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The effect of bicarbonate on the microbial dissolution of autunite mineral in the presence of gram-positive bacteria

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ABSTRACT

Bacteria are key players in the processes that govern fate and transport of contaminants. The uranium release from Na and Ca-autunite by *Arthrobacter oxydans* strain G968 was evaluated in the presence of bicarbonate ions. This bacterium was previously isolated from Hanford Site soil and in earlier pre-screening tests demonstrated low tolerance to U(VI) toxicity compared to other *A. oxydans* isolates. Experiments were conducted using glass serum bottles as mixed bioreactors and sterile 6-well cell culture plates with inserts separating bacteria cells from mineral solids. Reactors containing phosphorus-limiting media were amended with bicarbonate ranging between 0 and 10 mM and meta-autunite solids to provide a U(VI) concentration of 4.4 mmol/L. Results showed that in the presence of bicarbonate, *A. oxydans* G968 was able to enhance the release of U(VI) from Na and Ca autunite at the same capacity as other *A. oxydans* isolates with relatively high tolerance to U(VI). The effect of bacterial strains on autunite dissolution decreases as the concentration of bicarbonate increases. The results illustrate that direct interaction between the bacteria and the mineral is not necessary to result in U(VI) biorelease from autunite. The formation of secondary calcium-phosphate mineral phases on the surface of the mineral during the dissolution can ultimately reduce the natural autunite mineral contact area, which bacterial cells can access. This thereby reduces the concentration of uranium released into the solution. This study provides a better understanding of the interactions between meta-autunite and microbes in conditions mimicking arid and semiarid subsurface environments of western U.S.

1. Introduction

Uranium (VI) is a key contaminant of concern at many former U.S. Departments of Energy nuclear processing facilities and is well known as a health hazard for its toxicity and radioactivity. In neutral or basic pH conditions, uranium undergoes hydrolysis in aqueous solutions and can readily complex with a wide variety of ligands (Grenthe et al., 1992). Common ligands in the environment that form a diverse suite of aqueous uranyl complexes include: hydroxyl, phosphate, carbonate, silicate and organic substances (Davis, 2001). These complexation reactions often result in the formation of mobile aqueous species or precipitation of U-bearing minerals.

Soil properties, groundwater and pore water characteristics have a tremendous effect on uranium speciation and the formation of mineral phases (Sowder et al., 2001). Surveys of the uranium-contaminated areas suggested that carbonate and hydrogen carbonate (known as bicarbonate) are the major ions in the groundwater composition and the pore water is saturated with Ca and Mg-carbonates. Aqueous carbonate is considered one of the major variables affecting the dissolution of actinides from soil and sediments, thus increasing uranium mobility in natural waters (Clark et al., 1995; Zachara et al., 2007). In a bicarbonate-rich environment, carbonate anions are an important complexing agent for U(VI), forming highly soluble and stable uranyl dicarbonate $UO_2(CO_3)_2^{2-}$ and uranyl tricarbonate $UO_2(CO_3)_3^{4-}$ complexes, which

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became the predominant aqueous species at pH 7.0–8.0 (Langmuir, 1978; Bernhard et al., 2001; Guillaumont et al., 2003; Gorman-Lewis et al., 2008). In the presence of Ca ions, the mobile calcium uranyl carbonate species, $CaUO_2(CO_3)_3^{2-}$, influence the speciation of uranium (Clark et al., 1995; Kalmykov and Choppin, 2000; Bernhard et al., 2001).

Uranium readily forms strong and stable complexes with phosphate, promoting the formation of autunite group minerals, $X_{3-n}^{(n)+}[(UO_2)_2(PO_4)_2]\cdot xH_2O$, that can persist under intense weathering conditions over a long period of time (Giammar, 2001). One of the remediation strategies to mitigate the mobility of uranium contamination in the subsurface is an injection of sodium tripolyphosphate solutions in water-saturated sediments to form low solubility precipitates, autunite, $Na[(UO_2)(PO_4)]\cdot 3H_2O$ (Wellman et al., 2006, 2007). The autunite mineral group is very diverse and permits the substitution of a wide range of cations and varying degrees of hydration. In a calcium-rich soil, typical for arid and semi-arid environments of the western U.S, rapid ion-exchange reactions promote the replacement of sodium for calcium and the formation of calcium-autunite, $Ca[(UO_2)(PO4)]_2 \bullet 3(H_2O)$. In these reactions, two sodium cations are exchanged for the calcium ion to maintain the charge balance of the structure; the exchange is also associated with an increase in waters of hydration (Wellman et al., 2005). The solubility constant of the calcium form of autunite, log K_{sp}, has been measured as −48.36 with 2σ uncertainty values of ±0.03 (Gorman-Lewis et al., 2009). The associated solubility products are presented in eq. (1).

$$Ca(UO_2)_2(PO_4)_2\cdot 3H_2O = 3H_2O + Ca^{2+} + 2PO_4^{3-} + 2UO_2^{2+} \quad (1)$$

Autunite is a phosphorus-containing mineral; bacteria can liberate phosphorus, meeting their nutrient requirements and causing U mobilization back into the environment as a result of a breakdown of the mineral structure (Smeaton et al., 2008). The presence of indigenous bacterial populations that rapidly adapt to environmental conditions in soil and sediment can strongly influence the stability of uranyl phosphate phases. These bacteria can affect the dissolution and U(VI) complexation reactions due to the secretion of protons, various ligands and organic acids in their immediate habitats. In addition, bacterial cell walls, proteins and lipids contain functional groups such as carboxyl and phosphate, which are able to bind with uranium and are ultimately capable of affecting U(VI) mobility in aqueous systems (Fredrickson et al., 2004; Beazly et al., 2007). Therefore, understanding the role of bacteria on the stability of uranyl phosphates minerals is very important.

A number of studies have been carried out with a variety of the bacterial species in relation to their interaction with radionuclides in groundwater (Lloyd and Renshaw, 2005; Nedelkova et al., 2007). As previously described, the significance of bacteria–uranium interactions has been illustrated by focusing on three Gram-positive *Arthrobacter* species isolated from Hanford Site, which is the former the United State plutonium producing facilities, located along the Columbia River in eastern Washington State (Katsenovich et al., 2012b).

Arthrobacter is one of the most common groups of bacteria in soils and are found in large numbers in Hanford soil as well as other subsurface environments contaminated with radionuclides (Balkwill et al., 1997; Boylen, 1973; Van Waasbergen et al., 2000; Crocker et al., 2000). According to Balkwill et al. (1997), the microbial ecology of vadose zone sediments contaminated with high U(VI) at the U.S. DOE Hanford Site exhibits a high frequency of *Arthrobacter* species. In addition, *Arthrobacter*-like bacteria were the most prevalent in the highly radioactive sediment samples collected underneath leaking high-level waste storage tanks and accounted for about one-third of the total isolatable bacterial population from vadose zone soils (Fredrickson et al., 2004). Furthermore, a prior study conducted using the *Arthrobacter oxydans* strain G975 illustrated a bio-enhanced release of U(VI) from natural Ca-autunite in the presence of various concentrations of bicarbonate (Katsenovich et al., 2012a). The current research was performed to investigate the effect of *A. oxydans* strain G968 obtained from the SMCC on stability of the autunite mineral in carbonate-rich oxidized conditions typical for the western U.S. This strain was found to possess low resistance to U(VI) toxicity in bicarbonate-free media solutions (Sepúlveda et al., 2013).

The microbial effect of strains that possess low tolerance to U(VI) toxicity on the dissolution of U(VI)-bearing minerals has not been evaluated in publications. Additional research is necessary to understand the effect of bacterial cells with low resistance to U(VI) on the biodissolution of autunite in bicarbonate-amended media solutions mimicking subsurface conditions typical for arid and semi-arid environments of the western U.S. The objective of this research was (i) to evaluate the microbial dissolution of Na and Ca autunite in bicarbonate-amended solutions inoculated with *A. oxydans* strain G968, that possesses a low tolerance to U(VI) toxicity, (ii) to compare the results of autunite dissolution with data obtained in previous tests using uranium-resistant subsurface isolate *A. oxydans* strain G975, and (iii) to determine whether bacterial tolerance to uranium can affect autunite biodissolution in the presence of bicarbonate.

2. Materials and methods

2.1. Arthrobacter strains and culture conditions

Environmental sources and isolation of the Gram-positive aerobic *A. oxydans* strain G968 used for the research were described in recent publications (Katsenovich et al., 2012a,b). Identification of the *A. oxydans* strains was previously confirmed by 16S rDNA phylogeny (Balkwill et al., 1997; Van Waasbergen et al., 2000) and detailed descriptions of sample collection procedures and methods of bacterial isolation are available from Van Waasbergen et al. (2000). The bacterial culture was grown in 5% peptone, tryptone, yeast extract, glucose (PTYG) liquid culture media and agar plates consisting of 5 g/L peptone, 5 g/L tryptone, 10 g/L yeast extract, 10 g/L glucose, 0.6 g/L $MgSO_4\cdot 7H_2O$, and 0.07 g/L $CaCl_2\cdot 2H_2O$. The media was prepared in deionized water (DIW) (Barnstead NANOpure Diamond Life Science (UV/UF), Thermo Scientific), autoclaved at 121 °C and 15 psi for 15 min, then allowed to cool before being used. Bacterial cells were harvested in the late logarithmic phase of growth and washed; the cell stock suspensions were counted using a glass hemocytometer (Fisher Scientific, Pittsburgh, PA) or INCYTO C-Chip disposable hemocytometer (SKC America). Once the average cell count was obtained, it was multiplied by the dilution factor and the volume factor (10^4) in order to calculate the final concentration of cells per mL. The number of cells/mL in the stock suspension was used to calculate the desired volume of cell solution to inoculate the experimental bottles.

To account for viable bacteria, a well-mixed homogeneous aliquot (0.01–0.1 mL) of the suspension from each test vial was uniformly spread on the sterile Petri dishes containing a 5% PTYG growth media mixed with 15 g/L of agar. Inoculated plates were kept inverted in an incubator at 29 °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 and to maintain colonies from each stage of the enrichment for the duration of the experiment.