A Study of Cell Viability on DOE Hanford Soil Isolates: Effect of U (VI) and Bicarbonate

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Background

- In 2005, a study began to identify and evaluate remedial strategies to sequester uranium contamination in the subsurface.
- The injection of a polyphosphate amendment in hot spot areas significantly reduces the inventory of available uranium that contributes to the groundwater plumes.
- This *in situ* remediation process via polyphosphate injections into contaminated groundwater results in the formation of uranyl phosphate solid phases in the soil and groundwater, such as autunite.
- Because autunite sequesters uranium in the oxidized form, U(VI), rather than forcing reduction to U(IV), the possibility of subsequent re-mobilization of uranium is prevented.
- In addition, formed autunite, as a phosphorus-containing mineral, can attract bacteria to liberate phosphorus, meeting their nutrient requirements and causing U release back into the environment.
- The significance of bacteria-uranium interactions has been illustrated by focusing on three bacterial strains of *Arthrobacter* sp, isolated from Hanford Site soil.
- This research was extended to investigate the stability of the autunite mineral in oxidized conditions pertaining to the Hanford Site, and to study the effect of the *Arthrobacter* oxydans SMCC G968 strain on the U (VI) release from autunite.

Objectives

- Inspect bacterial surfaces after exposure to U (VI) in the bicarbonate-bearing synthetic groundwater solution via atomic-force microscopy (AFM)
- Investigate cell viability via Live/Dead Fluorescent assay

Methodology

- Bacterial cell growth
  - 5% PTYG liquid culture media
  - Two days
- Log 7 cells/mL of the bacterial stock solution was incorporated with uranyl nitrate and SGW media to create individual samples for analysis.
- Samples for viability assessment were similar to samples used for AFM imaging.
  - 3 µL of the dye mixture was added for each mL of the bacterial suspension.
  - Equal parts of SYTO9 and propidium iodide
- The samples were incubated at room temperature in the dark for 15 minutes and washed 3 times to prevent a bright background when imaging.
  - Five microliters of the stained bacterial suspension was placed on a microscope slide and allowed to dry for 1 hr before being imaged via a fluorescence microscope.

Results

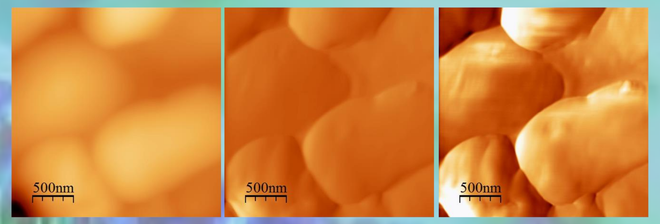

Figure 1. G968 control sample (scan size 2.5 x 2.5 µm^2) illustrating smooth bacterial surface. The topographic image on the left, deflection image in the middle and friction image on the right.

- Adhesion forces are sensitive to modifications in the surface; so, a force spectroscopy analysis was performed to gain a better understanding of the interactions at the atomic level.
- There is an inverse relationship between the adhesion forces and the concentration of uranium; as the concentration of uranium increases, the adhesion forces will decrease exponentially.
- When bicarbonate is present within the solution, the adhesion forces showed similar values to that of the control sample when no uranium is present.

	Adhesion (nN)	SD
Control	11.6*	1.68
5ppm U, 0 mM HCO3	7.14*	0.26
5ppm U, 5 mM HCO3	9.51	1.2
10ppm U, 0 mM HCO3	5.54	4.3
10ppm U, 5 mM HCO3	4.88	2.3

Table 1. Adhesion Forces for Arthrobacter sp. G968
*Data was obtained from the 2010 Year End Report and recent publications (Katsenovich et al. 2012a, Katsenovich et al. 2012b)

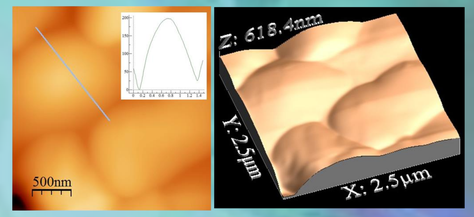

Figure 2. G968 control sample with a maximum height of 198. The profile plot is on the left with the 3D topographic image on the right.

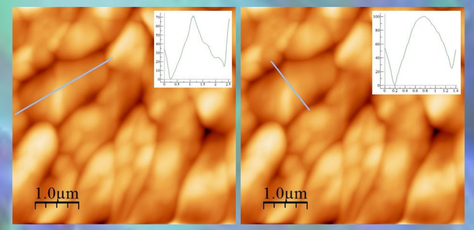

Figure 3. 10 ppm U (VI) and 0 mM bicarbonate. The profile heights: 70-90 nm.

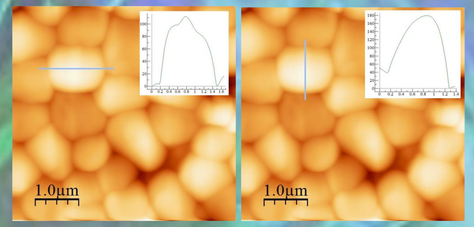

Figure 4. 10 ppm of U (VI) and 5 mM of bicarbonate. The profile heights: 110-180 nm.

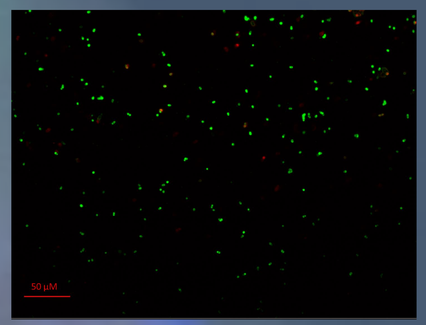

Figure 5. Live/Dead assay sample containing 10 ppm of U (VI) with no bicarbonate. This sample illustrates a higher concentration of dead cells compared to the figure below.

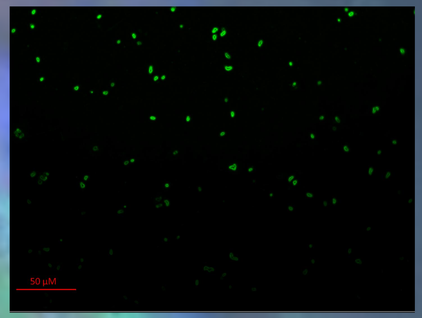

Figure 6. Live/Dead assay of sample containing 10 ppm of U (VI) with 5 mM bicarbonate. This sample illustrates a large concentration of live cells.

- When calculating the viability of cells for each sample, it has been found that there is not much difference between the varying concentration of uranium and bicarbonate.
- Each sample exhibited a ratio of live cells greater than 95% and when making a comparison between the sample containing 10 ppm of U (VI) with and without bicarbonate, it is apparent that the sample containing bicarbonate contains a higher ratio of live cells.

Discussion

- Live/Dead analysis shows that despite the concentration of uranium and bicarbonate present in the solution, each sample exhibited a ratio of live cells greater than 95%.
- Performing a cell viability assessment via culture plates, results demonstrated that although the bacterial cells established intact cytoplasmic membranes, resulting in viable cells for live/dead analysis, the cells that are exposed to uranium with no bicarbonate experienced a viable but nonculturable state, that is, experienced low levels of colonies when plated.
- Force spectroscopy results demonstrate that as uranium is added to the media, the adhesion force parameter decreases.
- Furthermore, the height provided from profile plots reveal that samples containing bicarbonate have a higher profile height, resulting in a much smoother surface. Thus, samples exposed to uranium with no bicarbonate are mostly viable, but are not alive. In contrast, samples containing bicarbonate have a reduced height and small cellular size but are alive and have acclimated to withstand uranium toxicity.

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