



Applied Research Center
FLORIDA INTERNATIONAL UNIVERSITY

A collage of four images: a green forest, a person in a blue lab coat holding a device, a yellow industrial tower, and a blue electronic circuit board.

DOE-EM Cooperative Agreement – Year 3 Research Review

Project 2: Rapid Deployment of Engineered Solutions to Environmental Problems

Presented: April 30, 2013
to the U.S. Department of Energy
by Dr. Yelena Katsenovich,
Research Scientist

Worlds
Ahead

Advancing the research and academic mission of Florida International University.



Outline

- Project Description
- Task 1.1- Subsurface pH manipulation using NH_3 gas
- Subtask 1.1-Characterization of uranium bearing precipitates
- Task 1.2- Microbial meta-autunite interactions
- Kinetic rate law parameters of the dissolution of meta-autunite in the presence of bicarbonate
- Work Proposed for FIU Year 4



Project Description

- FIU has been investigating U(VI) contamination in the VZ of the Hanford 200 Area and 300 Area saturated zone to protect the Columbia River by reducing the concentration of contaminants below the levels that can cause harm.
- Injection of reactive gases such as NH_3 is an innovative technology to decrease uranium mobility in the subsurface at Hanford's VZ (200 area). The injection causes the formation of ammonia hydroxide (NH_4OH) and a subsequent increase in pH, leading to silica and aluminum dissolution from soil minerals.
- The subsequent decrease in the pH to ambient conditions results in co-precipitation of U(VI) with mobilized Si and other constituencies released from soil minerals to pore water. This can potentially control the mobility of U(VI) since co-precipitated contaminants are less available for migration.
- Also, bacteria is an important environmental factor that affects the stability of minerals. FIU is investigating the effect of bicarbonate and Ca ions on the autunite mineral microbial dissolution by the *Arthrobacter* strains commonly found in Hanford Site soil.
- In addition, injection of a sodium tripolyphosphate amendment into the uranium-bearing saturated zone at the Hanford 300 Area has been shown to immobilize uranium through the formation of an insoluble uranyl phosphate mineral, autunite. FIU has researched the effect of bicarbonate on the rate of U(VI) release from autunite.



Staff and Students

Project Manager: Leonel Lagos, PhD, PMP®

Faculty/Staff: Dr. Yelena Katsenovich,
Dr. Ravi Gudavalli

DOE Fellows students (5): Claudia Cordona, Paola Sepulveda, Robert Lapierre, Joel McGill, Valentina Padilla.

PNNL Collaborator: Dr. Dawn Wellman



Overview of Project Tasks

Task 1.1: Sequestering uranium at the 200 Area by *in situ* subsurface pH manipulation using NH₃ gas. This task evaluates the role of major pore water constituents (Si, Al, Ca, Mg, HCO₃ ions) on the U(VI) sequestration process and studies the formation and solubility of uranium-bearing precipitates created after NH₃ gas injection.

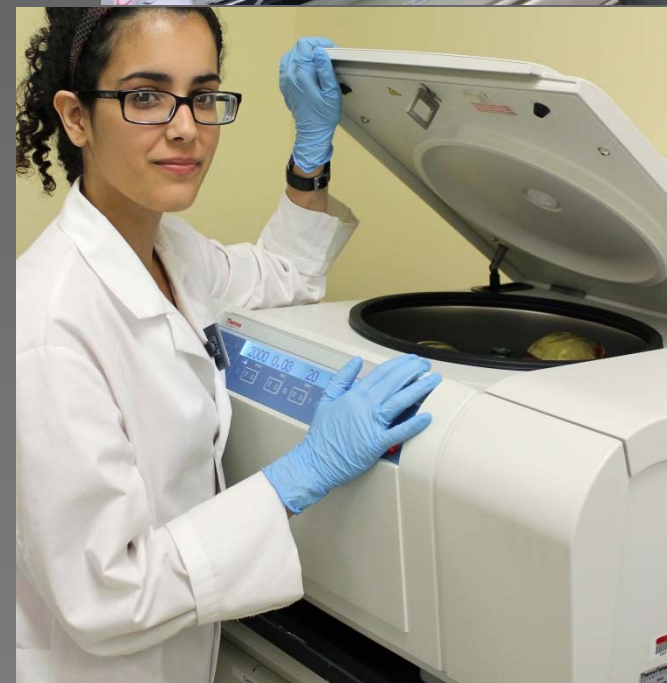
Task 1.2: Investigation of microbial meta-autunite interactions - the effect of bicarbonate and Ca ions. This task studies microbial dissolution of the autunite mineral and U(VI) biouptake by the *Arthrobacter* species, isolated from Hanford soil to provide insights into important microbiological processes affecting the fate and transport of uranium in Ca-rich carbonate-bearing subsurface environments.

Effect of bicarbonate on the dissolution of Ca- and Na-autunite. This task evaluates and compares important kinetic parameters and fundamental thermodynamic properties of the dissolution of synthetic and natural autunite in the presence of bicarbonate ions.



Instrumentation

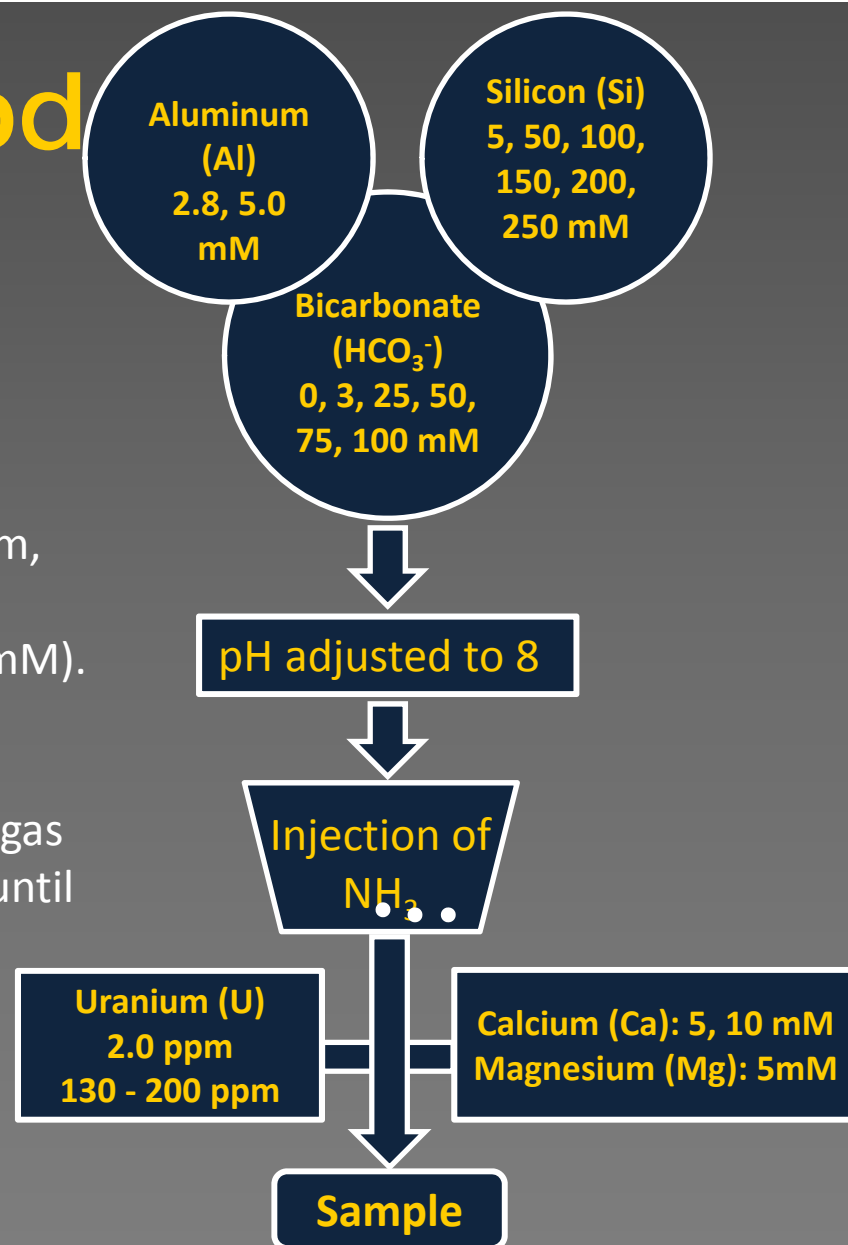
- ARC established a radiological laboratory that has the capability to process radioactive samples
 - **Kinetic Phosphoresce Analyzer (KPA)** from Chemchek Instruments
 - **Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES)** from PerkinElmer
 - **Sorvall ST 16R Centrifuge** from Thermo Scientific





Task 1.1-Method

- Evaluated literature data on the pore water composition for major cations, anions, and pH (Serne et al. 2002).
- Four elements were used in the initial test solutions to evaluate the effect of pore water constituencies on the removal of U(VI): uranium, silica, aluminum and bicarbonate. Later incorporated Ca (5mM and 10mM) and Mg (5mM).
- Each set contained 36 samples.
- Injected NH_3 gas (5% NH_3 in 95% N_2) via metal gas sparger (Mott Corporation) with 20 μm pores until the pH of the solution reached 11.





Task 1.1 Methodology

Kinetic Phosphorescence
Analyzer (KPA-11)

ICP-OES

Total Organic Analyzer
(TOC)

Visual MINTEQ



Scanning Electron Microscope -
Energy Dispersive Spectroscopy
(SEM-EDS)

Raman/FTIR Spectroscopy

X-ray Diffraction (XRD)

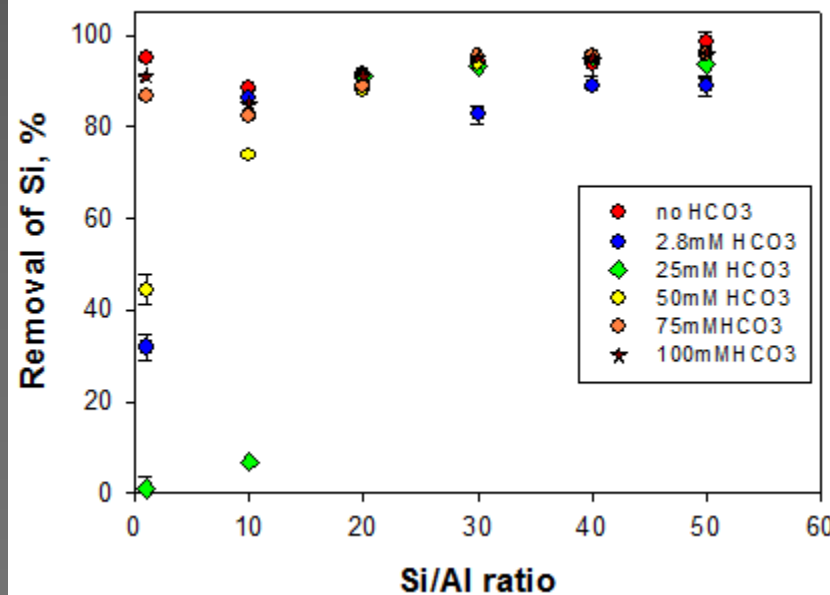
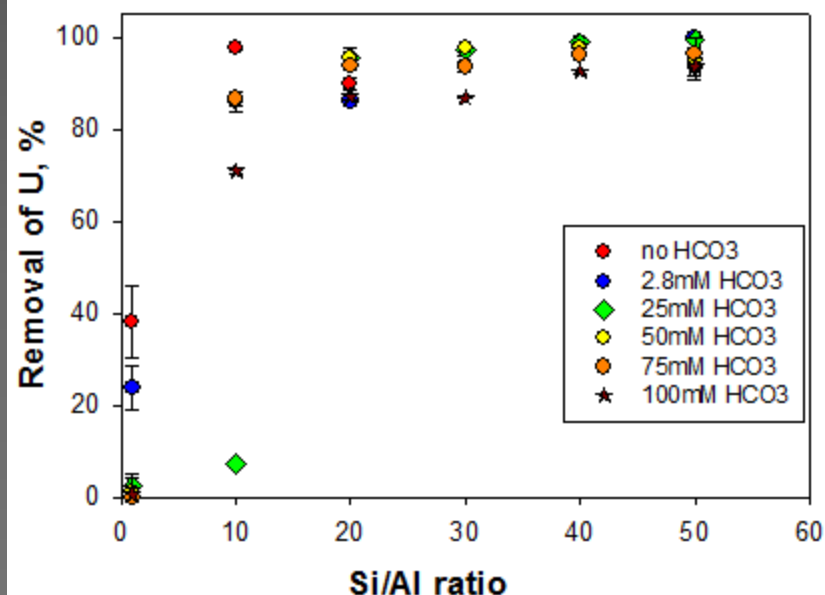
Transmission electron
microscopy (TEM)

Visual MINTEQ





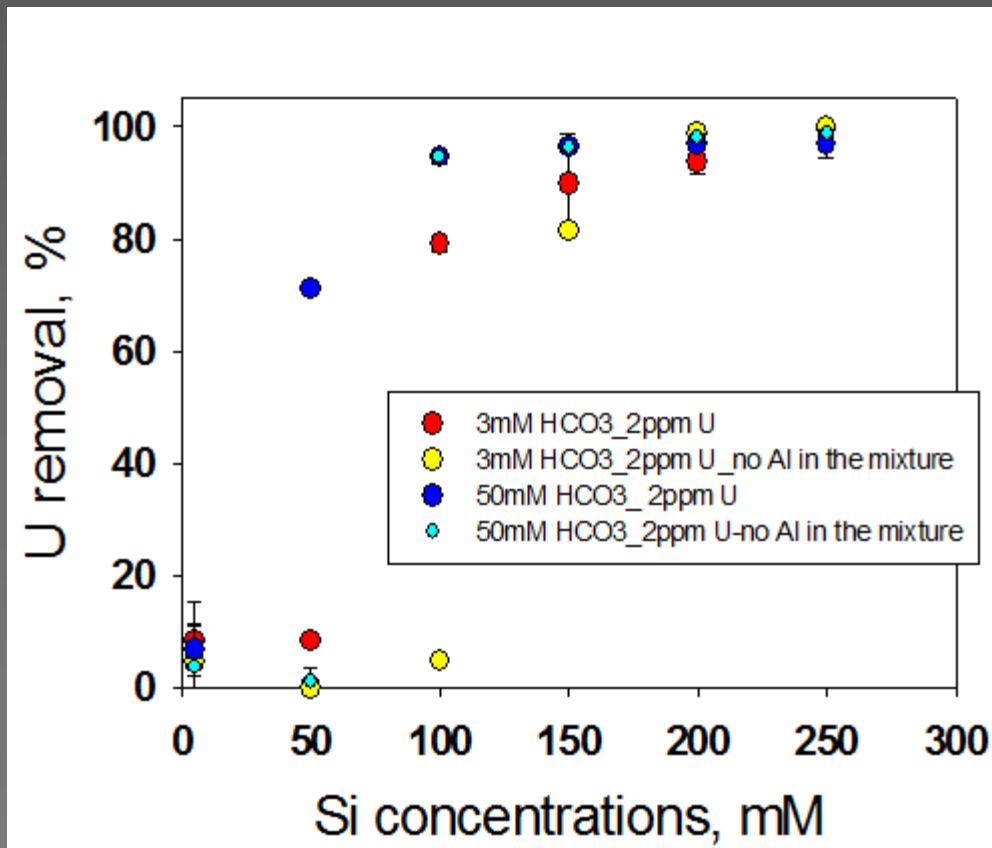
Task1.1 Results- Al 5mM



- Si:Al ratios > 10 showed removal efficiencies for U and Si reaching up to 97%.
- The results for Al removal were consistent with the results on Si removal.



Task 1.1 Results- No Al



- If no Al in the system, U(VI) removal was delayed.
- At 3 mM HCO₃⁻ U(VI) removal was observed at Si ≥ 150 mM;
- At 50 mM HCO₃⁻, Si ≥ 100 mM).

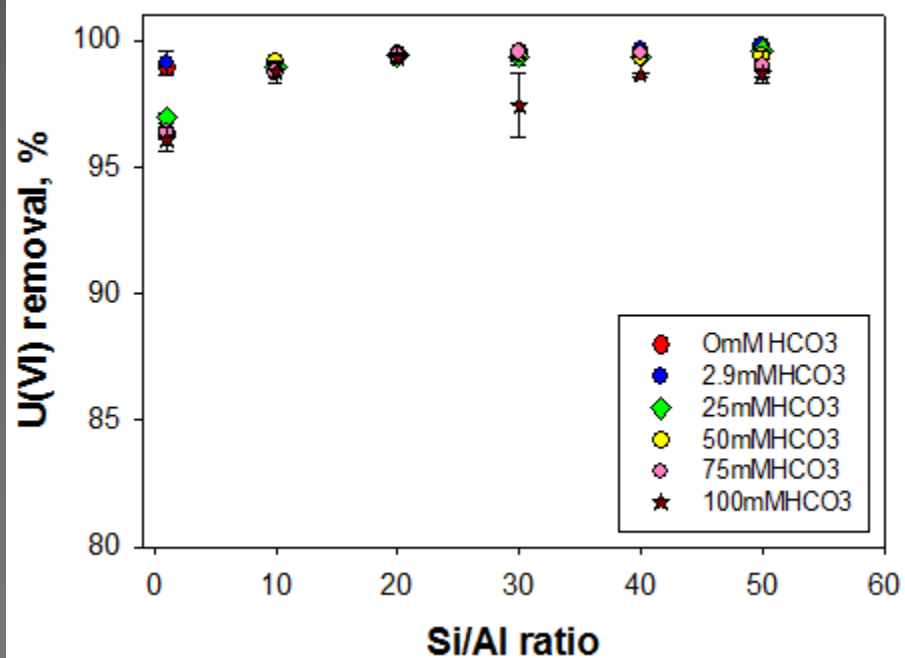
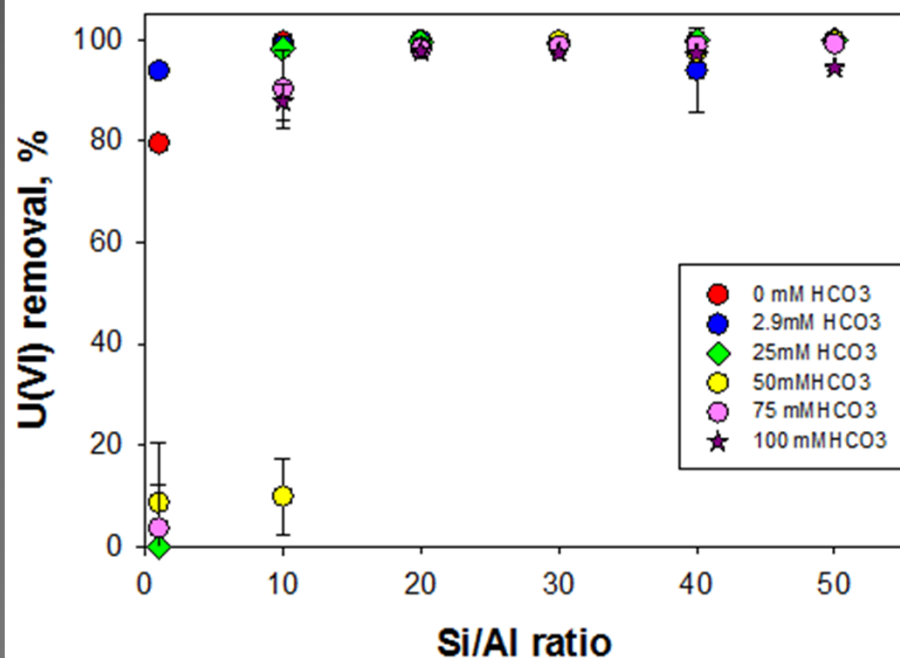


Task1.1 Results

- Si:Al molar ratio and HCO_3 concentration affect U(VI) removal.
- Si:Al over 30:1 (150 mM Si) showed >98% U(VI) removal.
- Both concentrations of U(VI) tested (0.5ppm and 2ppm) followed a similar path with relatively small deviations between samples.
- Completed experiments with a total of 250 test solutions to investigate U(VI) removal in the presence of various Si/Al molar ratios, HCO_3 in the range of 0-100 mM, 5 mM Al, and 10 mM of Ca ions or 5 mM Ca+5 mM of Mg. The removal of U(VI) yielded 86-99%, that overshadowed previous results without Ca ions. Repeated some ratios to get more consistent results.



Task 1.1 The addition of Ca

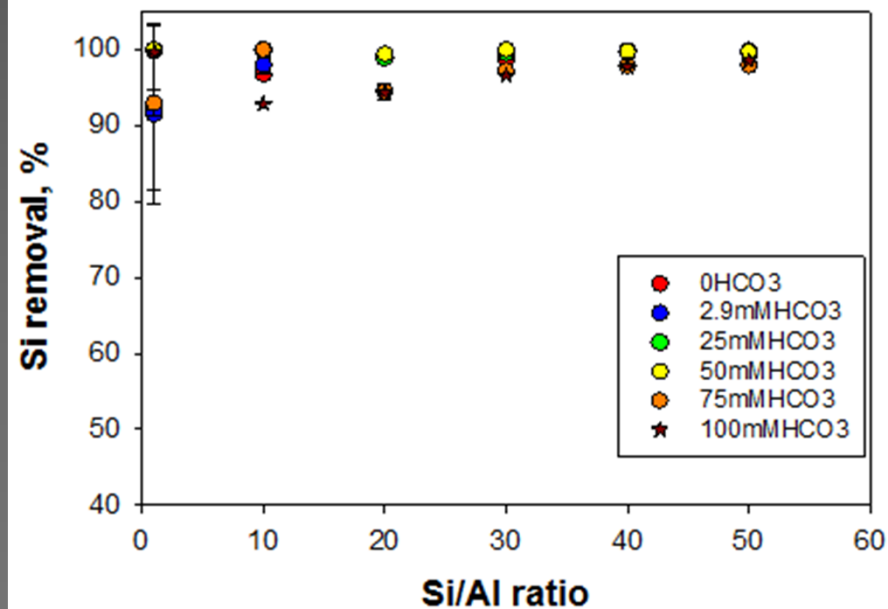
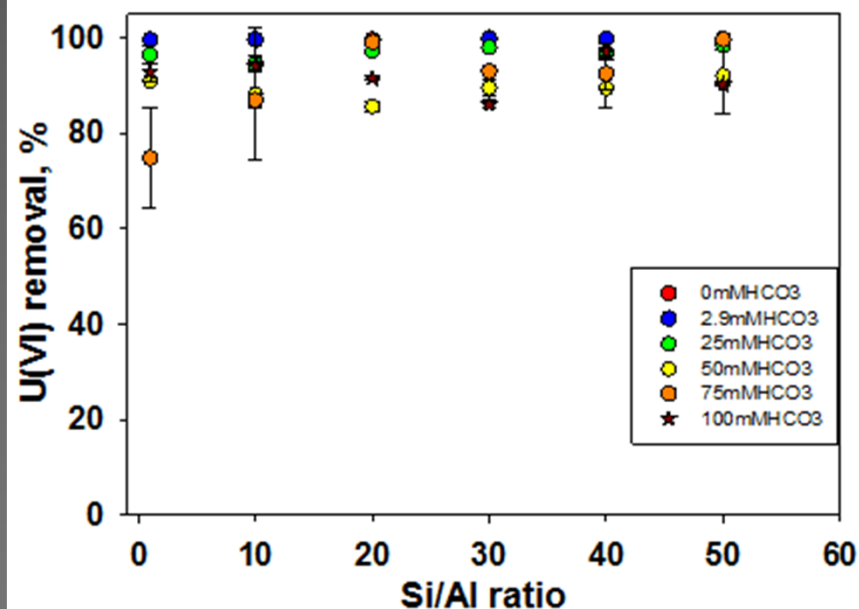


5mM Ca

10mM Ca



Task 1.1-The addition of Ca& Mg



5mM Ca+5mM Mg



Task1.1 Results

- The formation of Si gel always correlated with the removal of U(VI), Si, and Al from the solution.
- If no Si polymerization and gel formation was observed, there was no U removal.
- U(VI) removal efficiency was affected by the Si:Al molar ratio in solution. At the Si:Al molar ratios $\geq 20:1$, a removal efficiency of U(VI) is relatively high; (approximately $\geq 93\%$).
- The addition of Ca & Mg = higher removal of U(VI) at lower Si concentrations where removal was not observed or was very little without Ca & Mg addition.



SubTask 1.1-Characterization of uranium bearing precipitates produced by the NH₃ injection method

- Analyzed mineralogical and morphological characteristics of precipitates by means of X-ray diffraction (XRD), scanning electron microscopy and energy-dispersive-spectrometry (SEM-EDS), initiated Raman spectroscopy and TEM analysis to identify U(VI) mineral phases and structural features .
- Recent experiments focused on optimizing the sample preparation procedures for future studies.
 - Prepared 4 sets of sacrificial samples with total 40 samples for the experiments;
- Evaluated the effect of time kept in the “mother” solution on precipitate morphology;
 - Conducted SEM/EDS for 35 samples.

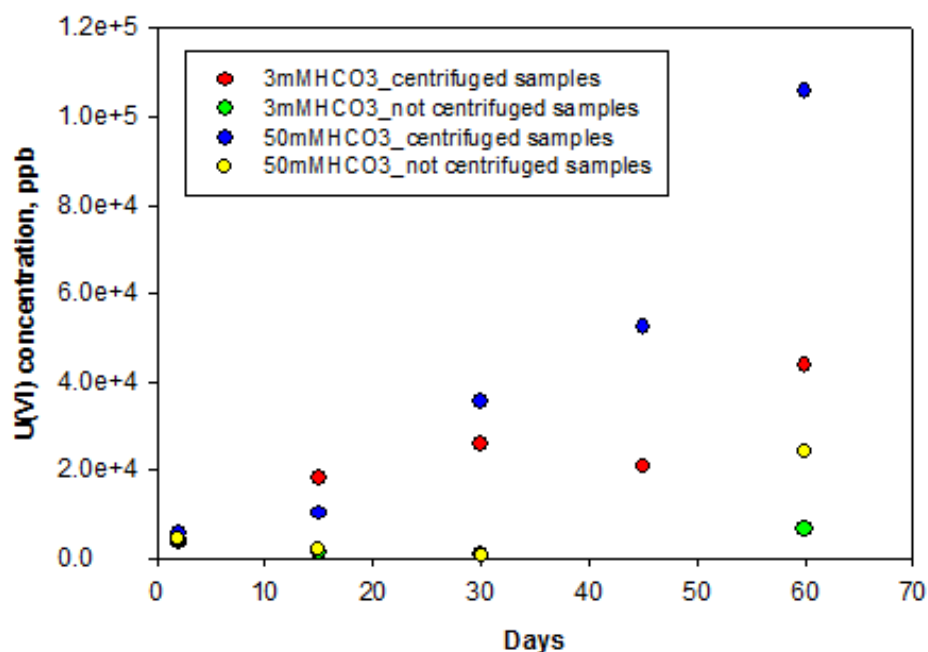


SubTask 1.1- Methods

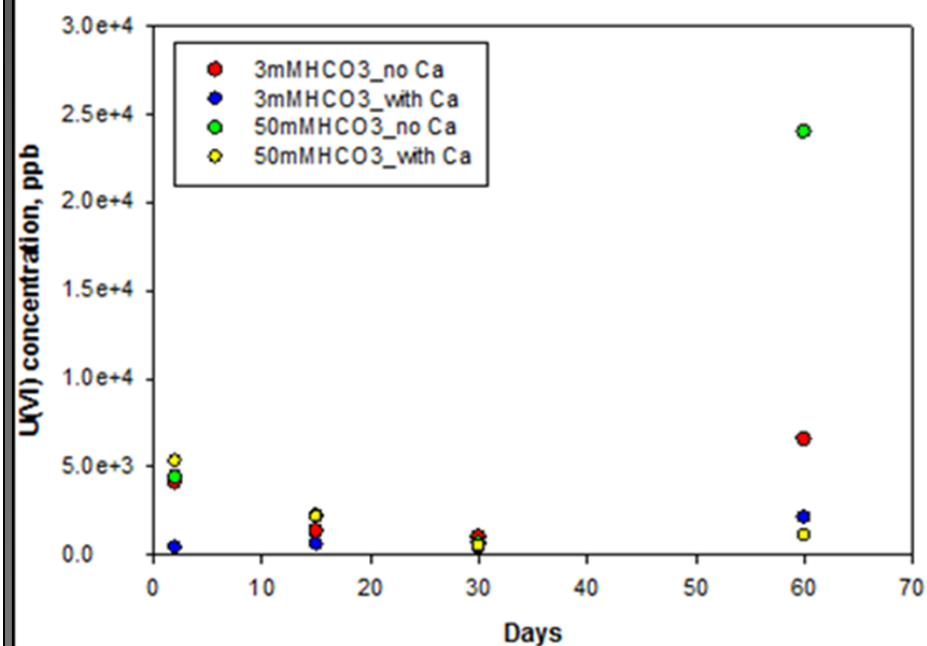
- Methodology:
- Synthetic pore water prepared to mimic the Hanford Site's 200 Area vadose zone conditions
 - Solutions were amended with 200 ppm of U(VI)
- Variables changed to test their impact on precipitate morphology
 - Centrifuging vs. without centrifuging
 - Low (3mM) vs. high (50mM) bicarbonate [HCO₃⁻]
 - With vs. without calcium
- Supernatants were analyzed by kinetic phosphorescence analyzer (KPA) for uranium (VI) content
- Dried samples mounted and analyzed by scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS)



SubTask 1.1- Results



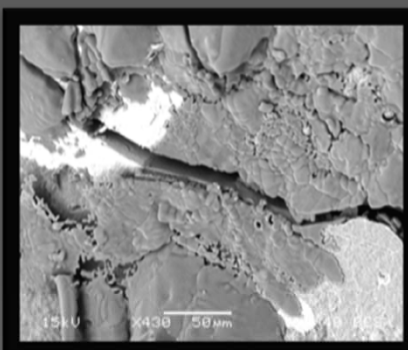
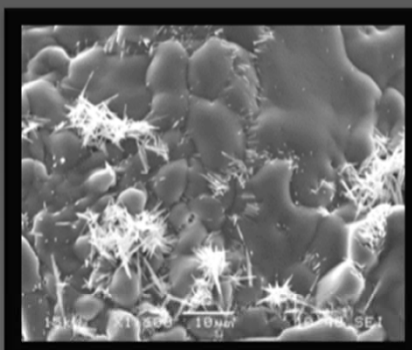
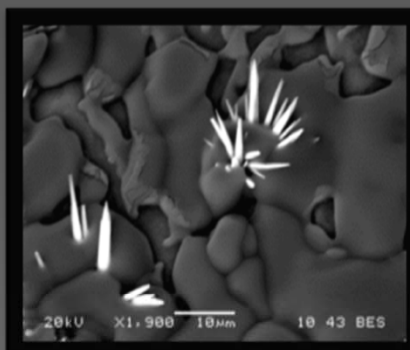
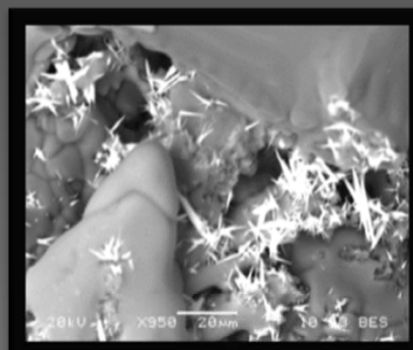
Effect of sample centrifugation on the concentration of U(VI) in the supernatant solution



Effect of 5mM Ca on the concentration of U(VI) in the supernatant solution



SubTask 1.1-Morphological Changes



2 weeks



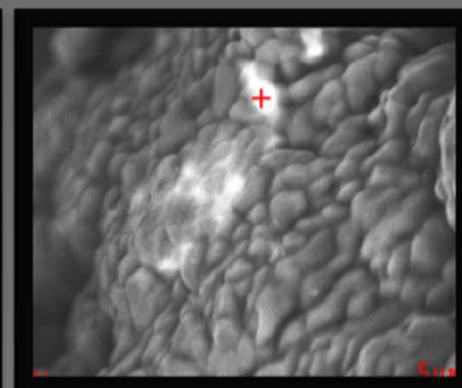
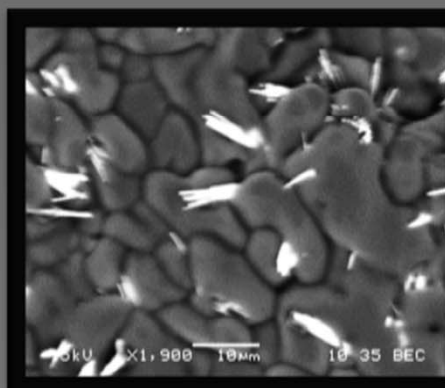
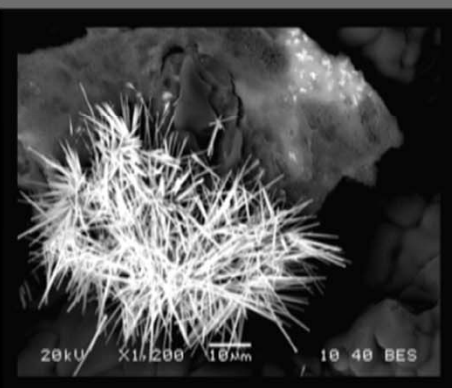
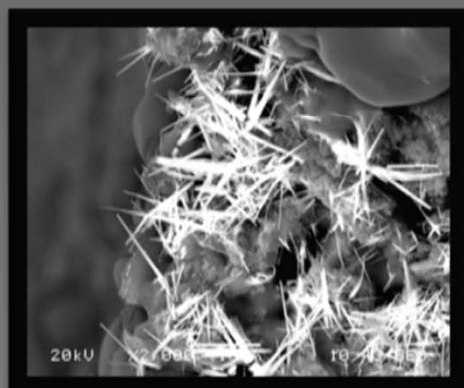
1 month



2 months



3 months



Timelines showing evolution of U-rich areas on precipitates, prepared using with (top) and without (bottom) calcium, over 3 months



SubTask 1.1-Morphological Changes

- The comparison of SEM micrographs on centrifuged and non-centrifuged samples showed little differentiation between the two
 - The KPA analysis of the supernatants solutions showed that uranium content increased with centrifugation
- The crystal-like and amorphous uranium-rich phases were exclusive to the samples prepared with high bicarbonate concentrations
 - No appreciable difference between with and without calcium
- Over 3 months, uranium "hot spots" changed from the crystal-like structures present on the 2- weeks sample to the amorphous uranium-rich areas seen in the 3 month samples
- Qualitative analysis of uranium-bearing precipitates suggested sample preparation procedures would best be limited to without centrifugation using high bicarbonate concentration and calcium.
- Further analysis to characterize the U-bearing phases are ongoing via XRD, FT-IR/RAMAN



Task 1.2 Investigation on microbial meta-autunite interactions: the effect of bicarbonate and Ca ions

- Autunite stability under vadose and saturated zone environmental conditions can help to determine the long-term effectiveness of the polyphosphate injection remediation strategy
- In addition, formed autunite, as a phosphorus-containing mineral, can attract bacteria to liberate phosphorus, meeting their nutrient requirements and causing U mobilization back into the environment
- Increased microbial activity that influences meta-autunite stability is an important geochemical factor affecting the uranium dissolution and transport in the specific environmental conditions present at the Hanford Site's subsurface
- Bicarbonate is a predominant complexing agent for U(VI) in oxidized environments, forming several carbonate complexes with uranium (VI)
- Their mobility in aquifers under circumneutral pH conditions is explained by the formation of highly soluble and stable uranyl-carbonato complexes, UO_2CO_3 , $\text{UO}_2(\text{CO}_3)_2^{2-}$ and $\text{UO}_2(\text{CO}_3)_3^{4-}$ (Langmuir 1978; Guillaumont et al. 2003)



Task 1.2-Objectives

- Examine the effect of bicarbonate on the dissolution of U(VI) from autunite in the mixed reactors using a *Arthrobacter* strain that is less tolerant to U(VI) - G968.
- Evaluate G968 microbial growth and changes in the cell surface morphology under U(VI) exposure in bicarbonate-bearing media.



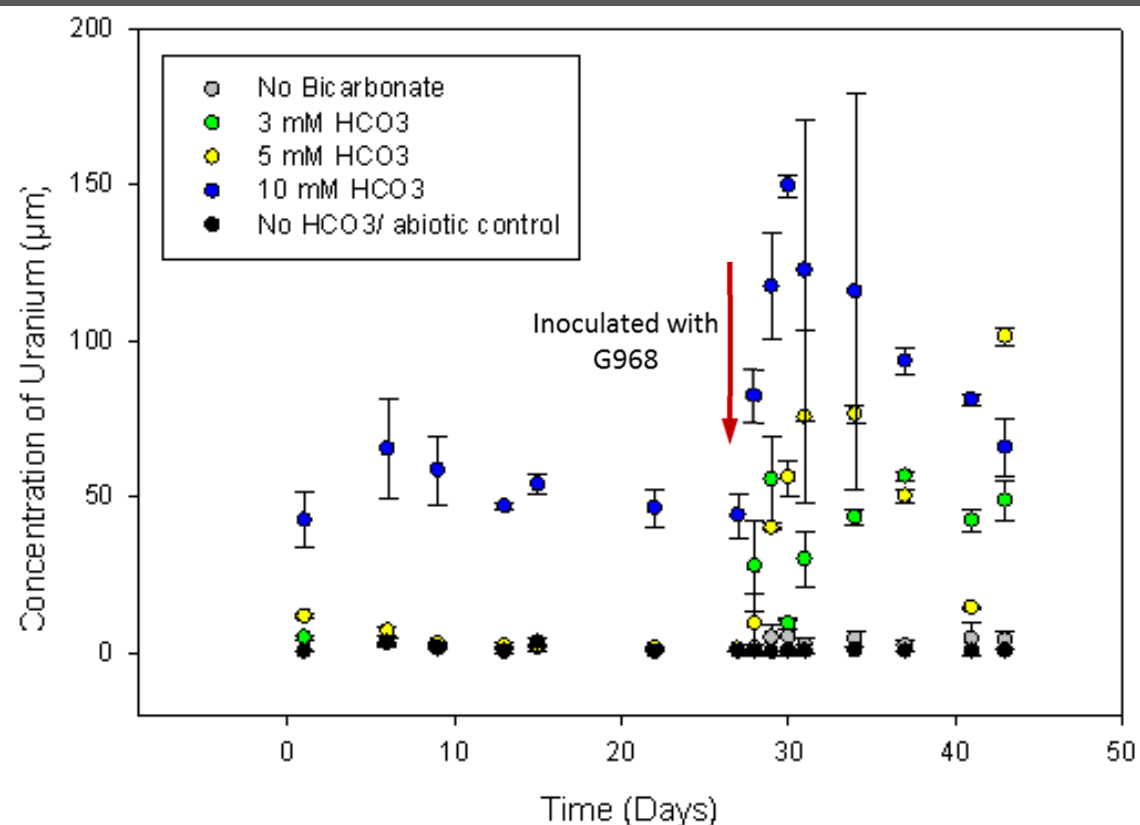


Task 1.2- Methodology

- The media solution: 0.25 g/L peptone, 0.25 g/L tryptone, 0.5 g/L glucose, 0.6 g/L MgSO_4 , and 0.07 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (no yeast)
- Autunite-containing bioreactors were injected with bacterial cells after the autunite equilibrated with the media solution amended with 0 mM, 3 mM, 5 mM and 10 mM concentrations of 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
- Periodically, 0.3 mL samples of the solution was aseptically withdrawn from each bottle, filtered (0.2 μm), and then analyzed for dissolved U(VI) by means of a kinetic phosphorescence analyzer KPA-11



Task 1.2 Results



After bacteria inoculation, U(VI) measured in the reactors increased 8.9 ± 23.34 , 101.0 ± 108.97 , 72.1 ± 123.86 , 2.3 ± 8.65 fold, respectively, compared to the corresponding bicarbonate-bearing controls at steady state.



Task 1.2 Results

- Even though there is an increased amount of U(VI) released into the solution driven by the presence of bacteria, the effect of bacteria on autunite dissolution is reduced as the concentration of $[\text{HCO}_3^-]$ increased;
- Higher bicarbonate concentrations correlate with an increase in autunite surface bacterial colonization;
- Viability of cells was conducted after 24 hours of cell incubation with the appropriate U(VI) and bicarbonate concentration treatment. The number of cells was greater in the sample containing 10 mM bicarbonate compared to 0 mM bicarbonate;
- As expected, the cells started to reduce after day 41 due to the nutritional exhaustion of the media. Moreover, viable bacteria accounted for more than 94% in the presence of 10 mM bicarbonate.

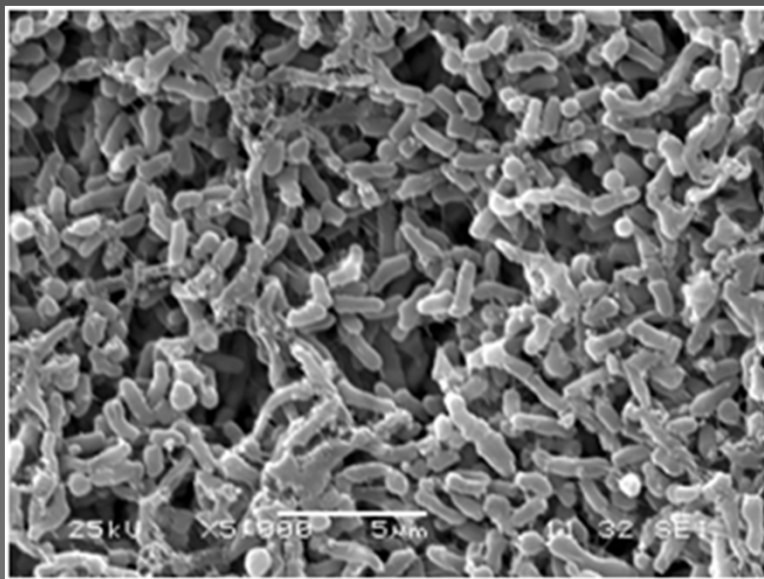


Task 1.2 Results

- Experiments showed that despite differences between the G975 and the G968 bacterial strains resistance to U(VI), in the presence of bicarbonate ions, they are able to cause dissolution of uranium from autunite minerals at the same capacity
- The effect of both bacterial strains on autunite dissolution is reduced as the concentration of bicarbonate increases while the increase in soluble U(VI) concentration induced by G968 and G975 is dwarfed, for larger $[\text{HCO}_3^-]$



Task 1.2 SEM/EDS Analysis

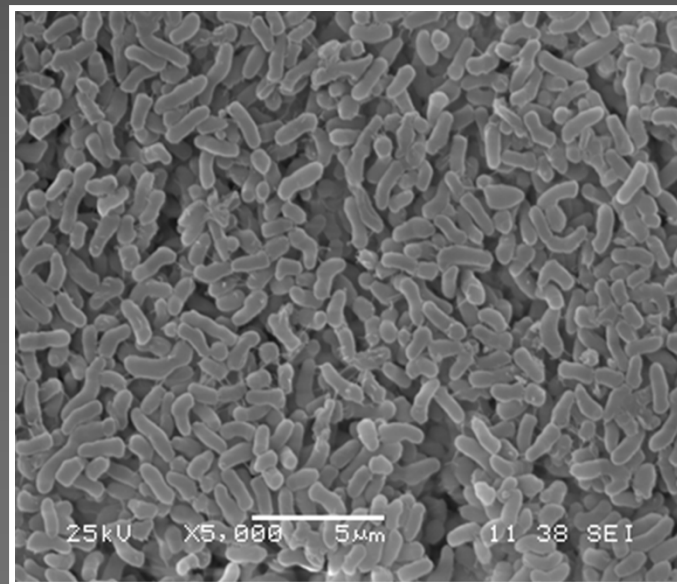
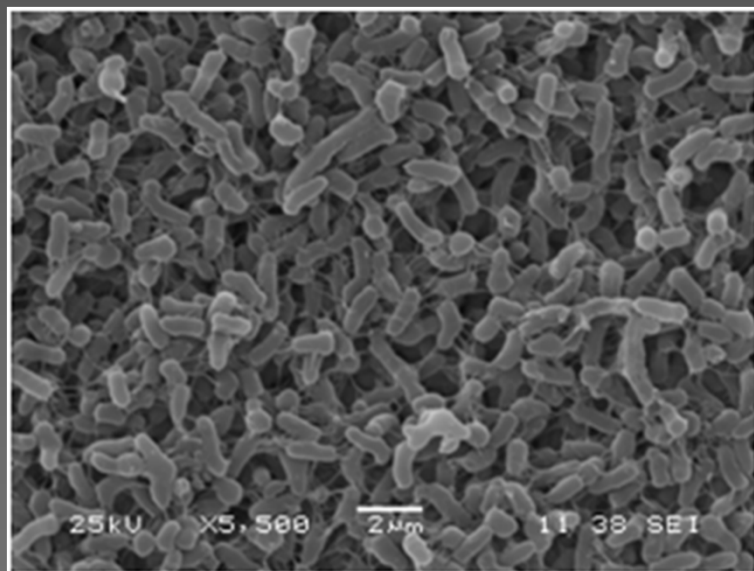


SEM images of G968 grown on autunite surface in the presence of 0mM KHCO_3 with 5ppm U(left image) and 10ppm (right image).

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



Task 1.2 SEM/EDS Analysis



- SEM images of G968 grown on autunite surface 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



Accomplishments

- Biodissolution with inserts
 - Collected and processed about 600 samples for 4 trials
- Biodissolution with synthetic autunite
 - Collected and processed ~170 samples
- SEM Imaging
 - 10 samples
- AFM Imaging
 - 5 samples



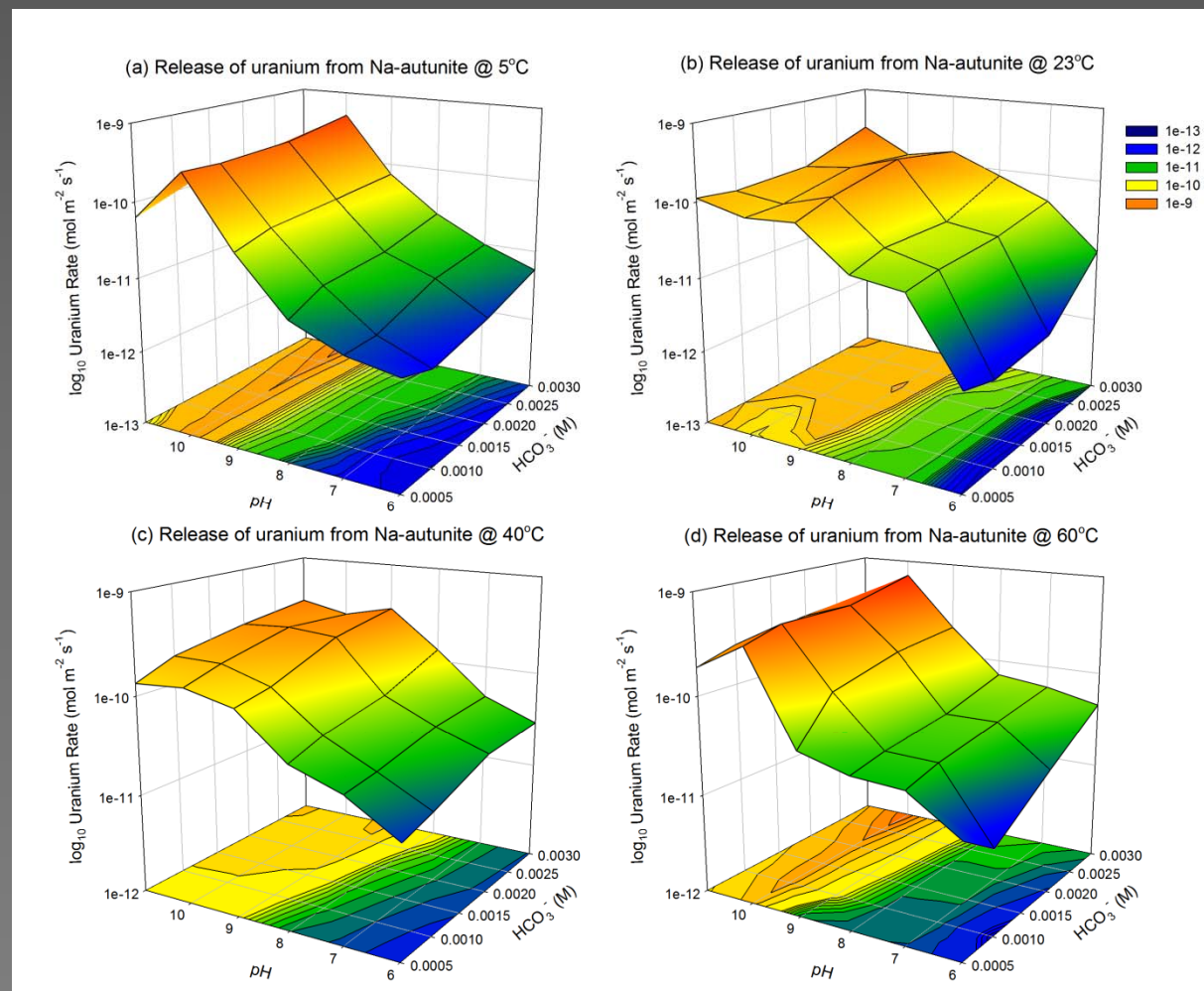
Effect of bicarbonate on the dissolution of meta-autunite

- Studied dissolution of natural and synthetic autunite subjected to various environmental conditions
 - pH: 7 - 11
 - Temperature: 23 - 60°C
 - HCO_3^- : 0.5 - 3.0 mM
- Conducted single-pass flow-through (SPFT) experiments using a programmable V6 syringe pump;
- Collected samples were processed via wet and dry ashing and were analyzed using KPA and ICP-OES;
- Determined rate constant and kinetic rate law parameters of the Na-autunite dissolution in the presence of bicarbonate ions;
- Compared the kinetic rate law parameters for the dissolution of natural and synthetic autunite in the presence of aqueous bicarbonate ions.



Results

The release of U(VI) at various environmental conditions





Results

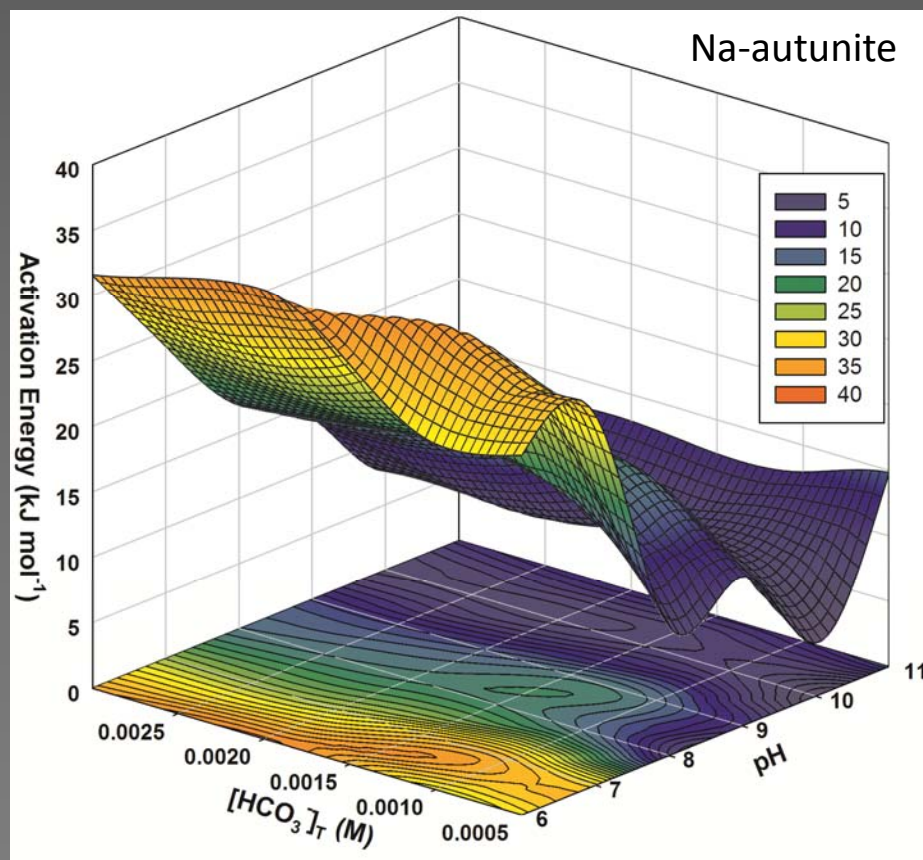
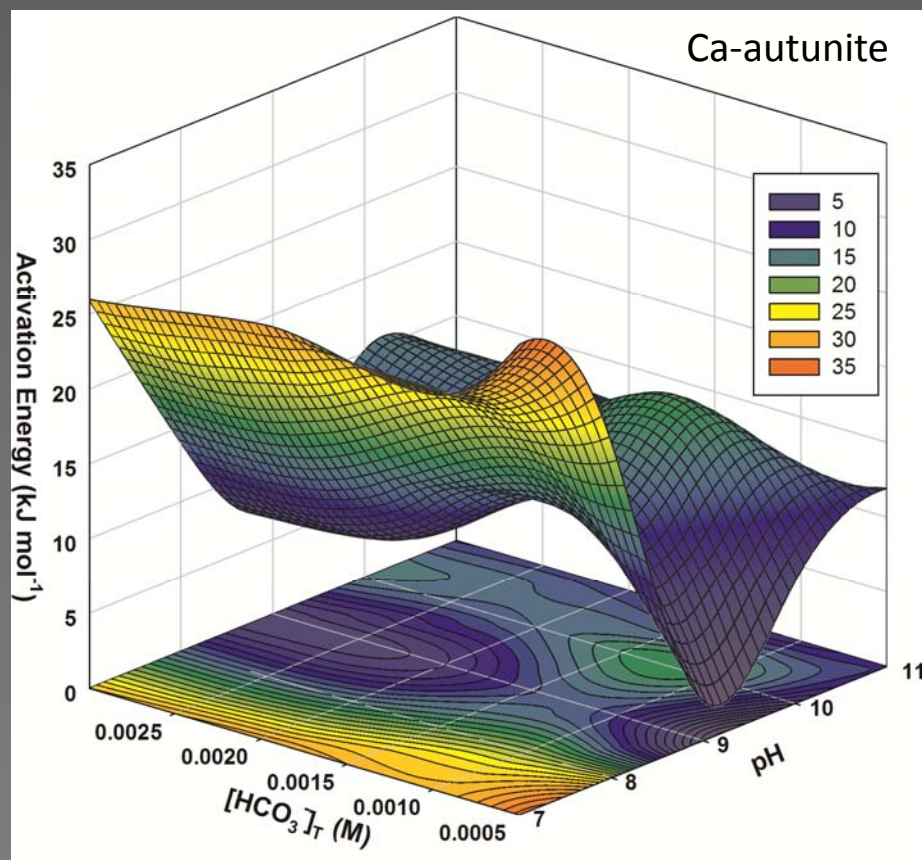
- Determined the intrinsic rate constant and power law coefficient (η , η) for Na, Ca-autunite.
- Applied transition state theory to estimate the activation energy, pseudo equilibrium constant and enthalpy of the system at different pH and bicarbonate values.
- At pH 7, the aqueous bicarbonate resulted in up to 370 fold increases in the rate of uranium release relative to the rate of uranium release in the absence of bicarbonate.

pH	Ca-autunite					Na-autunite				
	η	K (mol m ⁻² s ⁻¹)	E _a (kJ mol ⁻¹)	K _g	ΔH (kJ mol ⁻¹)	η	K (mol m ⁻² s ⁻¹)	E _a (kJ mol ⁻¹)	K _g	ΔH (kJ mol ⁻¹)
7	0.50 ± 0.12	1.09 x 10 ⁻⁰⁹	25.5	0.65	28.58	0.42 ± 0.13	3.62 x 10 ⁻¹⁰	26.87	0.16	19.39
8	0.52 ± 0.10	3.15 x 10 ⁻⁰⁹	18.16	0.79	11.83	0.57 ± 0.04	1.48 x 10 ⁻⁰⁹	15.63	0.35	17.24
9	0.79 ± 0.08	5.60 x 10 ⁻⁰⁸	7.99	4.2	11.9	0.48 ± 0.12	2.86 x 10 ⁻⁰⁹	14.2	1.39	24.8
10	1.09 ± 0.11	1.58 x 10 ⁻⁰⁶	13.77	25.97	15.88	0.39 ± 0.03	3.26 x 10 ⁻⁰⁹	7.07	1.34	12.44
11	0.94 ± 0.06	6.99 x 10 ⁻⁰⁷	11.4	27.75	13.84	0.51 ± 0.02	5.51 x 10 ⁻⁰⁹	13.06	1.3	9.41



Results

Variation of activation energies with pH and HCO_3^-





Major Accomplishments

- Data from Na- autunite dissolution experiments are incorporated into the Hanford model that simulates the application of polyphosphate injection technology to determine if the treatment is a feasible solution for uranium remediation at the 300 area of the Hanford site.
- Manuscript to be submitted to the Environmental Chemistry Journal:
 - Gudavalli, R.P., Katsenovich, Y., Wellman, D., Lagos, L., and B. Tansel, Effect of bicarbonate on the dissolution of sodium meta-autunite.



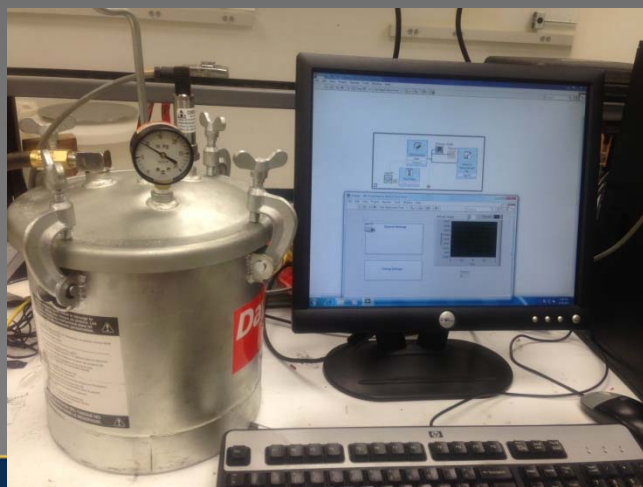
Major Accomplishments

- Manuscript under revision after peer-review:
 - Gudavalli, R.P., Katsenovich, Y., Wellman, D., Idarraga, M., Lagos, L., and B. Tansel. Comparison of the kinetic rate law parameters for the dissolution of natural and synthetic autunite in the presence of bicarbonate ions.
- Gave an oral presentation, published a proceeding paper and presented 2 student posters at the WM 2013 conference.
- Completed progress and year end reports on time according to schedule.
- Ravi Gudavalli completed his PhD from this research. Claudia Cardona has been accepted to the PhD program. Three students are currently performing master level research based on this topic.



Work Proposed for FIU Year 4

- **Task 1.1: Sequestering Uranium at the Hanford 200 Area Vadose Zone by In Situ Subsurface pH Manipulation Using NH_3 Gas**
 - Sequestering uranium – continue characterization of precipitates and examine the solubility of uranium-bearing solid phase created after NH_3 injection to manipulate of synthetic solutions mimicking the vadose zone at the Hanford Site 200 Area via isopiestic measurements and investigate the effect of environmental factors relevant to the Hanford vadose zone.



Completed a fabrication process of the isopiestic chamber using nickel crucibles placed in the aluminum block to facilitate heat exchange. The pressure transducer is connected to the acquisition system to monitor changes in vapor pressure.



Work Proposed for FIU Year 4

- **Task 1.2 U(VI)-microbial interactions** - examine the bacterial interactions with uranium of a less U(VI)-tolerant strain under oxidizing conditions and compare microbial dissolution of synthetic autunite versus natural autunite.
 - Finish bioleaching experiment using synthetic autunite instead of natural autunite.
 - Evaluate the U(VI) bioaccumulation by G968 in SGW with varying calcium and bicarbonate concentrations.
 - Complete AFM assessment on bacteria exposed to U(VI) in bicarbonate-bearing solutions to obtain quantitative data such as roughness analysis and adhesion forces.



Work Proposed for FIU Year 4

- **Task 2: Remediation research and technical support for Savannah River Site**
- **Subtask 2.1: Groundwater remediation at SRS F/H Area** - evaluate the effect of Si solutions injected directly into the groundwater on the removal of U and other radionuclides.
 - There are some concerns with the injection of silica solutions: high pH and potential clogging of the injection well. Continue discussion on the experimental matrix.
- **Subtask 2.2: Monitoring of U(VI) bioreduction after the ARCADIS demonstration at F/H- area**- collect field samples and analyze using ICP-OES and KPA instruments to assess the bioreduction of U(VI).
 - Take core samples and analyze changes.



Acknowledgment

- Funding for this research was provided by US DOE Grant Number: DE- EM0000598
- Drs. P. Sobecky and Robert J. Martinez, University of Alabama, for providing us with *Arthrobacter* sp. strains from the SMCC collection
- Rakesh Guduru, Vishal Musaramthota and Tom Beasley for their assistance with the AFM and SEM



Project Clients & Collaborators





Questions and Discussion

