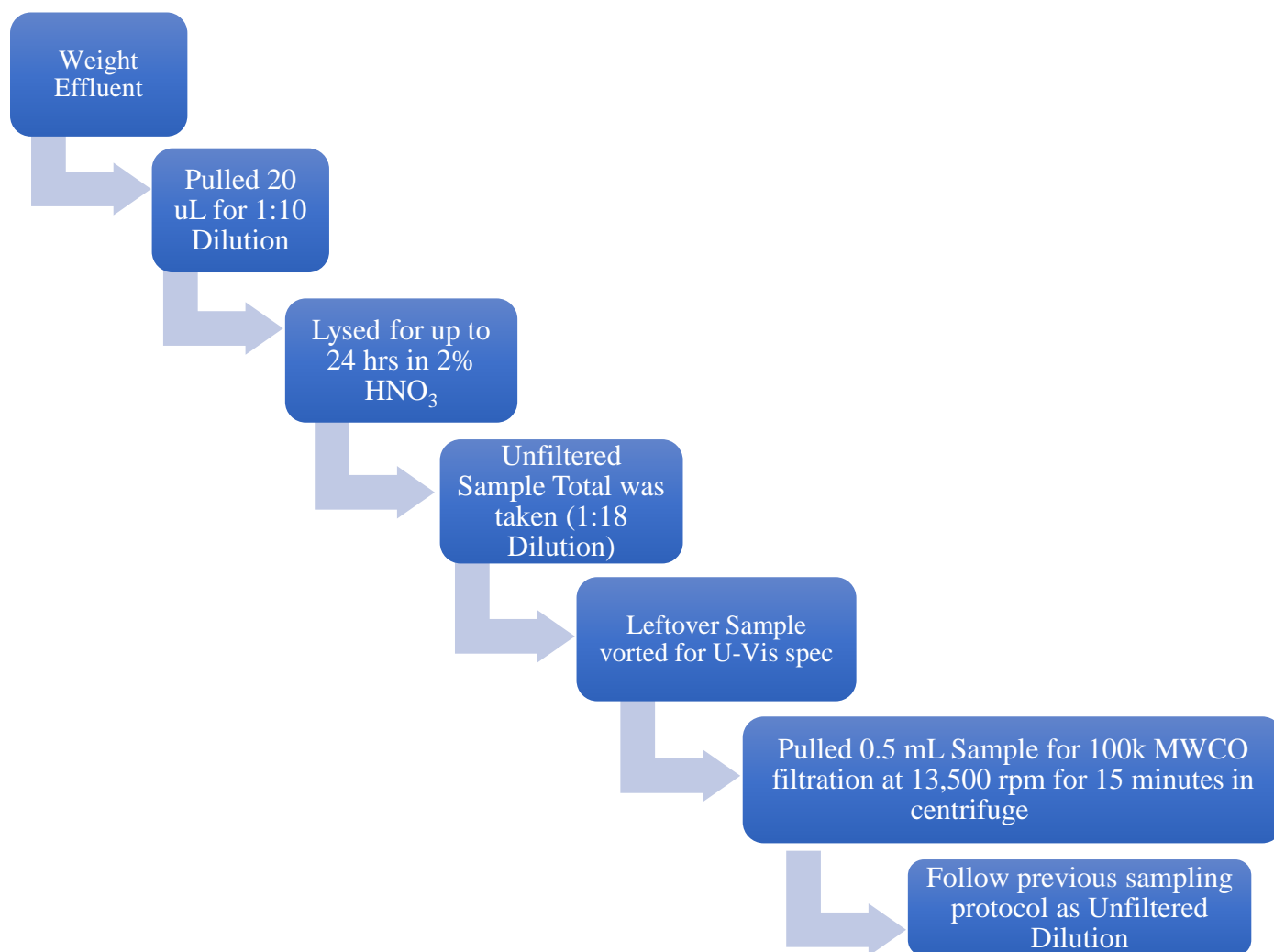
1. Microbes + 5000 ppb Cs + 5 g/L Dolomite + GHB
2. Microbes + 5 g/L dolomite + GHB
3. Microbes + 5000 ppb Cs + GHB
4. Microbes + GHB
5. Dolomite + GHB
6. GHB





Figure 8. Growth experiment in the incubator shaker (left); the batch samples (right) demonstrate a strong turbidity with the *Chromohalobacter* over time in terms of growth.



*Overall Sampling Protocol*

**Figure 9. General step-by-step sampling protocol.**

*ICP-MS Methodology*

All Cs measurements were carried out using an Agilent (Santa Clara, CA; USA) 7900 ICP-MS equipped with an Agilent ASX-500 series ICP-MS auto-sampler. System control and operation were facilitated via the vendor's MassHunter 4.1 software platform (Version C.01.01) as installed (Build 423.16) on an HP (Palo Alto, CA; USA) Z230 Workstation. Calibration of the ICP-MS against prepared Cs solutions consistently employed six points of response

measurement and resultant linearity values were frequently better than 0.9999. Additionally, blank and continuing calibration verification measurements were routinely interspersed throughout measurements of collected samples to ensure proper system functioning after initial startup.

ICP-MS system tuning was performed each day prior to batch measurements which employed a multi-element tuning solution (10 µg/mL Ce, Co, Li, Tl, and Y in 2% nitric acid) from Agilent Technologies (Santa Clara, CA; USA). System pulse-to-analog gain calibration, also performed prior to all batch analyses, was likewise accomplished using a multi-element solution from High Purity Standards in 2% nitric acid, with trace HF. The instrument would warm-up for ~ 20 min prior to all tuning and calibration efforts.

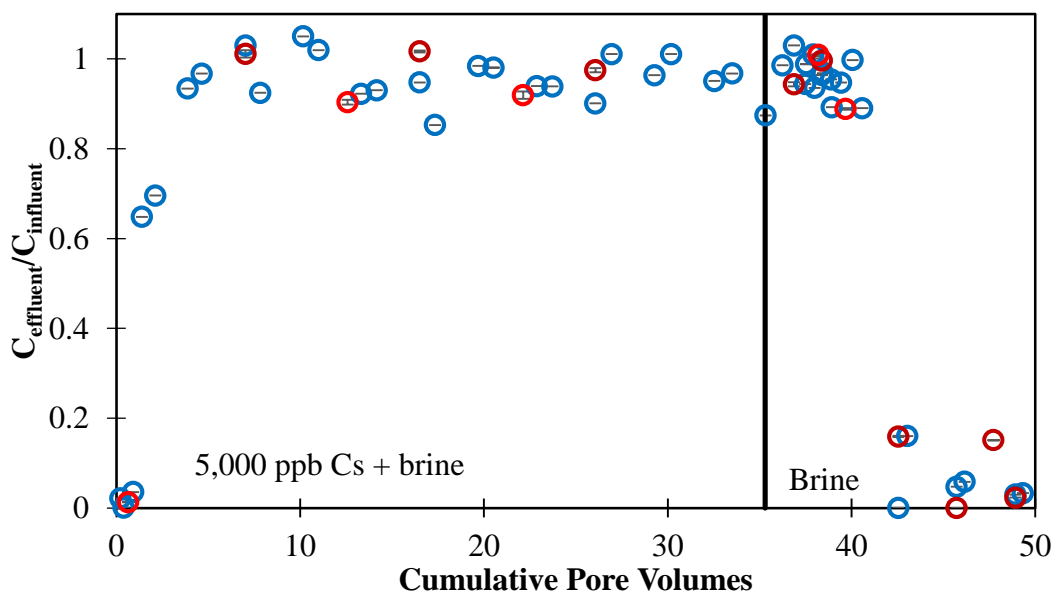
Prior to sample preparation, a large-volume indium solution was prepared at 300 ppb within a 2% nitric acid matrix to drive final sample dilution and preparation of calibration solutions. This internal standard solution was typically made in either 0.5 L or 1 L batch sizes depending on the expected number of samples to be collected in the associated experimental effort. Samples intended for Cs quantitation were diluted 1:18 (final volume 1.8 mL) with this solution before introduction to the ICP-MS system. Similarly, all Cs calibration solutions, ranging 0.1 ppb to 100 ppb throughout this effort, were prepared fresh prior to batch measurements at identical [In] employing the same internal standard solution used in the preparation of collected samples. This approach of pairing the internal standard solution used in the preparation of calibrants and samples was employed rigorously throughout this work. All solutions/samples were prepared and measured in short order, minimizing potential impacts from evaporative losses. The approximate limit of detection (LOD) across all analyses was 1.5 ppb.

## 4. RESULTS AND ANALYSIS

The results and analysis are still ongoing for all results presented in this work. Therefore, all discussion below is considered preliminary.

### *Miniature Column Results*

Cesium traveled through the dolomite columns without interacting with the solid phase in the absence of *Chromohalobacter* as shown in Figure 10. These results demonstrate that Cs did not adsorb onto the surface of the dolomite mineral and traveled unretarded with the water acting similar to a tracer.



**Figure 10.** Cesium recoveries are graphed with respect to cumulative pore volumes (one pore volume  $\sim 0.45$  mL) for initial injection of 5,000 ppb of Cs in 15% NaCl with 3 mM  $\text{NaHCO}_3$  at pH 8.3 into dolomite mini-column with a flow rate of 0.013 mL/min. Cs unfiltered recoveries are represented by (blue, open) markers while filtered is represented by (red, open) markers. Note: The solid line delineates the initial and secondary injections.

When Cs and *Chromohalobacter* were injected simultaneously (Figures 11-14), there was negligible interaction of Cs with both the microbes and dolomite. In previous research with stationary phase microbes, Cs did not interact with the microbes through surface adsorption or uptake (Zengotita *et al.*, 2017). However, there was a need to understand if Cs could be taken up into actively growing microbes searching for  $\text{K}^+$ . In the results in Figure 11, the microbial stock

was spiked with 5,000 ppb of Cs one hour prior to injection. However, filtered and unfiltered results overlap with each other suggesting that Cs is not associated with the microbes as they are larger than the 100k MWCO filter size. Cs also does not interact with *Chromohalobacter* when injected after Cs has been equilibrated with dolomite columns (Figure 12).

In Figures 13-14, *Chromohalobacter* was grown with Cs to consider if this might over-express the gene for  $K^+$  uptake. The final bio-colloidal column experiment (Figure 14) shows that there is no interaction of *Chromohalobacter* with Cs when grown with 5,000 ppb of Cs and then spiked additionally with 5,000 ppb Cs. Initially, the column results with *Chromohalobacter* grown with Cs were inconclusive due to the low concentrations of Cs, as Cs was lost during removal of GHB for injection into columns (Figure 13). Figure 13 could also suggest that the 5,000 ppb of Cs was too low of a concentration after washing of microbes for detection which resulted in subtle changes in the data. Therefore, an additional spike of Cs was added to observe whether or not there was any change. After growing the bacteria in the presence of Cs and adding more Cs, the genes for uptake of K/Cs could potentially be over-expressed. Like previous experiments, Cs did not interact with the microbes.

An important aspect is the low microbe recoveries (Figures 11-14) as they are lower than previous results with stationary phase *Chromohalobacter*. These data suggest that there may have been a loss of actively-growing microbes in the system, potentially due to entrapment and clogging in pores or attachment to the dolomite particle surfaces. Previous microbial recoveries found in Zengotita *et al.*, 2017 were around 80% to 100% break through vs the 20-40% to 80% breakthrough in these experiments. Thus, it could suggest that the microbial recoveries are greater in the stationary phase experiments. Additional work is ongoing to explain these results. However, we suggest that the actively growing bacteria are slightly larger than the stationary phase and, therefore, may be more likely to be trapped within the column.

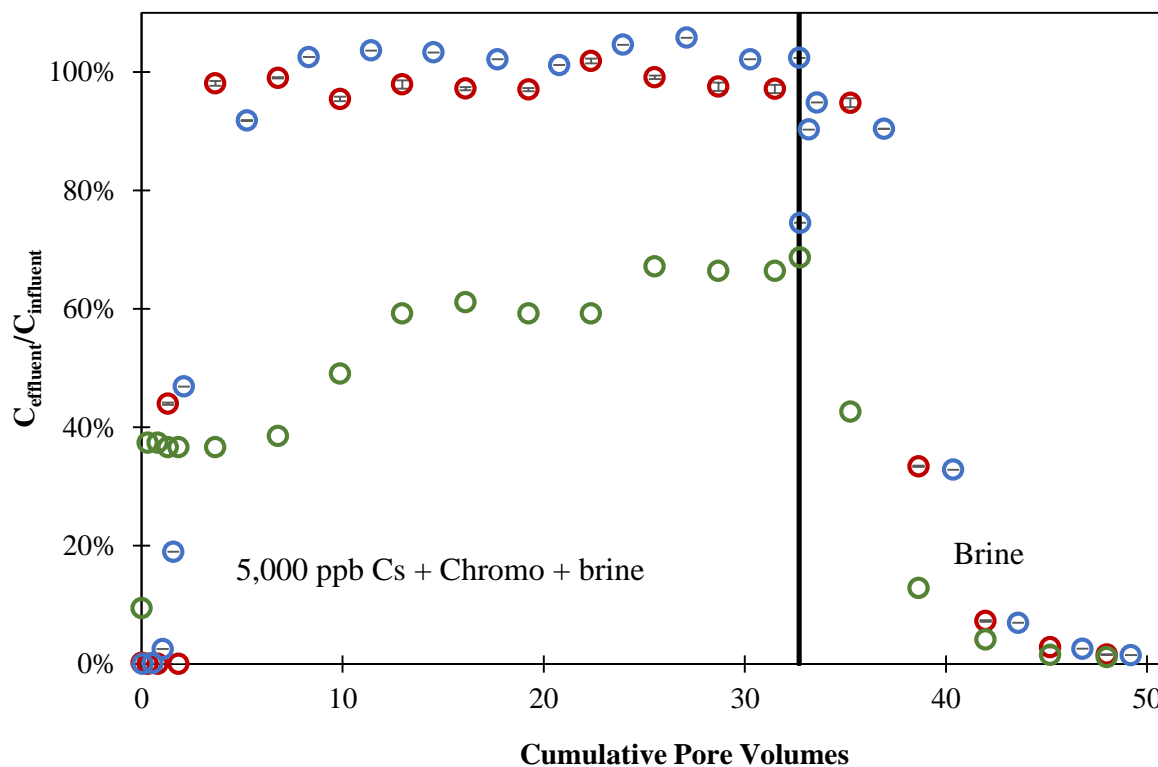


Figure 11. Cesium recoveries are graphed with respect to cumulative pore volumes (one pore volume ~ 0.43 mL) for initial injection of 5,000 ppb of Cs spiked in 15% NaCl with 3 mM  $\text{NaHCO}_3$  + *Chromohalobacter* solution at pH 8.30 into dolomite mini-column with a flow rate of 0.013 mL/min. Cs unfiltered recoveries are represented by (blue, open) markers while filtered is represented by (red, open) markers. Note: This aqueous solution was equilibrated one hour prior to injection and the solid line delineates the initial and secondary injections.

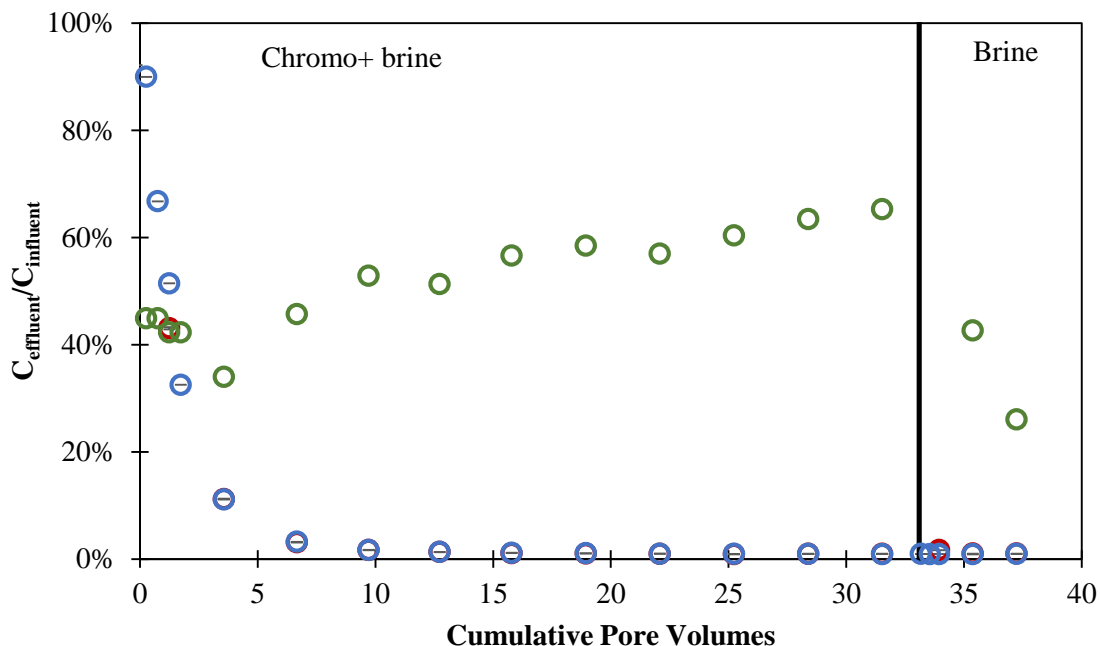


Figure 12. Cesium recoveries are graphed with respect to cumulative pore volumes (one pore volume  $\sim 0.42$  mL) for initial injection of 15% NaCl with 3 mM  $\text{NaHCO}_3$  + *Chromohalobacter* solution at pH 8.04 into dolomite mini-column with a flow rate of 0.013 mL/min. Cs unfiltered recoveries are represented by (blue, open) markers while filtered is represented by (red, open) markers. Note: The dolomite was saturated with 5,000 ppb Cs prior to injection.

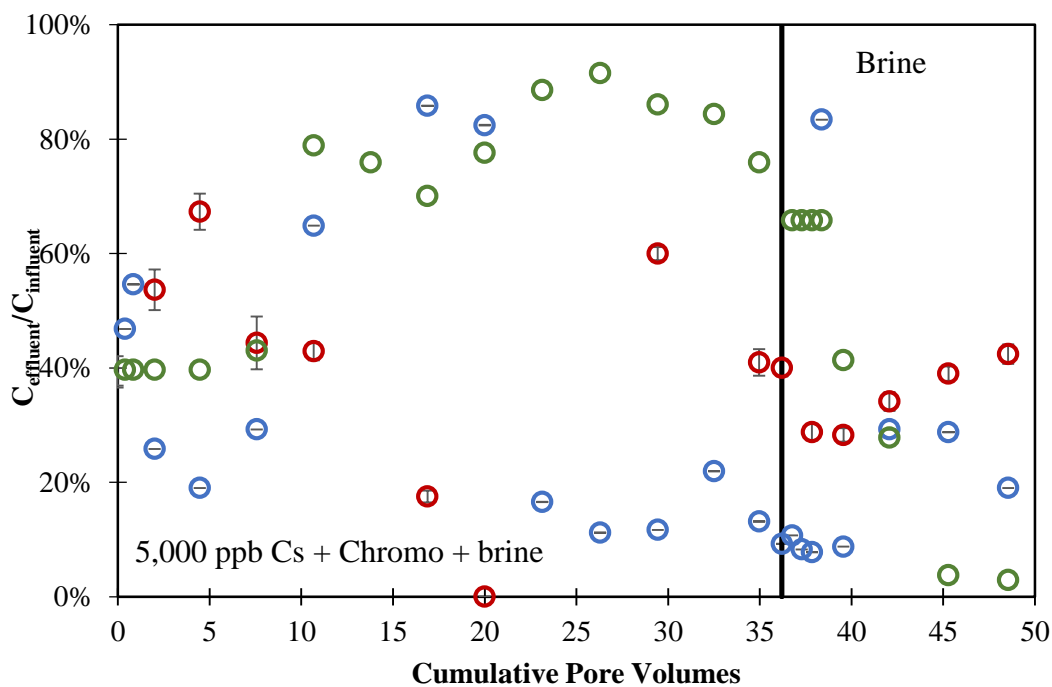


Figure 13. Cesium recoveries are graphed with respect to cumulative pore volumes (one pore volume  $\sim 0.43$  mL) for initial injection of *Chromohalobacter* grown with 5,000 ppb of Cs in 15% NaCl with 3 mM  $\text{NaHCO}_3$  solution at pH 8.05 into dolomite mini-column with a flow rate of 0.013 mL/min. Cs unfiltered recoveries are represented by (blue, open) markers while filtered is represented by (red, open) markers.

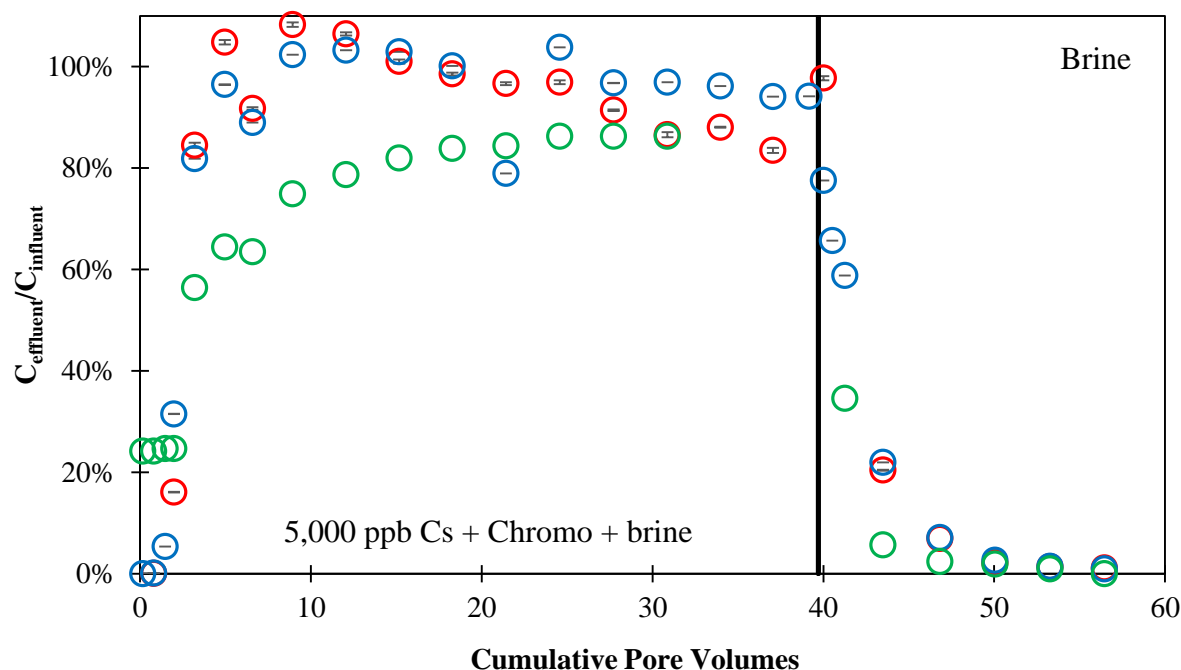


Figure 14. Cesium recoveries are graphed with respect to cumulative pore volumes (one pore volume  $\sim 0.42$  mL) for initial injection of *Chromohalobacter* grown with 5,000 ppb of Cs, spiked additionally with 5,000 ppb Cs in 15% NaCl with 3 mM  $\text{NaHCO}_3$  solution at pH 8.27 into dolomite mini-column with a flow rate of 0.013 mL/min. Cs unfiltered recoveries are represented by (blue, open) markers while filtered is represented by (red, open) markers.

#### Growth Kinetics Experiment Results

The results from batch growth experiments show that the unfiltered and filtered Cs concentrations overlap. The growing population of *Chromohalobacter* was monitored based on an increasing absorbance on the spectrometer. Growth began to decrease after 32 hours as shown by a decline in absorbance. This trend is relatively constant over time which implies that no Cs uptake is occurring. These results imply that despite active growing conditions, there is no Cs uptake; however, there was significantly more  $\text{K}^+$  present in this system due to GHB. Cs, therefore, may not have been taken up under these conditions. In the presence of greater  $\text{K}^+$  concentrations these results suggest that the *Chromohalobacter* selectively uptakes the  $\text{K}^+$  instead of  $\text{Cs}^+$ . This is confirmed in previous results in the column experiments, which implies that Cs does not interact with the microbes. However, these results may have been different if the growth broth had been depleted in  $\text{K}^+$ .

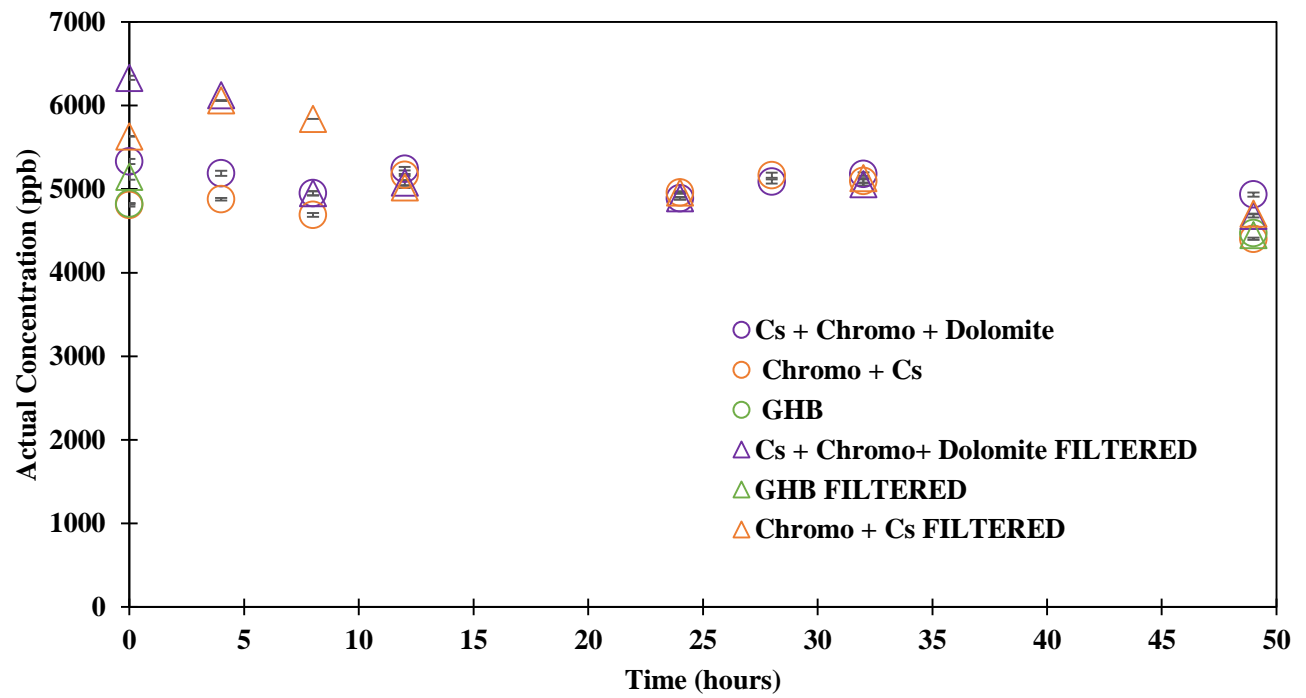


Figure 15. Growth Batch results that track Cs uptake over time. Note: The triangle markers represent filtered samples vs circles which represent unfiltered. Each marker represents an individual series with error bars representative of one standard deviation of triplicate analysis on ICP-MS.



## 5. CONCLUSION

Data analysis is still ongoing for batch experiments. However, preliminary conclusions can be drawn. In the column and growth batch experiments, Cs was not taken up or mobilized by *Chromohalobacter* despite being exposed to Cs for variable periods during and after growth. The Cs recoveries in the column based on the initial injection of *Chromohalobacter* were near 100% within just a couple of pore volumes, implying minimal interaction. In future experiments, representative batch and growth experiments would be conducted which would monitor the uptake of Cs, dissolution and mineralization of dolomite mediated by microbes. Further,  $K^+$  depleted media would be utilized in future experiments since the  $Cs/K^+$  ratio in the media may affect the potential for uptake.

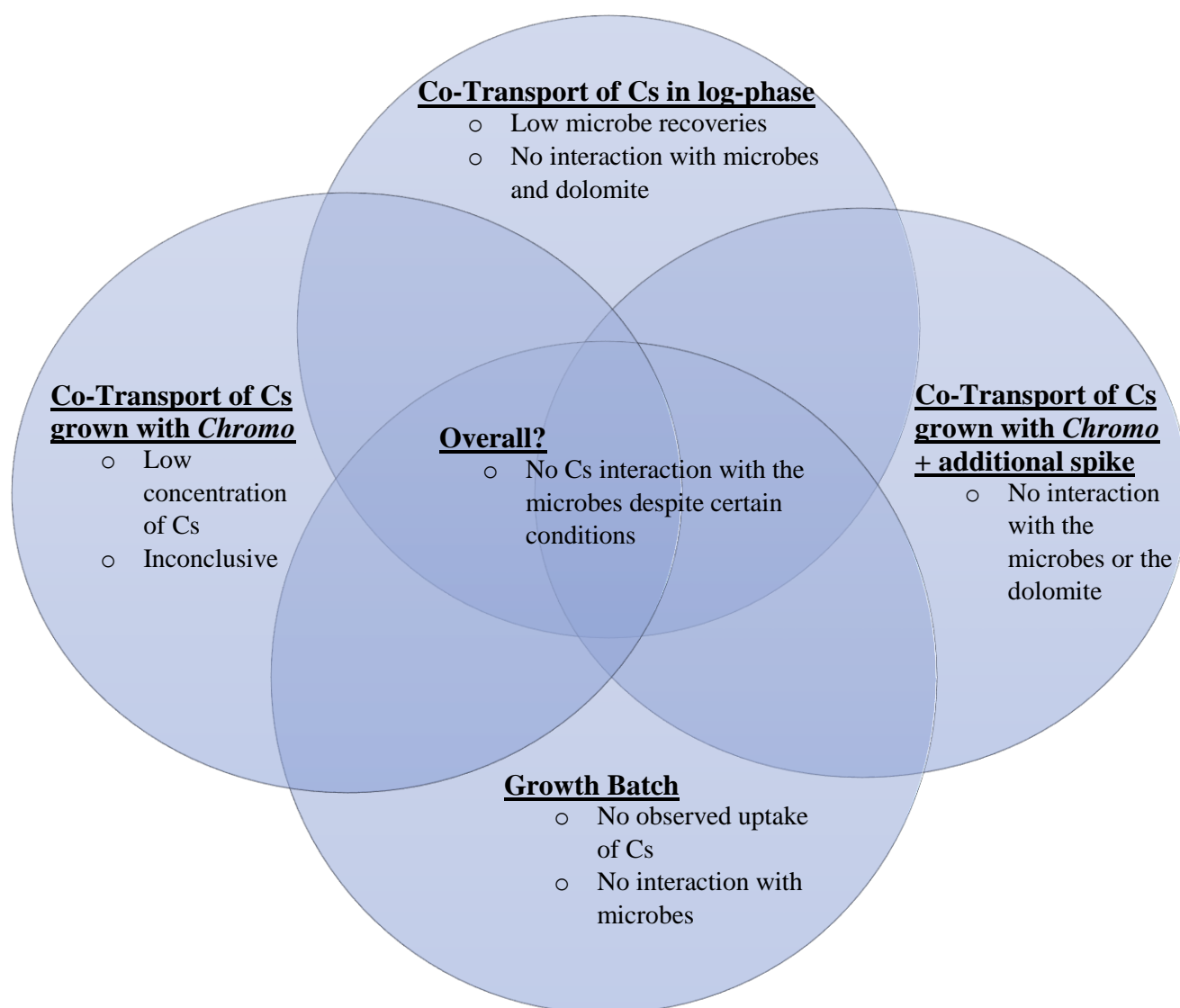


Figure 16. Comparison of overall results for experiments.

## 6. REFERENCES

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1. Adachi, K., Kajino, M., Zaizen, Y., & Igarashi, Y. (2013). Emission of spherical cesium-bearing particles from an early stage of the Fukushima nuclear accident. *Scientific reports*, 3, 2554.
2. Ams, D.A., Swanson, J.S., Szymanowski, J.E.S., Fein, J.B., Richmann, M., Reed, D.T., The effect of high ionic strength on neptunium (V) adsorption to a halophilic bacterium, *Geochimica et Cosmochimica Acta*, Volume 110, 2013, Pages 45-57, ISSN 0016-7037, <https://doi.org/10.1016/j.gca.2013.01.024>.
3. Ashraf Labib, M.J. Harris, Learning how to learn from failures: The Fukushima nuclear disaster, *Engineering Failure Analysis*, Volume 47, Part A, 2015, Pages 117-128, ISSN 1350-6307, <https://doi.org/10.1016/j.engfailanal.2014.10.002>.
4. Avery, S.V. (1995b) Cesium accumulation by microorganisms: uptake mechanisms, cation competition, compartmentalization and toxicity. *J Industrial Microbiology*, 14, 76-84.
5. Burger, A., Lichtscheidl, I., Stable and radioactive cesium: A review about distribution in the environment, uptake and translocation in plants, plant reactions and plants' potential for bioremediation, *Science of The Total Environment*, Volume 618, 2018, Pages 1459-1485, ISSN 0048-9697, <https://doi.org/10.1016/j.scitotenv.2017.09.298>.
6. Chen, G., Flury, M., Harsh, J. B., & Lichtner, P. C. (2005). Colloid-facilitated transport of cesium in variably saturated Hanford sediments. *Environmental science & technology*, 39(10), 3435-3442.
7. Chegbeleh, L.P., Nishigaki, M., Akudago, J.A., Alim, A., Komatsu, M., 2008a. Concepts of repository and the functions of bentonite in repository environments: a state-of-the-art review. *Journal of the Faculty of Environmental Science and Technology*, Okayama University 13 (1), 1–5 March, 2008.
8. Chino, M., Nakayama, H., Nagai, H., Terada, H., Katata, G., Yamazawa, H., 2011. Preliminary estimation of release amounts of <sup>131</sup>I and <sup>137</sup>Cs accidentally discharged from the Fukushima Daiichi Nuclear Power Plant into the atmosphere. *J. Nucl. Sci. Technol.* 48, 1129–1134.
9. Dittrich, T.M., Ware, S.D., Reimus, P.W. (2016) Mini-columns for Conducting Breakthrough Experiments: Design and Construction, in: *Technologies*, U.S. DOE. Los Alamos National Laboratory, Los Alamos, NM. LA-UR-15-24392
10. Emerson, H., Zengotita, F., Richmann, M., Katsenovich, Y., Reed, D.T., Dittrich, T., (2018) Retention of neodymium by dolomite at variable ionic strength as probed by batch and column experiments. *Journal of Environmental Radioactivity*. Miami, Florida
11. Flury, M., Czigány, S., Chen, G. and Harsh, J.B., 2004. Cesium migration in saturated silica sand and Hanford sediments as impacted by ionic strength. *Journal of contaminant hydrology*, 71(1-4), pp.111-126.
12. Kameník, J., Dulaiova, H., Buesseler, K. O., Pike, S. M., & Št'astná, K. (2013). Cesium-134 and 137 activities in the central North Pacific Ocean after the Fukushima Dai-ichi Nuclear Power Plant accident. *Biogeosciences*, 10(9), 6045-6052.
13. Kubo, A., Tanabe, K., Suzuki G., Ito, Y., Ishimaru, T., Kasamatsu-Takasawa, N., Tsumune, D., Mizuno, T., Watanabe, Y.W., Arakawa, H., Kanda, J., Radioactive cesium concentrations in coastal suspended matter after the Fukushima nuclear accident, *Marine*

- Pollution Bulletin*, Volume 131, Part A, 2018, Pages 341-346, ISSN 0025-326X, <https://doi.org/10.1016/j.marpolbul.2018.04.042>.
14. Mashal K., Harsh, J.B, Flury, M., Felmy, A.R., and, and Zhao, H., Colloid Formation in Hanford Sediments Reacted with Simulated Tank Waste, *Environmental Science & Technology* **2004** 38 (21), 5750-5756 DOI: 10.1021/es0349709
  15. Nuclear Energy Agency, Crystalline Club, *OECD* (November, 2017) Retrieved from <https://www.oecd-neo.org/rwm/crystallineclub/>
  16. Saiers, J.E., Hornberger, G.M., 1996. Migration of  $^{137}\text{Cs}$  quartz sand: experimental results and modeling approaches. *J. Contam. Hydrol.* 22, 255 – 270
  17. Swanson, Juliet S., Cherkouk, Andrea, Arnold, Thuro, Meleshyn, Artur, & Reed, Donald T. (2016). *The Microbiology of Subsurface, Salt-Based Nuclear Waste Repositories: Using Microbial Ecology, Bioenergetics, and Projected Conditions to Help Predict Microbial Effects on Repository Performance*. LA-UR-16-28895, Los Alamos National Laboratory, Carlsbad, NM.
  18. Thakur, P., Source term estimation and the isotopic ratio of radioactive material released from the WIPP repository in New Mexico, USA, *Journal of Environmental Radioactivity*, Volume 151, Part 1, 2016, Pages 193-203, ISSN 0265-931X, <https://doi.org/10.1016/j.jenvrad.2015.10.009>.
  19. U.S. EPA. (2006). Groundwater at WIPP. *Air and Radiation*.
  20. Ventosa, A., Gutierrez, M. C., Garcia, M. T. & Ruiz-Berraquero, F. (1989) Classification of *Chromobacterium marismortui* in a new genus. *International Journal of Systematic Bacteriology*, 39, 382–386.
  21. Ward AL, GW Gee, and MD White. 1997. A comprehensive analysis of contaminant transport in the vadose zone beneath tank SX-109. PNL-11463. Pacific Northwest National Laboratory. Richland, WA.
  22. Waste Isolation Pilot Plant (2016). Annual Transuranic Waste Inventory Report. DOE/TRU-16-3425. Revision 0. [http://www.wipp.energy.gov/library/TRUwaste/DOE-TRU-16-3425\\_Rev\\_0\\_ATWIR-2016.pdf](http://www.wipp.energy.gov/library/TRUwaste/DOE-TRU-16-3425_Rev_0_ATWIR-2016.pdf)
  23. Zengotita, Frances, Emerson, Hilary Palmer, Dittrich, Timothy M., Swanson, Juliet S., & Reed, Donald T. *The Role of Chromohalobacter on Transport of Lanthanides and Cesium in the Dolomite Mineral System*.

