Applied Research Center
FLORIDA INTERNATIONAL UNIVERSITY

U.S. DEPARTMENT OF ENERGY

ENVIRONMENT & ENERGY / ENVIRONMENTAL REMEDIATION

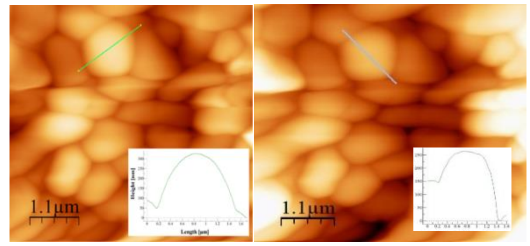
PROJECT: Rapid Deployment of Engineered Solutions to Environmental Problems: *Investigation of Microbial-Meta-Autunite Interactions - Effect of Bicarbonate and Calcium*

CLIENT: U.S. Department of Energy
PRINCIPAL INVESTIGATOR: Dr. Leonel Lagos
LOCATION: Hanford Site, WA

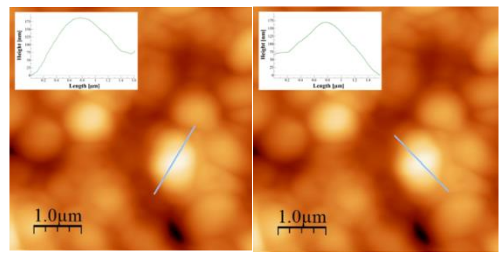
Description:

FIU's Applied Research Center (ARC) is supporting the U.S. Department of Energy's Hanford Site in developing a strategy to improve the efficiency of the uranium stabilization process through polyphosphate injection technology.

Tripolyphosphate injected to sequester uranium undergoes hydrolysis in aqueous solutions to orthophosphate forms, which serve as readily available nutrients for the various micro-organisms that thrive under these specific conditions and may even lead to an increase in their growth. The presence of rapidly adapting bacterial populations in sediment could strongly influence the migration/dissolution of uranium by dissolution and desorption due to the secretion of protons and various ligands. Therefore, understanding the role of bacteria in phosphate remediation technology and the interactions between meta-autunite and the microbes is very important. Of particular concern, however, is the long-term stability of the sequestered uranium in the subsurface that may undergo subsequent remobilization. Aqueous carbonate ions present in the soil and ground water are the predominant aqueous species affecting the dissolution of uranium-bearing solids and facilitating uranium desorption reactions from soil and sediments, thus increasing uranium mobility in soil and sediments. This task is designed to investigate bacteria-U(VI) interactions under oxidizing conditions and study the potential role of bicarbonate to influence U(VI) release from autunite minerals and reduce uranium adsorption by bacterial cells.



Arthrobacter oxydans strain G968 control sample (scan size 2.2 x 2.2 μm^2) illustrating smooth bacterial surface with no uranium, maximum cells height of 198 nm and length of 1.3 μm.



Arthrobacter oxydans strain G968 cultured in media amended with 5 mg/L U (VI) and 5 mM bicarbonate. The profile heights for this sample ranges from 125 to 130 nm. The effect of uranium could have caused these bacterial cells to shrink revealing rod-to-coccus shape change

The main objectives are to:

- Examine the ability of oligotrophic microbial species to influence the dissolution pathways of U (VI) present in the groundwater as stable meta-autunite.
- Investigate the bacterial interactions with uranium using a less U (VI)-tolerant strain and study the potential role of bicarbonate, which is an integral complexing ligand for U (VI) and a major ion in the pore water composition.
- Inspect bacterial surfaces after exposure to U (VI) in the bicarbonate-bearing synthetic groundwater solution via atomic-force microscopy (AFM) and investigate for cell viability.
- Determine the difference between microbial effect on uranium release from synthetic and natural autunite.
- Evaluate for bacterial viability in the presence of bicarbonate ions via a fluorescence microscopy Live/Dead assay.

ABOUT

Since 1995, the Applied Research Center (ARC) at Florida International University (FIU) has provided critical support to the Department of Energy's Office of Environmental Management (DOE-EM) mission of accelerated risk reduction and cleanup of the environmental legacy of the nation's nuclear weapons program. ARC's applied research is performed under the DOE-FIU Cooperative Agreement (under Contract # DE-EM0000598) and provides technical support to DOE EM in the area of environmental remediation and STEM workforce development and training.

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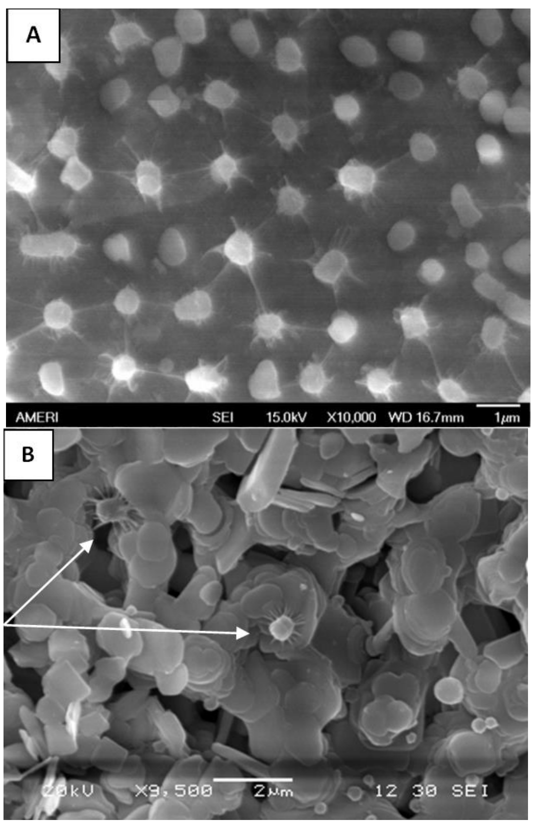
Benefits:

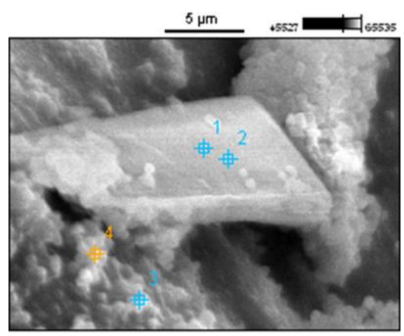
- Evaluates the role of bacteria in the bio-enhanced release of U(VI) from autunite in the presence of various concentrations of bicarbonate up to 10 mM, even while not in direct contact with the mineral.
- Evaluates the effect of bicarbonate on the bacterial viability in the presence of toxic U^{6+} ions.
- Yields insights on bacterial-U(VI) interactions under oxidizing conditions and the ability of low tolerant-to-U(VI) oligotrophic microbial species, such as Arthrobacter G968 to influence the dissolution of natural and synthetic autunite in the presence of bicarbonate.



SEM images of G968 grown on autunite surface in the presence of HCO_3^-. Clearly illustrating individual bacteria are deposited on the solid surface via special cell surface structures, fibrils; A) Natural autunite, 3mM HCO_3. B) Synthetic autunite, 10mM HCO_3 white arrow shows G968 cells attached to the surface.

Accomplishments:

- AFM analysis and Live/Dead fluorescent assay was used to qualitatively and quantitatively illustrate changes with bacterial cell surfaces when exposed to uranium in the presence and absence of bicarbonate.
- The Live/Dead analysis showed that despite the concentration of uranium and bicarbonate present in the solution, each sample exhibited a ratio of cells with intact membranes greater than 95%.
- Arthrobacter oxydans strain G968 cells grown in media containing bicarbonate have a reduced height and a smaller cocci-shape cellular size with no deformed surfaces.
- Arthrobacter strain G968 is a low uranium tolerant strain, but it can accelerate the release of uranium from autunite in the presence of bicarbonate through biodissolution of both natural Ca-autunite and synthetic Na-autunite, in conditions mimicking arid and semiarid subsurface environments of the western U.S.



SEM/EDS analysis of secondary minerals and biofilm created by G968 strain on natural autunite surface and compositional analysis (% weight) for each point at 10 mM HCO_3.

Point number	*Weight,%*							
	C	*N*	*O*	*Na*	*P*	*K*	*Ca*	*U*
pt1 (mineral surface)	24.1	3.9	29.4	0.08	4.3	0.9	1.9	35.4
pt2 (mineral surface)	25.2	2.8	29.6	0.2	4.2	1.3	1.8	35.0
pt3 (bacterial surface)	70.9	9.4	17.5	0.1	0.1	0.2	0.03	1.7
pt4 (bacterial surface)	67.3	12.9	18.3	0.1	0.2	0.2	0.09	0.9