



Applied Research Center
FLORIDA INTERNATIONAL UNIVERSITY

Investigation on Microbial Dissolution of Uranium (VI) from Autunite Mineral Using a Less U(VI)-Tolerant Strain, *Arthrobacter* Strain G968

Graduate Student: Paola Sepulveda

ARC Supervisor(s): Yelena Katsenovich

Major Professor/Department: Anthony McGoron (Biomedical
Engineering)

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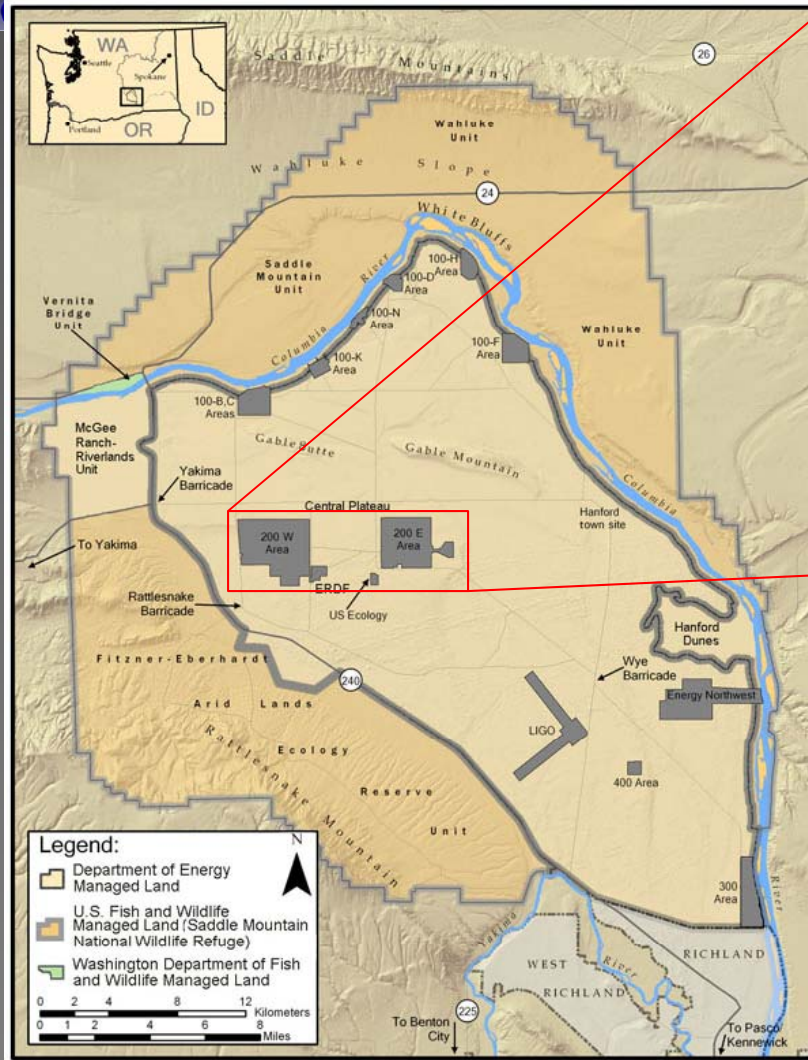


Overview

- Brief Background
- Project Topic
- Objectives
- Overview of Previous Research
- Research Progress for Current Semester
- Path Forward



Background



- Uranium fuel rods from the reactors were transported into the 200 area facilities
 - For removal and refinement of plutonium
 - Producing millions of gallons of radioactive waste



Background

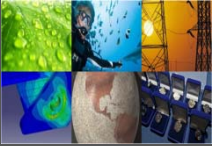


- 53 million gallons of high level radioactive waste were stored in 177 single and double shelled underground storage tanks
- Many of the single shelled tanks have been found leaking several tons of radioactive wastes into the vadose zone
 - Estimate of 1-2 million gallons
 - Creates a potential groundwater contamination



Project Topic

- Bacteria can play a significant role in the dissolution of minerals and the formation of secondary minerals.
- The *Arthrobacter* bacteria are one of the most common groups in soils and are found in large numbers in Hanford soil.
- Research is aiming to:
 - investigate the bacterial interactions of a less U(VI)-tolerant strain under oxidizing conditions with uranium and
 - study the potential role of bicarbonate, which is an integral complexing ligand for U(VI) and a major ion in groundwater compositions



Objectives

- Investigate the effect of bicarbonate on the microbial dissolution of the autunite mineral and U(VI) adsorption by *Arthrobacter* G968
- Make a comparison between G975 and G968 strains
- Inspect bacterial surface in the presence of bicarbonate and uranium in the solution using atomic-force microscopy (AFM)
- Determine the difference between microbial dissolution of synthetic autunite versus natural autunite



Research Progress for Current Semester

- Autunite bioleaching experiment in culture ware with inserts
- AFM and SEM assessment on bacteria exposed to U(VI) in bicarbonate-bearing solutions.
- Bioleaching experiment with bottles using synthetic autunite instead of natural autunite

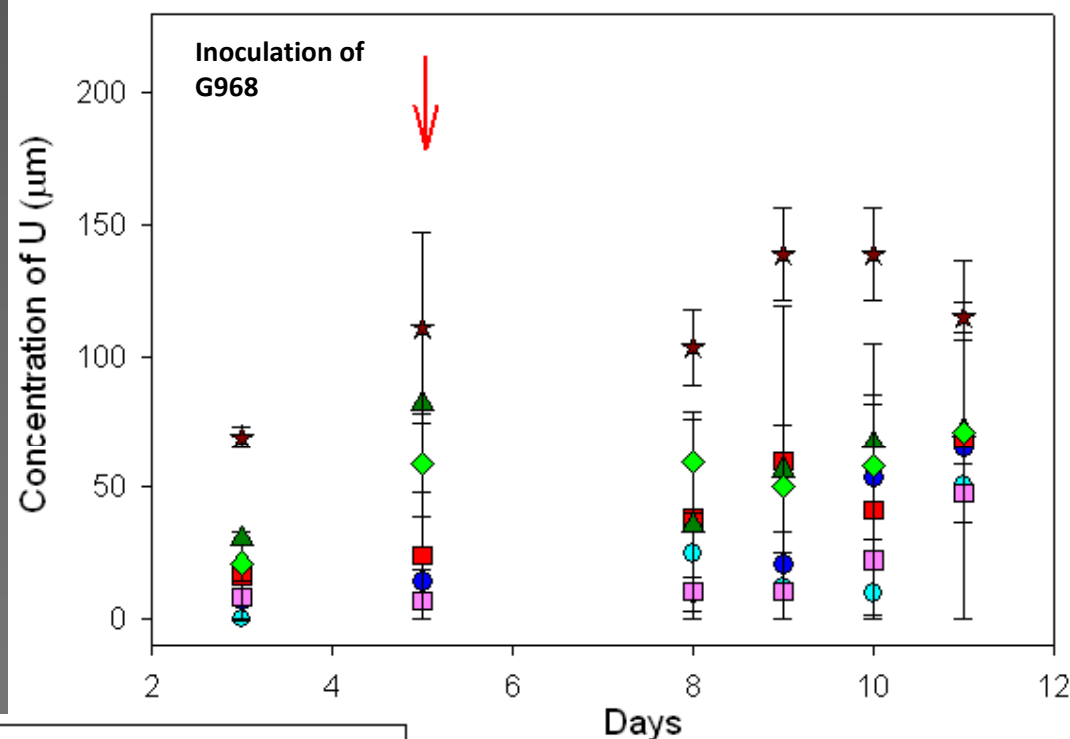


Insert Experiment

- Objectives
 - Conducted an autunite bioleaching experiment in culture ware with inserts to investigate how autunite mineral reacts with the bacteria separated from it
- Methods
 - Sterile 6-well cell culture plates with inserts were used where natural Ca meta-autunite and bacteria cells were kept separately.
 - The culture ware inserts have 0.4 μm cylindrical pores that transverse the membrane and only allow the diffusion of soluble uranium
 - These autunite-containing inserts were injected with bacterial cells after the autunite equilibrated with the media solution amended with bicarbonate.
 - G968 *Arthrobacter* cells in the amount of 10^6 cells/mL were injected into the reactors after a week, giving time for the autunite to reach steady state.



Insert Experiment



There is a slight increase in the dissolution of uranium as bicarbonate increases

- However, this is not as pronounced when compared to G975 or dissolution in bioreactors (when autunite, bicarbonate and bacteria have direct contact)

This may have been caused from

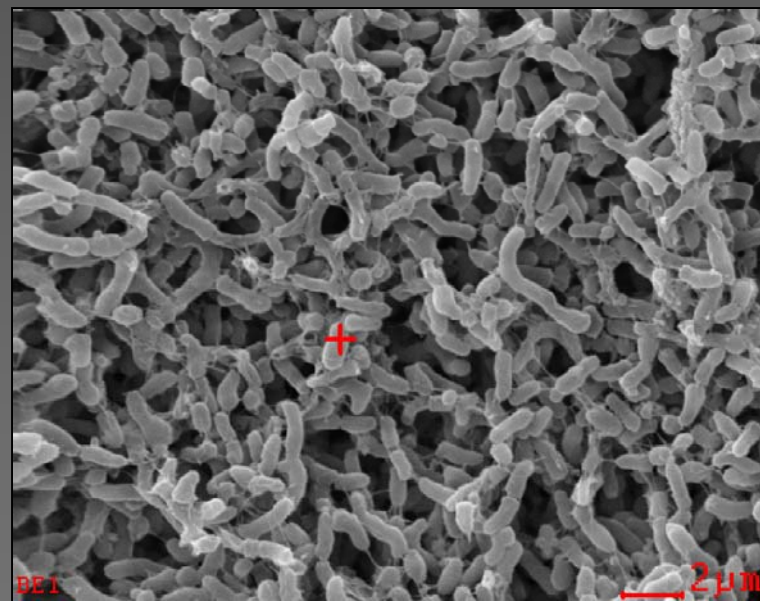
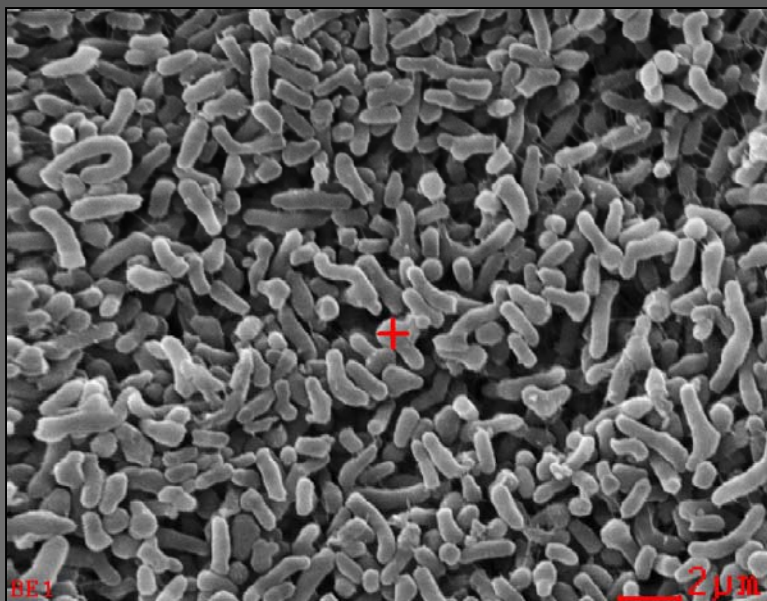
- Small volume of media
- Lower temperature
 - Methods used to prevent evaporation noted in previous experiments

Lower temperature to reduce evaporation were not optimum conditions for bacterial growth and proliferation

- 0mM HCO₃_abiotic control
- 0mM HCO₃
- 3mM HCO₃_abiotic control
- 3mM HCO₃
- ▲ 5mM HCO₃_abiotic control
- ◆ 5mM HCO₃
- ★ 10mM HCO₃_abiotic control
- ★ 10mM HCO₃



SEM/EDS Analysis

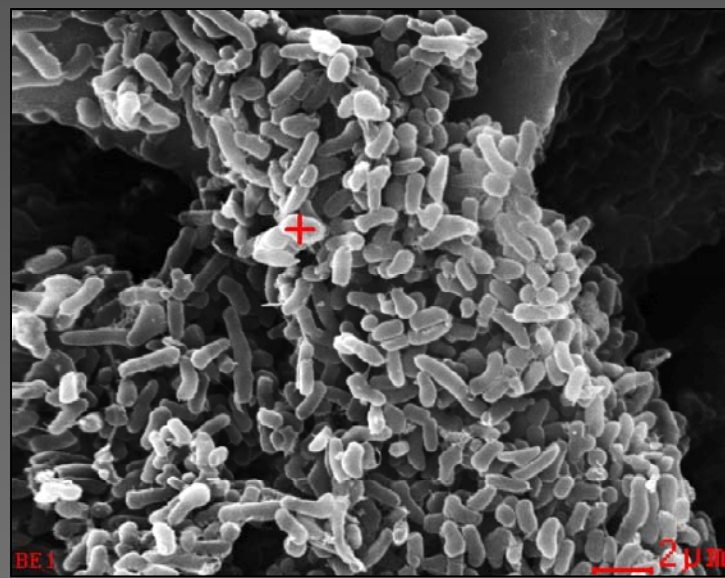
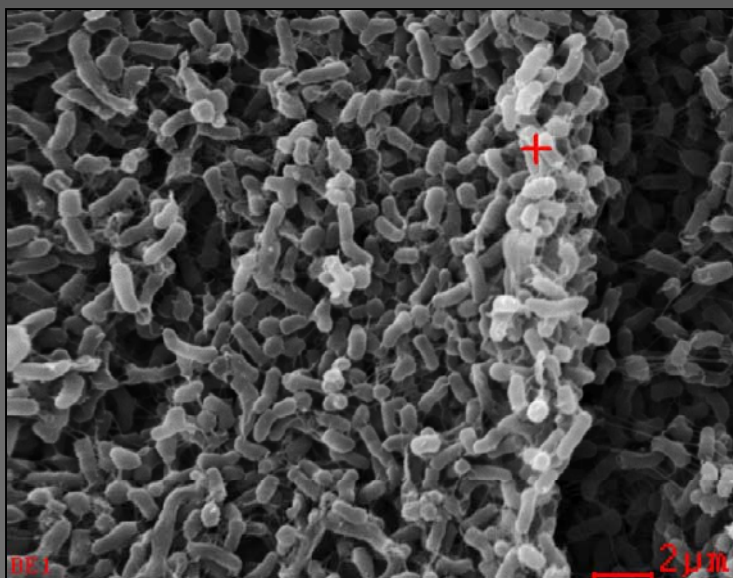


SEM images of G968 grown in the presence of 0mM KHCO_3 with 5ppm U(left image) and 10ppm (right image).

Purpose of SEM was to show surface morphology and illustrate that the cells look healthier in the presence of bicarbonate at this level of uranium



SEM/EDS Analysis



SEM images of G968 in the presence of 5mM KHCO_3 with 5ppm U(left image) and 10ppm (right image).

EDS analysis showed a small %weight of Uranium because the amount added was not sufficient to be detected by the SEM/EDS instrument

AFM analysis is required to perform a closer analysis on surface morphology

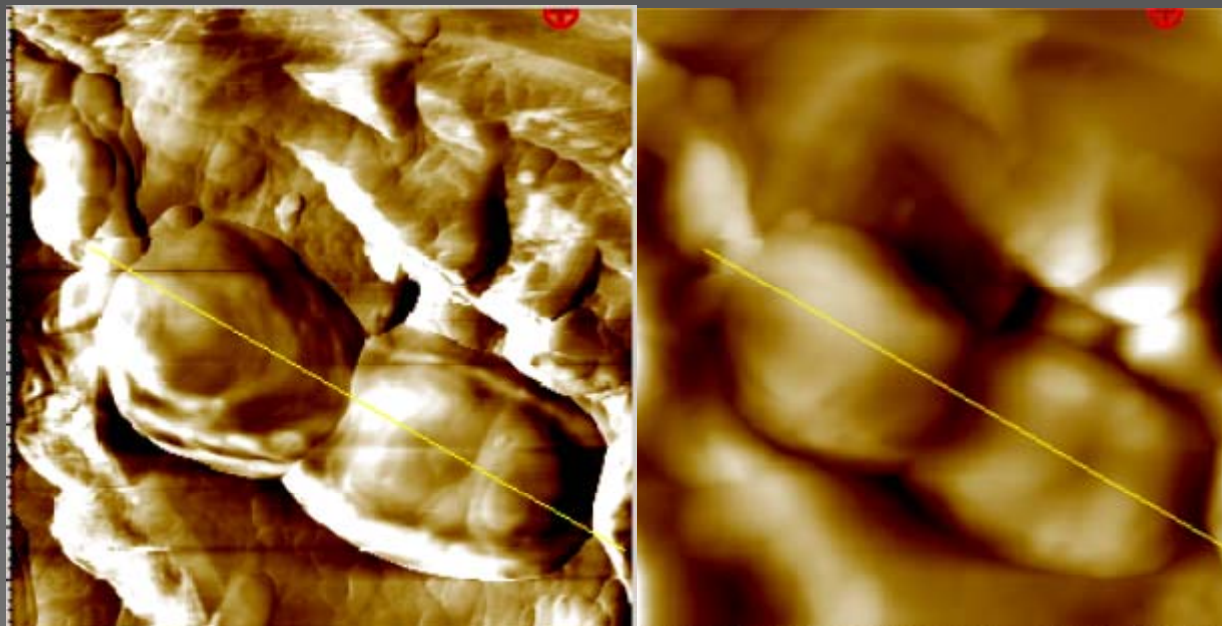


AFM Imaging

- Aim: To inspect bacterial surface in the presence of bicarbonate and uranium in the solution
- Synthetic Groundwater media contained: 5.22 mg/L of KCl, and 520.58 mg/L of HEPES.
 - Media was prepared in deionized water (DIW), autoclaved at 121°C, 15 psi for 15 minutes, and allowed to cool down to about 30°C.
 - Then it was equally distributed between four 250 ml bottles and separately amended to contain 0 mM 3 mM, and 5 mM KHCO_3 .
- Samples being analyzed included:
 - 0mM HCO_3 with 5 ppm U
 - 0mM HCO_3 with 10 ppm U
 - 5mM HCO_3 with 5 ppm U



AFM Imaging: 0mM HCO₃ with 5 ppm U



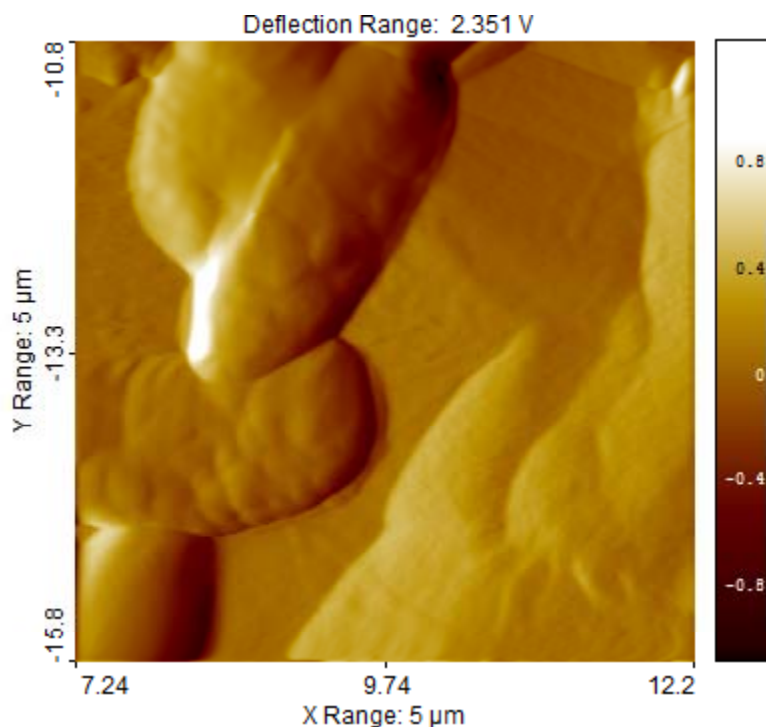
G968 cultured in media containing 5ppm uranium (scan size 6 x 6 μm^2 ; z range 260 mV). Friction image on the left and topography on the right.

Images were conducted using contact mode compared to previous methods of tapping mode

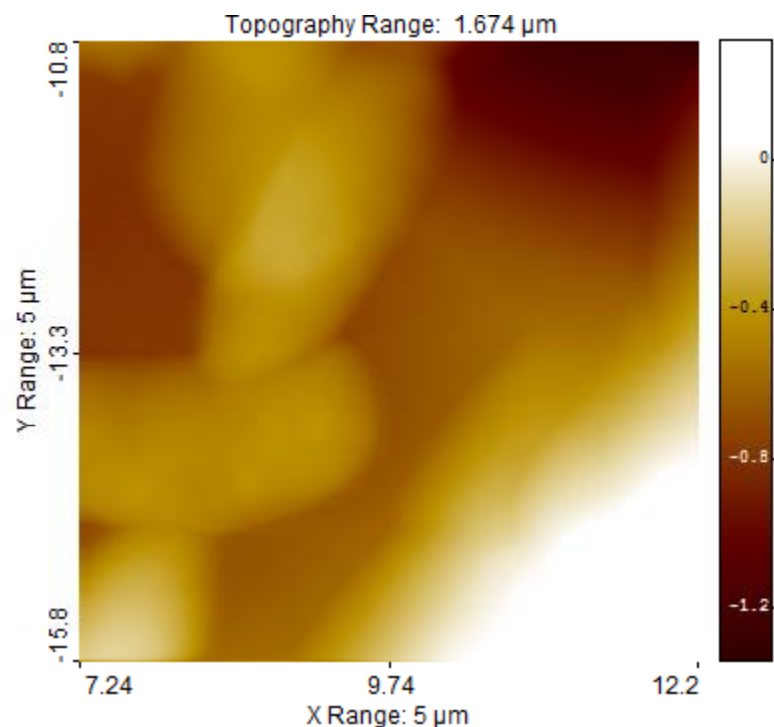


AFM Imaging: 5mM HCO₃ with 5ppm U

5mM_5ppm_5um.mi.drz-1



5mM_5ppm_5um.mi.drz-2





AFM Imaging: Force and Roughness Analysis

- Adhesion forces are sensitive to modifications in the surface
 - i.e.: physiological changes on the cellular membrane when exposed to uranium and bicarbonate
- Thus a force spectroscopy analysis was conducted to gain a full understanding of interactions in the atomic level
- Currently trying to organize the results to show in a later presentation



Bioleaching Experiment with Synthetic Autunite

- Aim: Determine the difference between microbial dissolution of synthetic autunite versus natural autunite
 - Synthetic autunite: smooth which could greatly affect dissolution
 - Natural autunite: cracked morphology which has a greater potential for bacteria to interact and attach to it
- Methods
 - Sterile 100 mL glass mixed reactors served as the major bioreactor for initial experimentation
 - These autunite-containing bioreactors were injected with bacterial cells after the autunite equilibrated with the media solution amended with bicarbonate
 - G968 *Arthrobacter* cells in the amount of 10^6 cells/mL were injected into the reactors after 27 days, giving time for the autunite to reach steady state
- Estimated time of completion: May 10



Path Forward

- If time permits: Evaluation on the Uranium (VI) bioaccumulation by G968 in SGW with varying calcium and bicarbonate concentrations
- Finish writing thesis



Acknowledgements

- Drs. P. Sobecky and Robert J. Martinez, University of Alabama, for providing us with *Arthrobacter* sp. strains from the SMCC collection.
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Questions?

