The Effect of Bicarbonate on Autunite Dissolution in the Presence of *Shewanella oneidensis* under Oxygen Restricted Conditions-16429

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ABSTRACT

Uranium is a key contaminant at many U.S. Department of Energy sites that served a leading role in the nation's defense for over 50 years. Uranium contamination of soil and groundwater is of great environmental concern due to the toxicological properties of the uranyl species. In an effort to decrease the concentration of soluble uranium, tripolyphosphate injections were identified as a feasible remediation strategy for sequestering uranium *in situ* in contaminated groundwater at the Hanford Site (WA). The introduction of sodium tripolyphosphate into uranium-bearing porous media results in the formation of uranyl phosphate minerals (autunite) of general formula $\{X_{1-2}[(UO_2)(PO_4)]_{2-1}\cdot nH_2O\}$, where X is a monovalent or divalent cation. The stability of the uranyl phosphate minerals in the subsurface is a critical factor since it determines the long-term effectiveness of the sodium tripolyphosphate injection as a remediation strategy.
The behavior of uranium and its mobility in the subsurface are affected by various factors such as the chemistry of porewater, groundwater and soil minerals, the presence of complex-forming ligands, and micro-organisms that thrive under these conditions. Bicarbonate presence in the aqueous phase facilitates uranium desorption from soil and sediments and promotes the release of U(VI) into the aqueous phase, thus increasing uranium mobility in natural waters. In neutral or basic pH conditions, uranium can readily complex with a wide variety of ligands such as carbonate and form soluble uranyl-carbonato complexes such as $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$. Bicarbonate complexes have been identified in contaminated pore water at the Hanford Site. Furthermore, the presence of bacteria can significantly affect uranium mobility since bacteria may bio-enhance the release of U (VI) from uranyl-phosphate minerals, thus liberating uranium in the aqueous phase in an effort to obtain phosphorous, a vital nutrient for their metabolism.
The Columbia River at the site exhibits large stage variations, causing fluctuations in the water table. These water table fluctuations and multiple rise-and-fall cycles in the river created an oxic-anoxic interface in this region. Previous assessments of Hanford sediment samples collected from this area noted a decline in cultivable aerobic bacteria and suggested the presence of facultative anaerobic bacteria. *Shewanella oneidensis MR1* is a common bacterial group in soils and *Shewanella* species have been found in the Hanford soil. *Shewanella oneidensis* is a facultative anaerobic species which also belongs to a group known as dissimilatory metal-reducing bacteria (DMRB), due to their ability to reduce metals as a part of their metabolic pathway. Therefore, understanding the role of *Shewanella* is a critical factor affecting the outcome of environmental remediation.

The objective of this research was to demonstrate the significance of bacteria–uranium interactions and investigate the effect of bicarbonate in the aqueous phase on Ca-autunite dissolution in the oxygen-restricted conditions. Sterile 100-mL glass mixed bioreactors with weighed amounts of ground Ca-autunite particles were used for initial experimentation. These autunite-containing bioreactors were injected with bacterial cells after the autunite equilibrated with the media solution amended with 0 mM, 3 mM 5 mM and 10 mM concentrations of bicarbonate. Additional abiotic samples were prepared without bacterial inoculation to provide a control for the biotic samples. The concentration of U(VI) determined in the aqueous phase was in direct correlation to the concentration of bicarbonate present in the solution. It was noted that an increase in bicarbonate concentration led to higher U(VI) concentration in the aqueous phase. A sharp decrease in U(VI) concentration was observed in the bicarbonate-free samples, probably due to a microbe mediated reduction of U(VI) to U(IV). In the case of samples amended with 3 mM bicarbonate, a slight, yet significant, decrease in U(VI) was also observed compared to the abiotic control. However, compared to the abiotic controls, there was no change in U(VI) levels in the aqueous phase as a result of bacterial inoculation for the samples amended with 5 and 10 mM bicarbonate for a period of time as long as 50 days. These results imply that the uranyl-carbonate complexes ($UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$) that are present in carbonate-rich environments might be interfering with the microbial reduction in the oxygen- restricted conditions; however, this conclusion requires further investigation. Finally, the data indicate that release of U, P and Ca from autunite was non-stoichiometric.
In conclusion, the high stability of uranyl-phosphate complexes makes them a strong candidate for the remediation efforts to sequester uranium in the subsurface. Nevertheless, in a bicarbonate-rich environment, Ca-autunite has a high probability of dissolution and the presence of metal-reducing bacteria does not seem to impede this process in the presence of higher bicarbonate concentrations under oxygen restricted conditions.

INTRODUCTION

Uranium is a key contaminant at many U.S. Department of Energy sites that served a leading role in the nation's defense for over 50 years. Uranium contamination of soil and groundwater is of great environmental concern due to the toxicological properties of the uranyl species. The behavior of uranium and its mobility in the subsurface are affected by various factors such as the chemistry of porewater, groundwater and soil minerals, the presence of complex-forming ligands, and micro-organisms that thrive under these conditions. Uranium exists in four oxidation states but, under oxidizing conditions, it dominates as a highly soluble and stable uranyl ion, UO_2^{2+}. In neutral or basic pH conditions, uranium undergoes hydrolysis in aqueous solutions and can readily complex with a wide variety of ligands such as carbonate, nitrate and phosphate. In a bicarbonate-rich environment, carbonate anions are an important complexing agent for U(VI), leading to the formation of negatively charged soluble uranyl-carbonate complexes such as $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$, as well as neutral complexes such as $UO_2CO_3^0$ [1]. The presence of carbonates in the aqueous phase facilitates uranium desorption from soil and sediments and promotes the release of U(VI) into the

aqueous phase, thus increasing uranium mobility in natural waters [2]. Uranyl-bicarbonate complexes have been identified in contaminated pore water and groundwater at the Hanford Site, Washington State, [3, 4].

In an effort to decrease the concentration of soluble uranium, tripolyphosphate injections were identified as a possible remedial alternative for sequestering uranium *in situ* in contaminated groundwater at the Hanford Site (WA). The introduction of sodium tripolyphosphate into uranium-bearing porous media results in the formation of uranyl phosphate minerals (autunite) of general formula $\{X_{1-2}[(UO_2)(PO_4)]_{2-1}\cdot nH_2O\}$, where X is a monovalent or divalent cation. The stability of the uranyl phosphate minerals in the subsurface is a critical factor since it determines the long-term effectiveness of the sodium tripolyphosphate injection as a remediation strategy.

The presence of soil bacteria can significantly affect uranium mobility since bacteria may bio-enhance the release of U (VI) from uranyl-phosphate minerals, thus liberating uranium in the aqueous phase in an effort to obtain phosphorous, a vital nutrient for their metabolism. In addition to the biological activity, the presence of bicarbonate ions seems to enhance the release of U(VI) into the aqueous phase [5]. Natural systems are complex and their behavior is dictated by the synergistic and/or antagonistic effect of both biotic and physico-chemical factors.

The Columbia River at the site exhibits large stage variations, causing fluctuations in the water table. These water table fluctuations and multiple rise-and-fall cycles in the river created an oxic-anoxic interface in this region. Previous assessments of Hanford sediment samples collected from this area noted a decline in cultivable aerobic bacteria and suggested the presence of facultative anaerobic bacteria [6]. *Shewanella oneidensis MR1* is a common bacterial group in soils and *Shewanella* species have been found in the Hanford soil [6, 7]. *Shewanella oneidensis* is a facultative anaerobic species and due to their ability to reduce metals as a part of their metabolic pathway, it's also belongs to a group known as dissimilatory metal-reducing bacteria (DMRB). Therefore, understanding the role of facultative and anaerobic bacteria (e.g., *Shewanella*) as one of the factors affecting the stability of autunite solids is very important for designing a successful environmental remediation strategy.

The objective of this research is to investigate the effect of bicarbonate on Ca-autunite biodissolution in the oxygen-restricted conditions by focusing on the bacterial strains of *Shewanella oneidensis* sp. MR1. There have been a few studies on the microbial dissolution of autunite in the anaerobic conditions examining a dissimilatory metal-reducing bacteria (DMRB) (*Shewanella putrefaciens* 200R) [8] and *Shewanella oneidensis* MR1 [9, 10]. Previous experiments with aerobic *Arthrobacter oxydans* strains illustrated a bio-enhanced release of U(VI) from natural Ca-autunite in the presence of various concentrations of bicarbonate. *Arthrobacter strains*, G968 and G975, that exhibited various degrees of tolerance to U(VI) toxicity, were able to bio-enhance the release of U(VI) from natural Ca-autunite at almost the same capacity [11]. Previous research by Bencheikh-Latmani and Leckie [12] and Katsenovich et al. [13] has also suggested that uranyl-carbonate complexes formed in the solution do not strongly interact with the negatively charged bacterial surface, which in turn can mitigate U(VI) toxicity on cells.

MATERIALS AND METHODS

Bicarbonate media solution preparation

The media solution was prepared in 1 L of DIW buffered with 0.02M Na-Hepes buffer with pH adjusted to 7.1 with 0.1 mol/L HCl or NaOH. Sodium lactate ($C_3H_5NaO_3$, 60% w/w) was added to the solution with a concentration of 0.024 mol/L. The solution was divided into four 250-mL bottles and sterilized by autoclaving at 121°C, 15 psi for 15 min and cooled at room temperature. As the experiment is based on the investigation of bacteria interactions in the presence of different bicarbonate concentrations, potassium bicarbonate salt was added to the autoclaved bottles to obtain one bottle each of 3 mM, 5mM and 10 mM bicarbonate; the remaining bottle was kept bicarbonate free. This accounts for a total of four concentrations of bicarbonate for the experiment tested. Next, the solutions were filter-sterilized into other sterile 250-mL bottles. Finally, the sterile bottles were stored in the anaerobic chamber until the beginning of the experiment.

Shewanella oneidensis MR1 culture growth conditions

Shewanella oneidensis MR1 strain was obtained from the Pacific Northwest National Laboratory (PNNL). Fresh culture was grown on sterile hard and liquid Luria-Bertani (LB) media prepared with 10.0 g of tryptone, 5.0 g of yeast extract, and 10.0 g of sodium chloride, with a pH of 7.0. Hard media required an addition of 15.0 g of agar. Then fresh culture was grown in 15-mL tubes placed in the incubator for two days at 30°C.

Bacterial cells were harvested in the late logarithmic phase of growth and cell density (cells/mL) was calculated with a glass hemocytometer (Fisher Scientific, Pittsburg, PA) to determine the concentration of a stock solution and estimate a desired volume (mL) of a bacterial suspension needed for the inoculation of each bottle. In addition, fresh bacterial culture was preserved in 25% glycerol at -80°C.

Sterile 100-mL serum bottles prepared in triplicate for each bicarbonate concentration tested were used as mixed bioreactors for experimentation. Each bottle contained 50 mL of sterile bicarbonate-bearing media buffered with 20 mM HEPES-Na and 90 mg of meta-autunite to provide a U(VI) concentration of 4.4 mmol/L. This concentration was used to compare results with previous data obtained in the autunite mineral dissolution experiments using *Arthrobacter* strains, G968 and G975 [11, 14] and in the research conducted by Liu et al. on the dissolution of Na-boltwoodite in anaerobic conditions by *Shewanella oneidensis* strain MR-1 [15]. After preparation, each bottle was crimp-sealed and left in the incubator shaker at 80 rpm with the temperature set at 20°C for the autunite equilibration with the bicarbonate-amended media solutions for about 30 days.

After autunite equilibration, when the dissolution was close to its solubility, the triplicate samples were inoculated with the desired volume of bacterial suspension to obtain an initial cell density of 10^6 cells/mL. In addition, abiotic control bottles were kept for each bicarbonate concentration and sampled in parallel with the experimental bottles. A total of 4 bottles per concentration with a final number of 16 bottles were prepared. All of the bottles were crimp-sealed to keep the cells under oxygen-restricted conditions and then placed in the incubator-shaker at 20°C.

To account for viable bacteria, a well-mixed homogeneous aliquot of 0.01 mL of the suspension from each biotic test bottle was uniformly spread on sterile LB-agar Petri plates. The inoculated plates were kept inverted in an incubator at 30°C. Viable microorganisms were calculated from the number of colony-forming units (CFU) found on a specific dilution. In addition, the agar plating was used to provide a quick visual check for contamination.

Sampling procedure and elemental analysis

Sterile 1-ml syringes (BD) were used to inject bacterial inoculum or extract liquid samples from the bottles. At certain time intervals dictated by the experimental schedule, aliquots of 0.2 mL were isolated from the supernatant solutions of the experimental and control bottles and filtered through 0.45 µm PTFE filters into a 20-mL scintillation vial for further uranium analysis by kinetic phosphorescence analysis (KPA-11, Chemchek Instruments Inc.) instrument. The presence of organic content in the solutions can interfere with KPA measurements; hence, samples collected during the experiments were pre-processed by wet ashing followed by dry ashing procedures. A modified ashing technique described by Ejnik et al. [16] was used for wet and dry ashing. Wet digestion was performed by the addition of 500 µL of concentrated nitric acid (HNO_3) and 500 µL of concentrated hydrogen peroxide (H_2O_2) to each vial; the vials were placed on a heating plate until full evaporation was achieved and a white solid residue was acquired. Occasionally, some samples turned yellow while ashing; 0.5 mL of peroxide was added to these samples and the process was continued until a white precipitate was obtained. The dry samples were placed in a furnace preheated to 450°C for 15 min and then allowed to cool at room temperature. Finally, precipitates obtained in the drying step were dissolved in 1 mL of 2 mol/L nitric acid and analyzed by means of the KPA instrument to determine uranium concentrations released into the aqueous phase as a function of time. In addition, calcium and phosphorous were determined by means of inductively coupled plasma – optical emission spectroscopy (ICP-OES 7300 Optima, Perkin Elmer) using calcium and phosphorous standards (Spex CertiPrep).
ANOVA statistics were used to examine the results on the release of U(VI) due to varying concentrations of bicarbonate ions. The significant levels were set at α= 0.05.

RESULTS AND DISCUSSION

Autunite dissolution experiments

The results of U(VI) concentration in the aqueous phase as a function of time for each condition studied (0, 3, 5 and 10 mM HCO_3^-) are presented in Fig. 1. Average values and relative standard deviations were calculated for the triplicate samples.

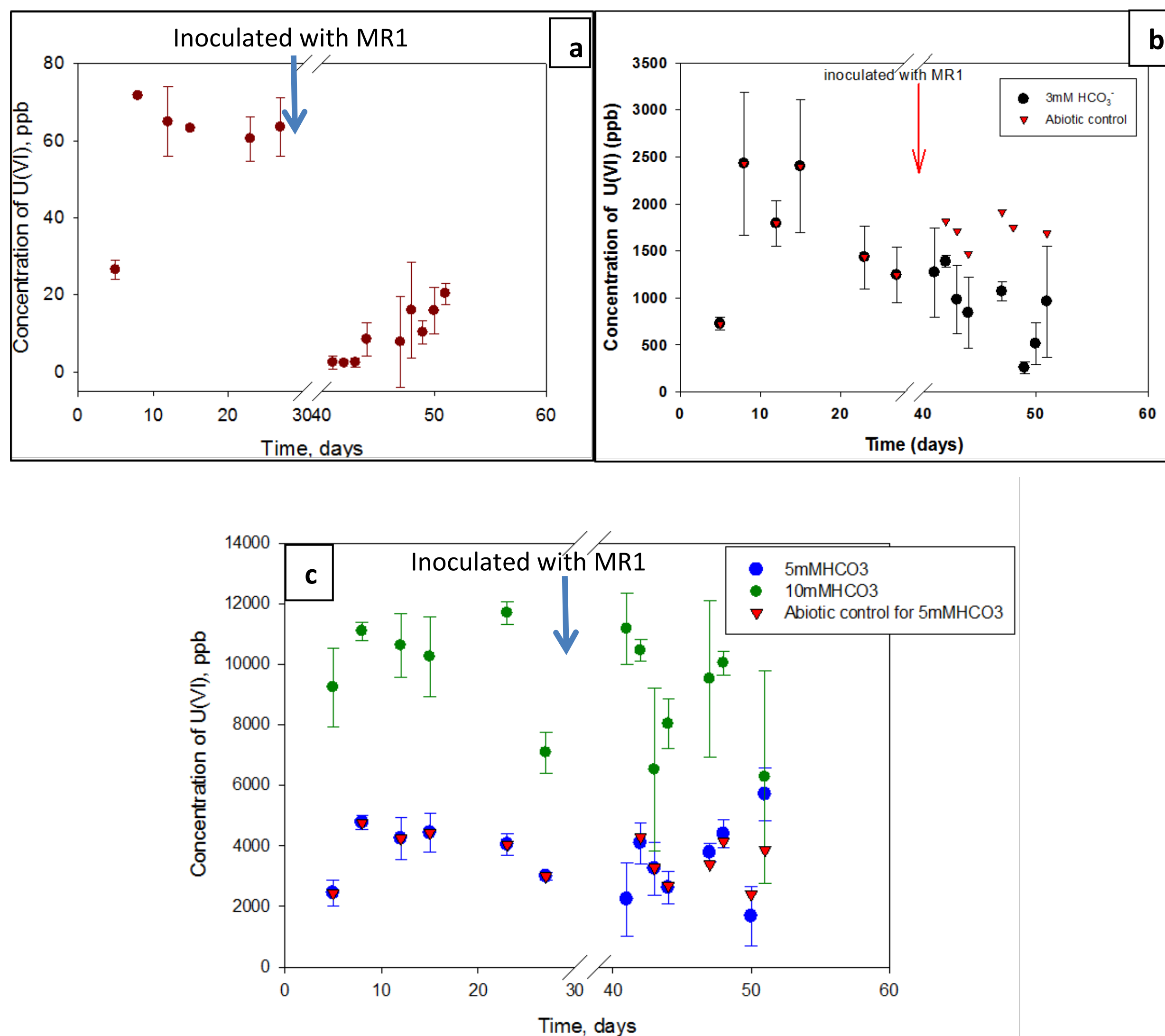

Fig.1. Uranium (VI) released in the aqueous phase as function of time: a) bicarbonate-free solution; b) 3 mM bicarbonate media solution, c) two different bicarbonate concentrations: 5 and 10 mM.

The data suggest that before the inoculation with bacteria, there was a significant amount of U(VI) released from autunite to the aqueous phase due to the presence of bicarbonate ions in the liquid media solution. Specifically, in the bicarbonate-free samples, the amount of U(VI) released into the supernatant was negligible; whereas, in samples amended with 3 mM, 5mM and 10 mM bicarbonate concentrations, U(VI) levels in the aqueous phase were found to be 2 ppm, 5 ppm and 10 ppm, respectively. These results are in agreement with the data analysis reported for the enhanced autunite dissolution in the presence of bicarbonates [5] and for the relatively fast extraction of uranium from contaminated soil [17].
The results of uranium monitoring in the aqueous phase after inoculation with bacteria are presented in Fig.1a for 0 mM bicarbonate, Fig.1b for 3 mM HCO3- and Fig.1c for 5 mM $HCO3^-$ and 10 mM $HCO3^-$. In the case of 0 mM bicarbonate, a sharp decrease in uranium concentration was observed (Fig.1a) in the first three days

after bacterial inoculation, making U(VI) difficult to detect due to very low, close to the detection limit, concentrations.

Results also showed that following the initial sharp decrease, U(VI) concentrations began to slowly rebound. This is probably due to the lack of organic substrate that was consumed faster in the presence of the remaining oxygen, dissolved and present in the headspace of the oxygen-restricted bioreactors.

In the case of the samples amended with 5 and 10 mM bicarbonate, the data revealed no change in the U(VI) concentration before and after bacterial inoculation. It is also clear that an increase in bicarbonate concentration leads to an increase in uranium release to the aqueous phase. This was expected since the uranium release from autunite is strongly influenced by the increase in bicarbonate concentrations, leading to the formation of aqueous uranyl bicarbonate complexes [5]. Bicarbonate can promote mineral dissolution by fast binding to the autunite surface, followed by a slow detachment of the U(VI) carbonate species from the surface into solution [18].

Results obtained by means of ICP-OES are presented in Fig.2a and Fig.2b for calcium and phosphorous, respectively.

Phosphorous is released to the aqueous phase from phosphate-bearing minerals through microbial dissolution. The primary mechanisms of P solubilization are H^+ excretion, production of low molecular weight organic acid, and acid phosphatase biosynthesis [19]. Furthermore, phosphorous is one of the major essential macronutrients for biological growth, playing a central role in the cellular activities for the synthesis of DNA, ATP, polyphosphates, and cell wall phospholipids [20]. In addition, speciation modeling conducted for the post-inoculation conditions predicts the formation of hydroxyl- apatite, which is a practically insoluble mineral. Therefore, a reduction in phosphorous levels after bacterial inoculation might have been expected. In addition, according to the statistical analysis by ANOVA, there is not a statistically significant difference (P=0.413) between phosphorus concentrations detected after the bioreactors' bacteria inoculation, justifying that the levels of phosphorous detected in the aqueous phase are similar for all different bicarbonate concentrations tested. Bacterial inoculation incurred a slight decrease in calcium concentration, noted for all of the bicarbonate-amended media. This decrease might be attributed to the formation of secondary minerals such as calcite and calcium phosphate. Similar to phosphorus, there is not a statistically significant difference (P=0.221) for calcium concentrations measured in the media solutions after bacteria inoculation for any of the bicarbonate concentrations tested.

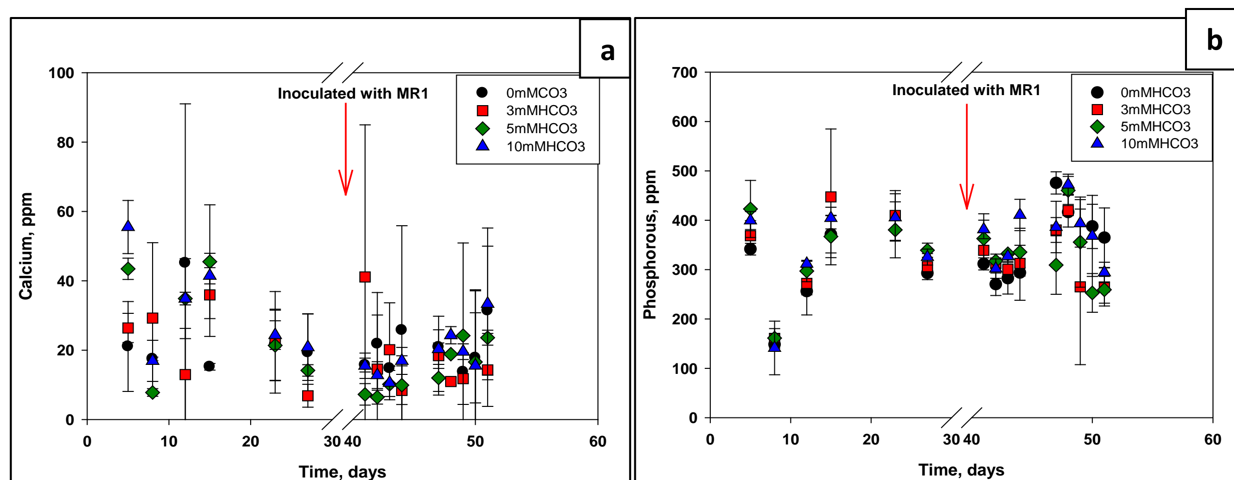

Fig.2. a) Concentration of calcium detected in the aqueous phase as a function of time in different bicarbonate concentrations: 0, 3, 5 and 10 mM; b) Concentration of phosphorous released in the aqueous phase as a function of time in different bicarbonate concentrations: 0, 3, 5 and 10 mM.

Agar plating

Agar plating was used to check for culture contamination with a visual inspection. For that, an aliquot of 0.01 mL was taken from each experimental bottle and spread on a sterile Petri dish containing LB growth media prepared with agar. At day 30, the experimental bottles were inoculated with facultative anaerobic bacteria; all control bottles were kept bacteria free. The sampling procedures were applied to all control and experimental bottles after inoculation with bacteria and uranium analysis was conducted on the collected samples via the KPA instrument.
Multiple sampling through septa resulted in contamination of some control bottles. Control samples were periodically prescreened under a light microscope and then examined by plating on the hard LB media. Abiotic control samples containing 0 mM and 10 mM of bicarbonate showed growth of microorganisms that suggest a contamination of the control bottles. However, the petri dishes inoculated from the control bottles amended with 3 mM and 5 mM of bicarbonate presented no evidence of bacterial contamination.
The experiment is currently being replicated following the same experimental procedures as described above with two notable differences: the introduction of sacrificial vials for each experimental point (as opposed to sampling periodically from the same batch sample) in order to eliminate cross-contamination.

CONCLUSIONS

The high stability of uranyl-phosphate complexes makes them a strong candidate for the remediation efforts to sequester uranium in the subsurface. However, in a presence of bicarbonate ions, Ca-autunite has a high probability of dissolution and the presence of facultative bacteria does not seem to impede this process under oxygen restricted conditions. Bacterial inoculation caused a slight decrease in calcium concentration, which might be attributed to the formation of secondary

minerals such as calcite and calcium phosphate. The statistical analysis by ANOVA suggested that there are not a statistically significant difference for phosphorus (P=0.413) and calcium (P=0.221) concentrations detected after the bioreactors' bacteria inoculation, justifying that the levels of phosphorous and calcium detected in the aqueous phase are similar for all different bicarbonate concentrations tested.

REFERENCES

1. Bachmaf, S., B. Planer-Freidrich, and B.J. Merkel, *Effect of sulfate, carbonate, and phosphate on the uranium(VI) sorption behavior onto bentonite.* Radiochimica Acta, 2008. **96**(6): p. 359-366.
2. Langmuir, D., *Uranium solution- mineral equilibria at low temperatures with application to sedimentary ore deposits.* Geochimica et Cosmochimica Acta, 1978. **42**: p. 547-569.
3. Bernhard, G., et al., *Uranyl(VI) carbonate complex formation: Validation of the Ca2UO2(CO3)3(aq.) species*, in *Radiochimica Acta International journal for chemical aspects of nuclear science and technology*. 2001. p. 511.
4. Brooks, S.C., et al., *Inhibition of Bacterial U(VI) Reduction by Calcium.* Environmental Science & Technology, 2003. **37**(9): p. 1850-1858.
5. Gudavalli, R.K.P., et al., *Comparison of the kinetic rate law parameters for the dissolution of natural and synthetic autunite in the presence of aqueous bicarbonate ions.* Chemical Geology, 2013. **351**: p. 299-309.
6. Lin, X., et al., *Distribution of Microbial Biomass and Potential for Anaerobic Respiration in Hanford Site 300 Area Subsurface Sediment.* Applied and Environmental Microbiology, 2012. **78**(3): p. 759-767.
7. Marshall, M., et al., *Hydrogenase- and outer membrane c-type cytochrome-facilitated reduction of technetium(VII) by Shewanella oneidensis MR-1.* Environmental Microbiology, 2008. **10**(1): p. 125-136.
8. Smeaton, C.M., et al., *Bacterially enhanced dissolution of meta-autunite.* American Mineralogist, 2008. **93**: p. 1858-1864.
9. Sheng, L. and J. Fein, *Uranium reduction by Shewanella oneidensis MR-1 as a function of $NaHCO_3$ concentration: surface complexation control of reduction kinetics.* Environmental Science & Technology, 2014. **1**(48): p. 3768-3775.
10. Sheng, L. and J. Fein, *Uranium adsorption by Shewanella oneidensis MR-1 as a function of dissolved inorganic carbon concentration.* Chemical Geology, 2013. **358**: p. 15-22.
11. Katsenovich, Y., et al., *Assessment of the Resistance to Uranium (VI) Exposure by Arthrobacter sp. Isolated from Hanford Site Soil.* Geomicrobiology Journal, 2012. **30**(2): p. 120-130.
12. Bencheikh-Latmani, R. and J.O. Leckie, *Association of uranyl with the cell wall of Pseudomonas fluorescens inhibits metabolism.* Geochimica et Cosmochimica Acta, 2003. **67**(21): p. 4057-4066.
13. Katsenovich, Y.P., et al., *Enhanced U(VI) release from autunite mineral by aerobic Arthrobacter sp. in the presence of aqueous bicarbonate.* Chemical Geology, 2012. **308–309**: p. 1-9.

14. Sepulveda-Medina, P.M., et al., *The effect of bicarbonate on the microbial dissolution of autunite mineral in the presence of gram-positive bacteria.* Journal of Environmental Radioactivity, 2015. **144**: p. 77-85.
15. Liu, C., et al., *Influence of Calcium on Microbial Reduction of Solid Phase Uranium(VI).* Biotechnology and Bioengineering, 2007. **97**(6): p. 1415-1422.
16. Ejnik, J.W., et al., *Optimal sample preparation conditions for the determination of uranium in biological samples by kinetic phosphorescence analysis (KPA).* Journal of Pharmaceutical and Biomedical Analysis, 2000. **24**(2): p. 227-235.
17. Mason, C.F.V., et al., *Carbonate Leaching of Uranium from Contaminated Soils.* Environmental Science & Technology, 1997. **31**(10): p. 2707-2711.
18. Gudavalli, R.K.P., et al., *Quantification of kinetic rate law parameters for the dissolution of sodium meta-autunite as a function of aqueous bicarbonate concentration.* Environmental Chemistry, 2013. **10**(6): p. 475-485.
19. Behera, B.C., et al., *Diversity, mechanism and biotechnology of phosphate solubilising microorganism in mangrove—A review.* Biocatalysis and Agricultural Biotechnology, 2014. **3**(2): p. 97-110.
20. Hirota, R., et al., *Bacterial phosphate metabolism and its application to phosphorus recovery and industrial bioprocesses.* Journal of Bioscience and Bioengineering, 2010. **109**(5): p. 423-432.

ACKNOWLEDGMENTS

Funding for this research was provided by U.S. DOE cooperative agreement DE-EM0000598.