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 solids 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 autunite SMCC 0968 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% PYTG liquid culture media

Two days

Log 7 cells/mL of the bacterial stock solution was incorporated with uranyl nitrate and SWO 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

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.

<table>
<thead>
<tr>
<th>Adhesion (nN)</th>
<th>SD</th>
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<tbody>
<tr>
<td>Control</td>
<td>11.4</td>
</tr>
<tr>
<td>10ppm U, 0 mM HCO3</td>
<td>7.18</td>
</tr>
<tr>
<td>5.54 µM U, 0 mM HCO3</td>
<td>9.81</td>
</tr>
<tr>
<td>5.54 µM U, 5 mM HCO3</td>
<td>5.54</td>
</tr>
<tr>
<td>5.54 µM U, 10 mM HCO3</td>
<td>4.80</td>
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</tbody>
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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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