Investigation on Uranium Biosorption by DOE-Hanford Site Soil Isolates: Effects of Calcium and Bicarbonate

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Background

- The Hanford Site (1,500 km2) in Washington was a recipient of radiological contamination as a result of spills and accidents associated with nuclear fuel production during 1943-1989.
- Despite extensive remediation efforts, the uranium (U) groundwater plume still persists in multiple locations at Hanford, exceeding the 30ppb EPA standard.
- Uranium - plutonium extraction and enrichment processes at the 200 Area have resulted in the release of ~200,000 kg of uranium to the ground surface (Simpson et al. 2006). These releases of radioactive materials onto the ground surface have directly impacted the vadose zone by creating a wide variety of hazardous waste streams that contained chemical and radiological constituents with potential risk to receptors through water uptake from contaminated wells or discharge to surface water.
- The protection of water resources across the DOE complex from contaminated groundwater resulting from past, present, and future operations at the Hanford Site is a key element of the overall Hanford cleanup efforts.
- Different factors can affect the sorption kinetic rates and bioavailability of soluble and complexed uranium (i.e. hydroxyl and carbonate complexes) in the environment.
- Understanding these interactions and the role of microbes capable of withstanding the toxic concentrations of uranium in carbonate bearing aqueous environments, such as carbonate-free synthetic groundwater and carbonate-containing groundwater is important for modeling and predicting U transport in subsurface.

Objectives

- Evaluate the uranium (VI) bioaccumulation by *Arthrobacter* G975 sp. in synthetic ground water (SGW) (pH 7.35) with varying calcium & bicarbonate concentrations.
- Determine significant differences, if any, between SGW presence of calcium & bicarbonate treatments
- Characterize biosorption with a sorption model
- Evaluate and compare MINTEQ models with experimental results

Methodology

- The total cell density (cell/mL) was determined with a hemocytometer
- SGW composed of: 0.07 mM KCl, 2 mM HEPES, 0.21 mM $MgCl_2$, 0.6 mM $CaCl_2 \cdot 2H_20$
- Four SGW treatments with the following additions were evaluated: 5mM Ca^{2+} & 2.5mM $KHCO_3$; 5mM Ca^{2+} & 0mM $KHCO_3$; 0mM Ca^{2+} & 2.5mM $KHCO_3$; 0mM Ca^{2+} & 0mM $KHCO_3$
- MINTEQ models created using SGW components
- KPA-11 (Chemcheck Instruments, Richland, WA) measurements were 24 hours after cell inoculation to determine U(VI) sorption by *Arthrobacter* G975 sp.
- After 24 hrs, homogeneous sample were transferred to a sterile 50 ml tube with fresh media to a concentrations of about 100,000 cells/ml to influence growth in 24 hour period. Samples were then measured with UV spectrophotometer and TOC (Figure 5).

Preliminary Results

Figure 1. Effects of Ca & $KHCO_3$ in G975 U(VI) biosorption in SGW, 24 hrs, pH 7.35, 25°C,n=3

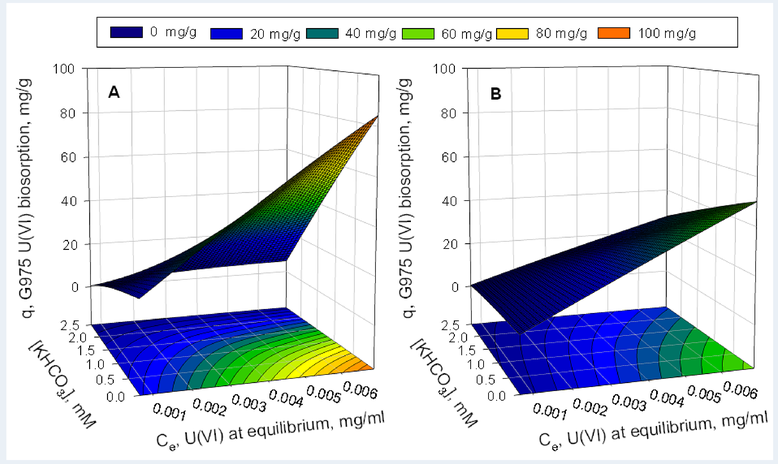


Figure 2. Effects of Ca in G975 U(VI) biosorption in SGW with 0mM added bicarbonate, 24 hrs, pH 7.35, 25°C,n=3

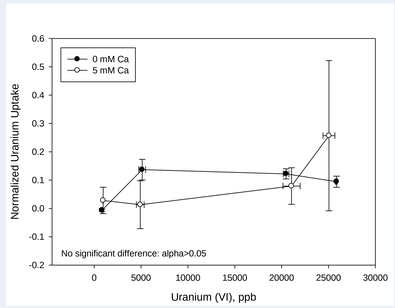


Figure 3. Effects of Ca in G975 U(VI) biosorption in SGW with 2.5mM added bicarbonate, 24 hrs, pH 7.35, 25°C,n=3

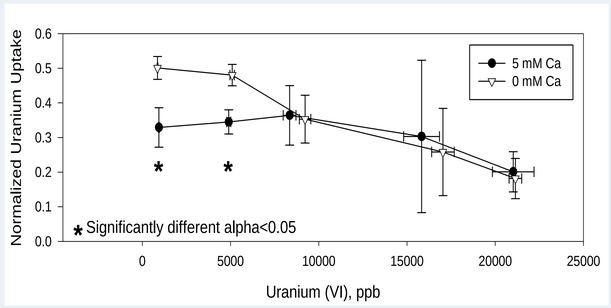


Figure 5. Cell growth 24 hours after treatment in 20ppm U(VI)

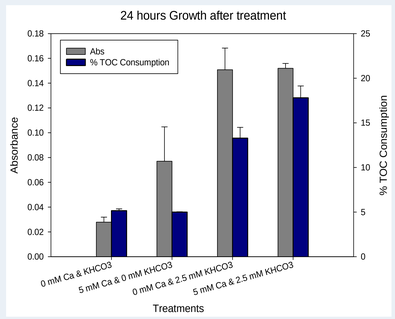
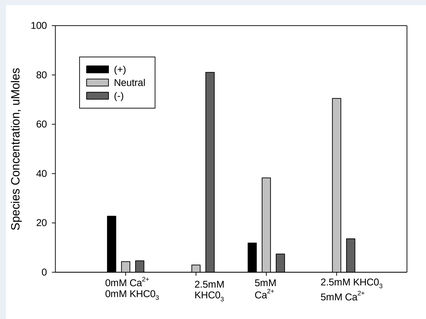


Figure 4. Total Charged Species at 30ppm U(VI)



Discussion

- The uranium uptake by *Arthrobacter* G975 sp. in Ca-free SGW(0 mM added bicarbonate, pH 7.35, 25°C, for 24 hrs) was significantly higher (P<0.05), by at least 14%, than samples containing 5mM Ca^{2+} when exposed to concentrations lower than 5ppm (Figure 1).
- Cell exposure to U(VI) concentrations higher than 10 ppm exhibited lower U(VI) uptake and no distinct difference (P>0.05) due to addition of 5mM Ca^{2+} (Figure 2).
- The uranium uptake of *Arthrobacter* G975 sp. in Ca-free SGW (2.5mM added bicarbonate, pH 7.35, 25°C, for 24 hrs) was not significantly higher (P>0.05) than samples containing 5mM Ca^{2+} when exposed to any uranium concentration within the constraints experimented, 1-40ppm U(VI) (Figure 3).
- In comparison to the treatment without bicarbonate (Figure 2) the treatment with bicarbonate (Figure 3) experiences lower uranium(VI) uptake throughout the U(VI) concentrations tested, 1-40ppm U(VI).
- In the absence of calcium and bicarbonate in SGW, MINTEQ models show positive species to be dominant: $(UO_2)_3(OH)^{5+}$ followed by $(UO_2)_4(OH)^{7+}$.
- In the presence of bicarbonate alone, the negative species $UO_2(CO_3)_2^{2-}$ prevails in the SGW treatment.
- In the presence of calcium alone, $Ca_2UO_2(CO3)_3$ (aq), a neutral species, increases the fastest, followed by positively charged species $(UO_2)_3(OH)^{5+}$.
- In the presence of both calcium and bicarbonate, MINTEQ model shows dominance of $CaUO_2(CO_3)_3^{2-}$, a negative species, and $Ca_2UO_2(CO3)_3$ a neutral species.
- Langmuir Isotherm provides the best fit for all SGW treatments.

Conclusions

- Addition of calcium to SGW containing 2.5mM $KHCO_3$ does not significantly alter the uptake of U(VI) by *Arthrobacter* G975 sp. (Figure 3).
- Based On MINTEQ models: Increasing the uranium concentration in solution yields more positive species in the absence of Ca^{2+} & $KHCO_3$, greater amounts of negative species in the presence of 2.5mM of $KHCO_3$ alone, more neutral species before 50 ppm U and more positive species thereafter in the presence of 5mM of Ca^{2+} alone, and mostly neutral species when both 5mM Ca^{2+} and 2.5mM of $KHCO_3$ are present in solution.
- Experimental results and MINTEQ models converge to show that it is possible for the high affinity between the (+) charged U(VI) species and (-) charged cell membrane of *Arthrobacter* G975 sp. account for the uranium sorption that is greatest in the absence of both Ca^{2+} and $KHCO_3$.
- The least amount of sorption occurs in the presence of bicarbonate, with or without calcium. Bicarbonate containing SGW solution will produce mostly negative and neutral charged species, which should not greatly interact with the negatively charged bacterial cell membrane. In the calcium & bicarbonate treatments, bicarbonate governs the biosorption of uranium by *Arthrobacter* G975 sp. (Figure 3)
- Experimental results converge with MINTEQ results to show highest cell viability in 5mM calcium and 2.5mM bicarbonate treatments. (Figure 5)

Future Experimentation

- Determine the cell viability of *Arthrobacter* G975 sp. in calcium & bicarbonate treatments at equivalent physiological conditions (pH 7.35, 25°C) with cell plates
- Justify results with MINTEQ models and tested results based on Figure 1 and Figure 2

References

[1] Simpson BC, RA Corbin, MJ Anderson, CT Kincaid, and JM Zachara. 2006. Identification and Classification of the Major Uranium Discharges and Unplanned Releases at the Hanford Site Using the Soil Inventory Model (SIM) Rev. 1 Results. NUV-06-21106-ES-001-DOC Rev. 1, Novotec, USA Inc., Cincinnati, Ohio

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