A Study of Uranium Biosorption by U.S. DOE Hanford Site Soil Isolates: Effect of pH and Carbonate

Denny Carvajal (Graduate Student, DOE Fellow)

Applied Research Center, Florida International University. dcarv006@fiu.edu

Background

The Hanford Site was a recipient of radiological contamination as a result of spills and accidents associated with nuclear fuel production during 1944-1980. Despite extensive remediation efforts, the groundwater plume still persists beneath the 300 Area and the concentration of U(VI) exceeds the EPA standard of 30ppb. Recently, the oligotrophic Hanford soil has been enriched with injections of a soluble sodium tripolyphosphate amendment, as a DOE remediation strategy designed to immobilize uranium by forming insoluble uranyl phosphate minerals. The injected polyphosphate undergoes hydrolysis in aqueous solutions to form orthophosphate, which serves as a nutrient and leads to growth of microorganisms that thrive under oligotrophic conditions [1]. However, there is limited information on bacterial-mineral interactions that may affect the stability of uranyl phosphates. Biosorption is a potential mechanism that affects mass transfer and the mobility of U(VI) in the subsurface. The focus of this study is to characterize the U(V) biosorption capabilities of *Arthrobacter* sp and determine if their increasing population can affect uranyl phosphate mineral behavior in the field. *Arthrobacter* sp., a genus of aerobic bacteria, accounts for about 25% of the microbial population in Hanford Site soil and sediments. They are also the most prevalent genera found underneath the leaking radionuclide storage tanks at Hanford [2].

Objectives

- Determine the maximum U(VI) concentration tolerable by *Arthrobacter* bacteria.
- Characterize cell morphology and mass/cell relationship.
- Conduct equilibrium biosorption experiments and evaluate the effect of environmental factors, such as pH and the presence of bicarbonate in the groundwater composition on U(VI) biosorption.

Experimental Approach

Arthrobacter strains, isolated from the Hanford subsurface, were obtained from the Subsurface Microbial Culture Collection (SMCC) (Florida State University). Strains were grown at 29°C under aerobic conditions in 3mL of 5% PTYG media amended with Hepes buffer to sustain pH in the 6.5-7 range. Bacterial tolerance was evaluated using different U(VI) concentrations in the range of 1-120ppm. The total cell density was determined with a hemocytometer, and viability was confirmed by colony forming units (CFU) using sterile petri dishes containing the 5% PTYG media mixed with 15g/L of agar. The weight-cell relationship was determined by weighing the oven-dried 0.2 micron filter paper used to collect the cells in a volume of known cell suspension.

Changes of the cell surface morphology were determined using a field emission scanning electron microscope (FE-SEM), EDS analysis, and atomic force microscope (AFM). Sample preparation procedures for SEM/EDS analysis included multiple treatment steps using glutaraldehyde. The cell surface composition was analyzed using a SEM-Energy-Dispersive-Spectrometry (SEM-EDS) Noran System Six Model 200 at a magnification of 1000.

Preliminary Results

Picture 1. G975 control sample in SEM magnified 5000 times.

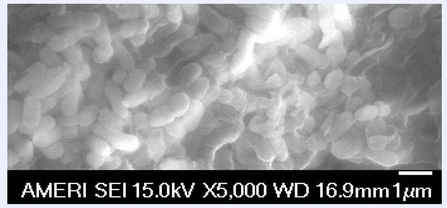


Table 1. EDS results for G975

Element	Weight % mean *Control*	*20ppm*	Element
Na	1.1	0.8	Na
O	10.4	10.3	O
P	1.3	1.1	P
U	0.0	0.4	U
K	0.4	0.8	Si
C	86.8	86.5	C
Total	100.0	100.0	Total

Picture 2. AFM images of G968 (Left to right: height image, phase image, zoomed in phase image). A) control sample; B) 1ppm U(VI) sample.

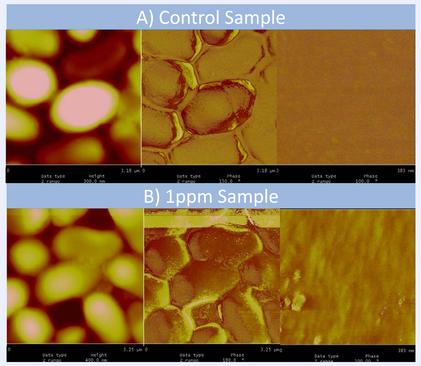


Figure E. Weight of *Arthrobacter* sp. Against cell number calculated via hemocytometer

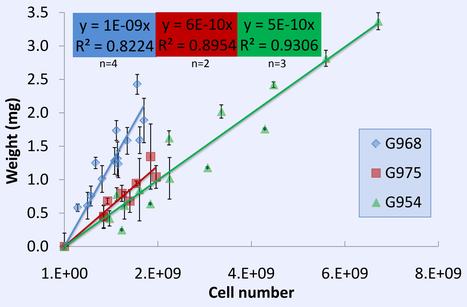


Figure A. Log Cells/mL growth of the three *Arthrobacter* strains over 7 days.

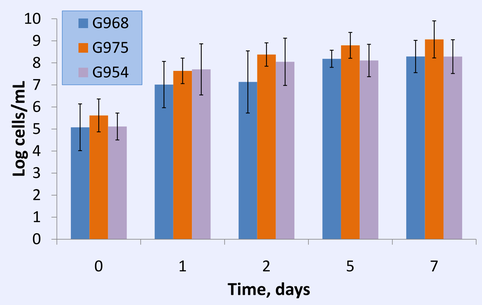


Figure B. Log Cells/mL growth of G954 over time with various concentrations of U(VI)

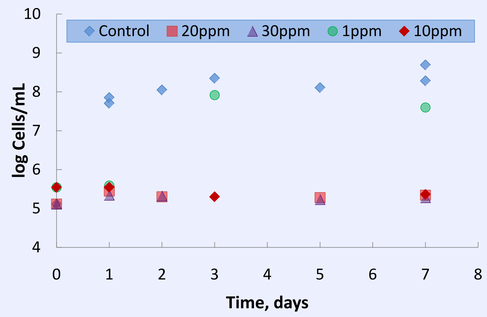


Figure C. Log Cells/mL growth of G968 over time with various concentrations of U(VI)

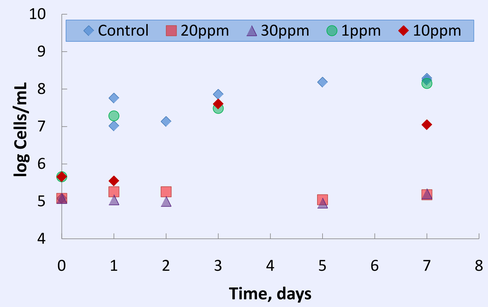
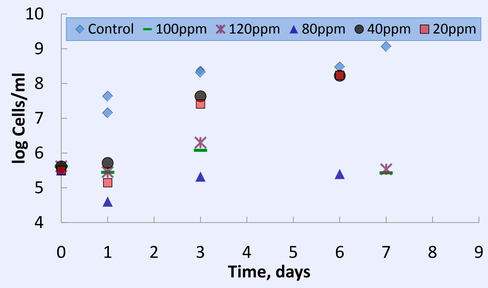


Figure D. Log Cells/mL growth of G975 over time with various concentrations of U(VI)



Discussion

Figure A shows the characteristic exponential growth of bacteria up to 7 days where growth plateaus. The hemocytometer method used to quantify growth is not sensitive enough to differentiate small differences among the strains. Figures B-D demonstrate bacterial uranium resistance to 1 ppm, 10 ppm and 100 ppm for G954, G958, and G975, respectively, verified by means of hemocytometer count, and colony forming units (data not shown). Their survival in high U(VI) concentration suggests that the much lower U(VI) concentrations found in the Hanford soil will not be a limiting growth factor. Figure E shows the non-identical weight/cell relationship for each strain mostly attributed to cell morphology. Picture 1 provides a FE-SEM image of the control for *Arthrobacter* strain G975 magnified 5000 times. The AFM micrograph of U(VI) containing bacterial sample shows precipitated uranium solids on bacterial surface confirmed by EDS analysis results (Table 1). Cells growing in media amended with 1 ppm of U(VI) (Picture 2B) have distinct surface morphological changes compared to the control (Picture 2A).

Conclusions

- Elemental analysis of bacterial cell surface confirmed the presence of uranium.
- Under normal conditions, cells have reached a stationary phase by day 5; to minimize cells division as a variable, and to maximize bacteria count and surface area, future biosorption experiments will use incubated bacteria at this growth stage.
- Studied strains have slight differences in morphology; their weight-cell correlation and abilities to tolerate the presence of U(VI) varied greatly.
- Based on our observations, we hypothesized that each strain will exhibit unique adsorption capabilities; these may single out a specific strain potentially affecting the stability of U(VI)-bearing precipitates.

Future Work

- Conduct biosorption experiments and evaluate process parameters under different pH & bicarbonate conditions.
- Fit the data to a statistical model and summarize results.

References

[1] I. T. Miettinen, T. Vartiainen, and P. J. Martikainen, Applied and Environmental Microbiology 63:3242 (1997)

[2] J. K. Fredrickson, J. M. Zachara, D. L. Balkwill, D. Kennedy, S. M. W. Li, H. M. Kostandarithes, M. J. Daly, M. F. Romine, and F. J. Brockman, Applied and Environmental Microbiology 70:4230 (2004).

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