



**Applied Research Center**  
FLORIDA INTERNATIONAL UNIVERSITY

# DOE-EM Cooperative Agreement – Year 4 Mid-Year Research Review Project 2: Rapid Deployment of Engineered Solutions to Environmental Problems

Presented: February 27, 2014  
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.*



# Project description

- Project 2 includes two tasks
- Task 1- performed to Hanford Site
- Task 2- performed to SRS



# Project Description- Task 1.1

- Vadose Zone (VZ) remediation is a significant challenge; in arid regions VZ can be very thick.
- Technology development needs deeper insight into important remedial and fate and transport processes of contaminants.
- FIU is investigating U(VI) contamination in the VZ of the Hanford 200 Area to reduce contaminant movement to groundwater below levels requiring control.
- Injection of reactive gases such as  $\text{NH}_3$  is a new technology to decrease U(VI) mobility in the VZ.



# Project Description- Task 1.1

- The injection of  $\text{NH}_3$  causes
  - formation of ammonia hydroxide ( $\text{NH}_4\text{OH}$ )
  - increase in pH
  - dissolution of Si and Al from soil minerals
- The subsequent decrease of pH to ambient conditions
  - results in co-precipitation of U(VI) with mobilized Si and other constituencies released from soil minerals.
- This can potentially control the mobility of U(VI) since co-precipitated contaminants are less available for migration.



## Project Description- Task 1.2

- Bacteria is an important environmental factor affecting the stability of soil minerals.
- FIU is investigating the effect of  $\text{HCO}_3$  on bacteria-uranium interaction
- This data supplements autunite mineral microbial dissolution studies using *Arthrobacter* strains commonly found in Hanford Site soil.





# Staff and Students

**Project Manager:** Leonel Lagos, Ph.D., PMP<sup>®</sup>

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

**Students/DOE Fellows:** Claudia Cardona  
Paola Sepulveda  
Vishal Musaramthota  
Robert Lapierre

**PNNL Collaborator:** Dr. Dawn Wellman



# Overview of Project Tasks

- **Task 1.1: Sequestering uranium at the Hanford 200 Area by *in situ* subsurface pH manipulation using NH<sub>3</sub> gas**
  - Evaluates the role of major pore water constituents (Si, Al, Ca, Mg, HCO<sub>3</sub> ions) on the U(VI) sequestration
  - studies the solubility of uranium-bearing precipitates created after NH<sub>3</sub> gas injection.
- **Task 1.2: Investigation of microbial meta-autunite interactions - the effect of bicarbonate and Ca ions**
  - Focuses on U(VI)-bacterial interactions using a less U(VI) tolerant strain of *Arthrobacter* species, isolated from Hanford soil
  - inspect the bacteria surface after exposure to U(VI) via AFM
  - investigate for cells viability
  - provide insight into microbiological processes affecting the fate and transport of U in carbonate-bearing subsurface environments.



## Task 1.1 - Progress

- Finalized experiments to evaluate the removal of elements from the U(VI)-Al-Si-HCO<sub>3</sub>-Ca solution mixtures.
- Summarized observations on U(VI) trapping in Si/Al-rich gels and possible mechanisms responsible for U(VI) removal in the FIU Year End Report.
- Stability of U-bearing precipitates is the next major question in the experimental plans.





## Task 1.1-Isopiestic measurements

- Solubility influences the behavior and fate of many contaminants.
- The importance of quantitative measurements of solubility resulted in numerous reviews of specific techniques to measure solubility.
- Isopiestic method is considered one of the most accurate methods for the solubility determinations.
- The basic feature-isothermal equilibration of samples of known mass and known initial concentration with reference standards in a common chamber.



## Task 1.1-Isopiestic measurements

- All of the solutions at equal vapor pressure or isopiestic equilibrium reach the same solvent activities.
- By equilibration, the samples with standard of known solvent activities under the conditions of experiment as a function of molality, the solvent activity of other solutions can be determined.
- The composition of all the solutions placed in the same vessel change during equilibration to reach a common vapor pressure at constant temperature.



# Task 1.1-Isopiestic measurements

- Isopiestic vapor-pressure chamber to conduct solubility experiments.
  - isopiestic chamber connected to the acquisition system
  - aluminum block with holes for the nickel crucibles





# Task 1.1-Isopiestic measurements

- An analytical balance with an uncertainty of  $\pm 1 \times 10^{-4}$  g was used to weigh all sample cups with lids.
- Pressure transducer is in the 0-30 Pisa range.





## Task 1.1-Isopiestic measurements

- Stock solutions were prepared by weight using distilled and deionized water.
- NaCl- reference solution (Alfa Aesar, Puratronic, 99.999% on metal basis).
- Conducted preliminary experiments to identify:
  - procedures for executing future deliquescence and phase changes experiments.
  - molecular weight of an unknown solute calculated according to Raoult's law.



## Task 1.1-Isopiestic measurements

- Six to eight samples with NaCl in 15cc nickel crucibles in the chamber (molalities 3 - 4 mol/kg).
- Degased chamber to 4-4.5 kPa and placed in an environmental chamber at 25°C.
- Monitored inside vapor pressure using a pressure transducer connected to a data acquisition system.
- The experiment was conducted for a period of a month and interrupted several times to record the weight of each crucible.





## Task 1.1-Isopiestic measurements

### Initial observations:

- Chamber is properly sealed, no vapor leakages.
- The water activity ( $a_w$ ) values of NaCl standard are in the range presented in literature.
- The difference between  $a_w$  values at equilibrium were  $\sim 0.3\%$  (same as in literature).
- Vapor pressure doesn't show values obtained by calculations.
- Working on reprogramming LabView to read the current pressure from the isopiestic chamber and automatically converting from psia to mm of Hg.



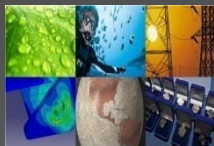
## Task 1.1-Isopiestic measurements

- Main goal is to investigate the relationship between deliquescence, relative humidity (RH) or  $a_w$  and T for a multicomponent salt system.
  - determine water activity (RH)
  - the occurrence of solid-liquid transitions in the multicomponent systems of U-bearing precipitates
- Started preparation of mixed samples containing Na-Si-Al-Ca-Cl-U-NO<sub>3</sub>-CO<sub>3</sub> ions
  - Solution compositions of known solute ratios are being formulated in crucibles



## Task 1.1 - Characterization of uranium bearing precipitates produced by the $\text{NH}_3$ injection method

- Analyzed mineralogical and morphological to identify the U(VI) mineral phases and structural features
  - X-ray diffraction (XRD),
  - scanning electron microscopy - energy dispersive spectrometry (SEM-EDS),
  - Raman Microscopy
- Initiated steps for continuing with elevated uranium content to enhance uranium presence during spectroscopic analyses



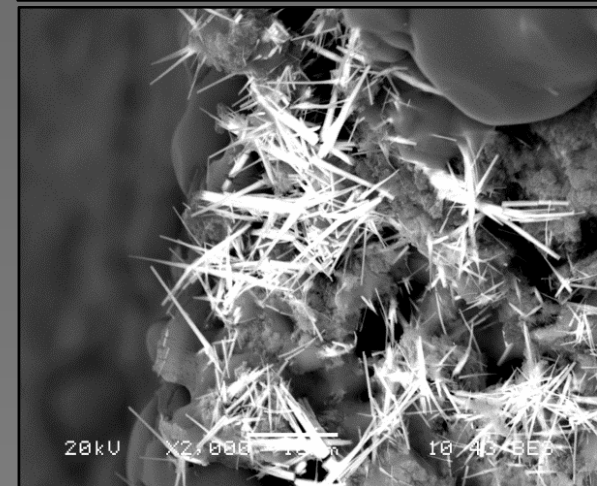
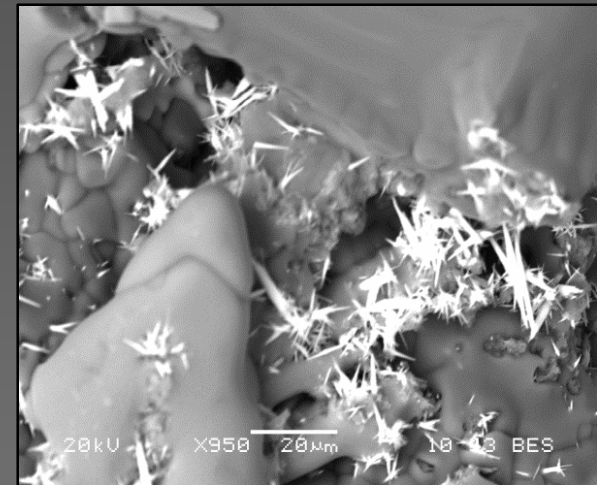
## Task 1.1 - Sample Preparation and Analysis

- Synthetic pore water prepared to mimic the Hanford Site's 200 Area vadose zone conditions
  - ~200 ppm of uranium
  - High (50 mM) bicarbonate [ $\text{HCO}_3^-$ ]
  - With (5 mM) and without calcium [Ca]
- Precipitates from prepared samples were dried and mounted to studs and slides for analysis by SEM/EDS and Raman Microscopy, respectively
- Samples were manually pulverized and analyzed by XRD along with method blanks



## Task 1.1 - Results

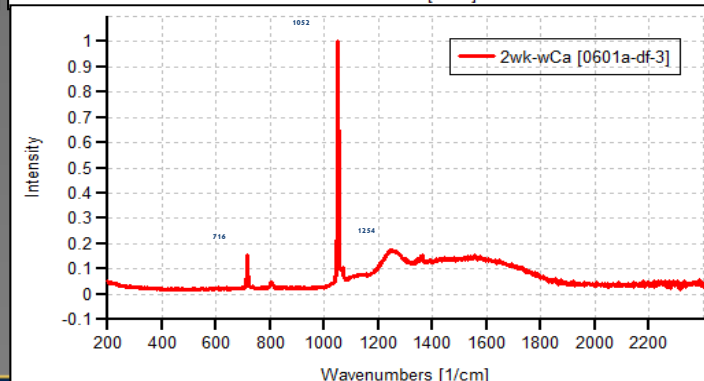
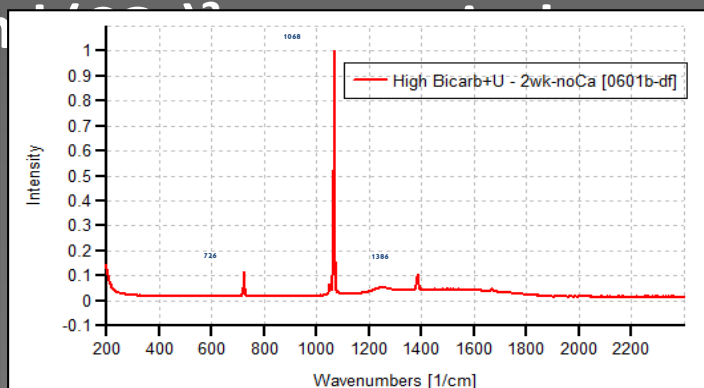
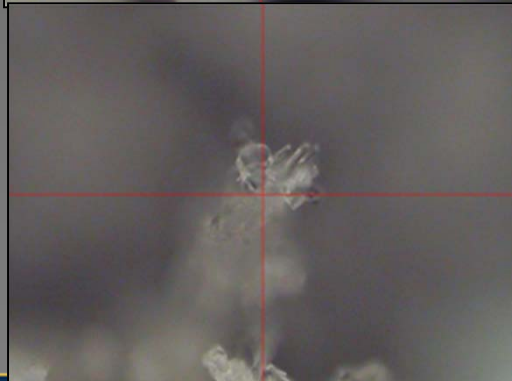
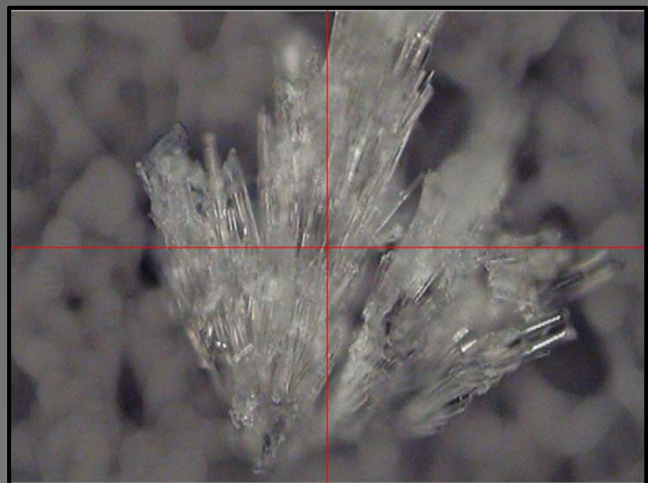
- Backscatter SEM images of samples prepared with (Top) and without (Bottom) calcium showing the crystal-like forms.
  - Significant presence of uranium was confirmed by EDS analysis
  - These U-rich samples were then selectively sent for subsequent analyses





## Task 1.1 - Results

- Micro-Raman image and spectra for samples prepared with (B) and without (T) calcium.
  - Bands at  $800\text{ cm}^{-1}$  &  $1000\text{ cm}^{-1}$  are consistent with reported values for the str

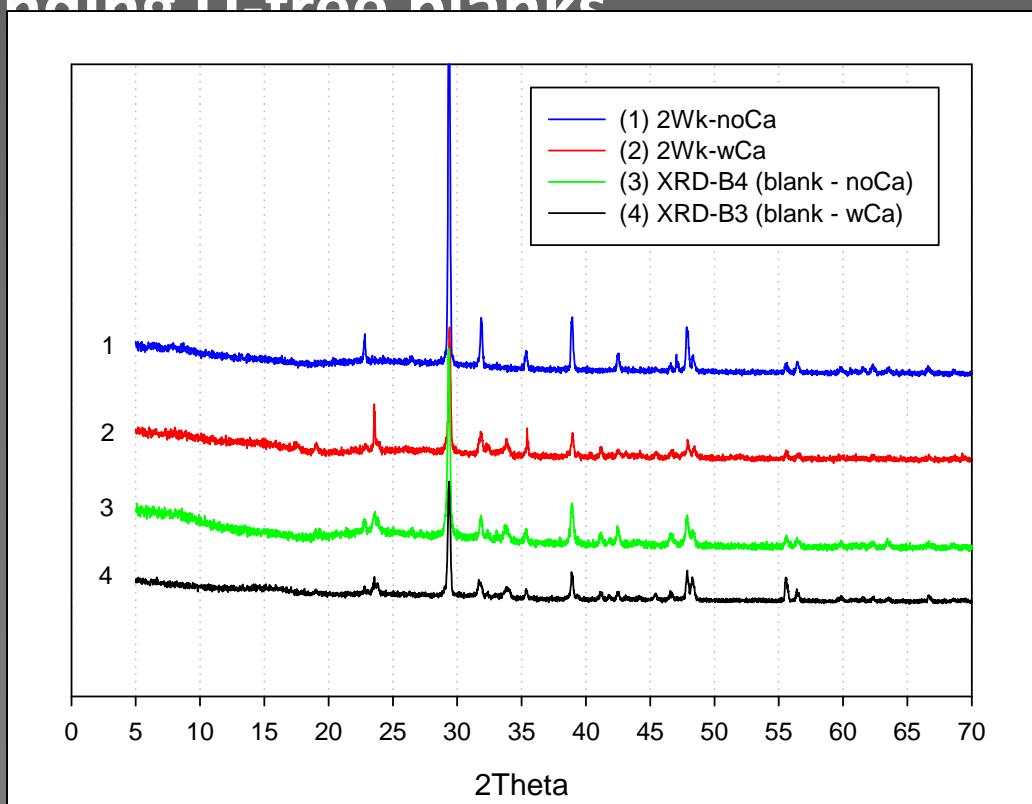






## Task 1.1 - Results

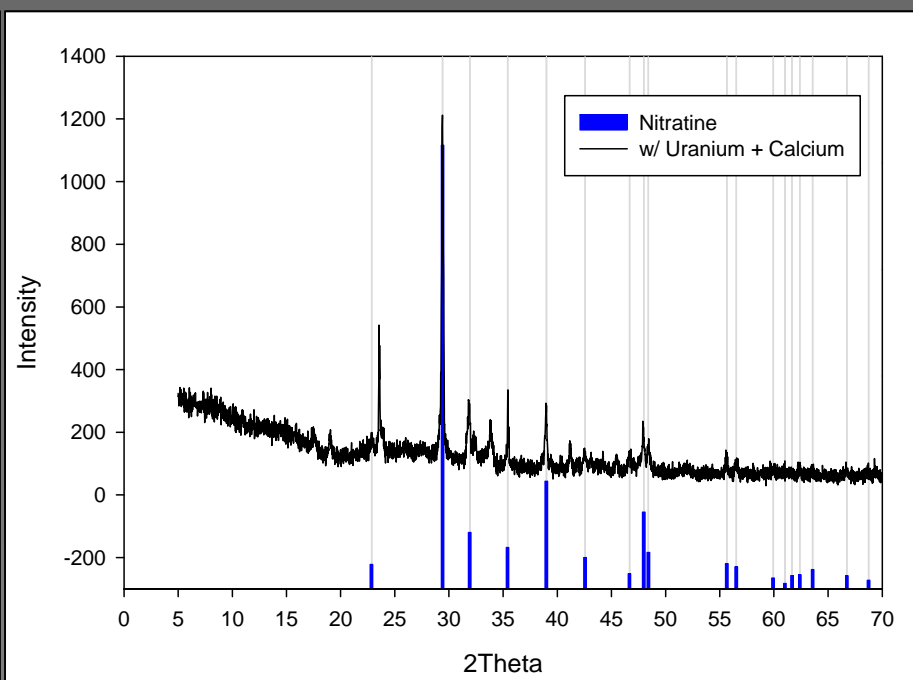
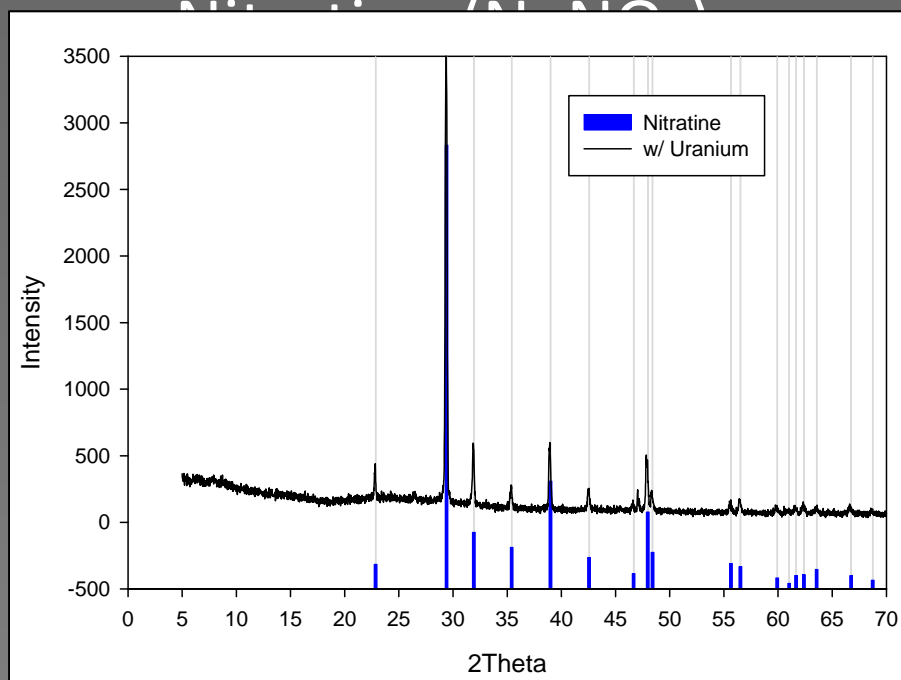
- Comparison of XRD patterns for samples prepared with and without calcium including their corresponding U-free blanks





## Task 1.1 - Results

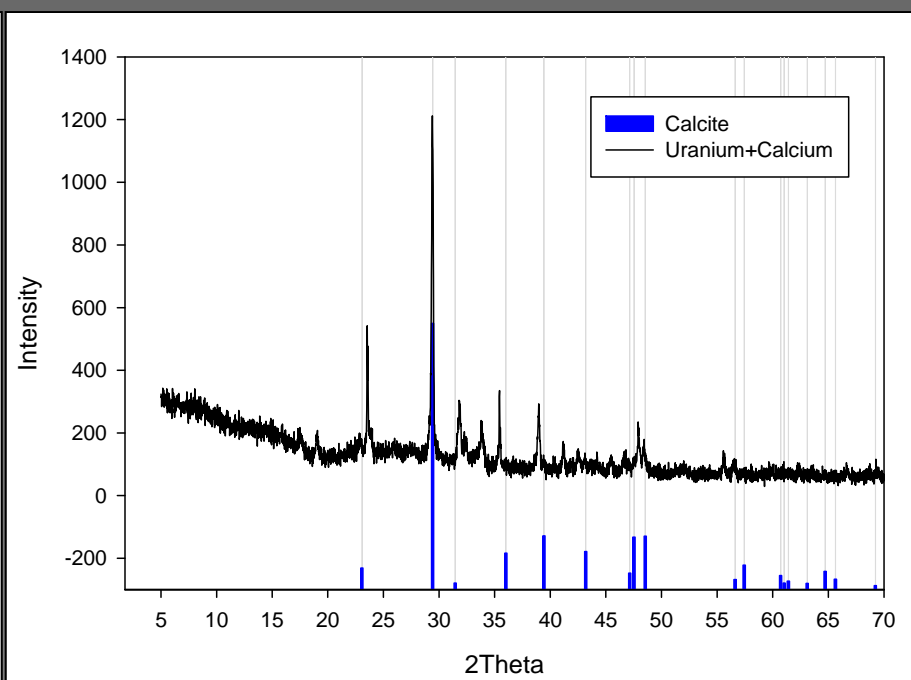
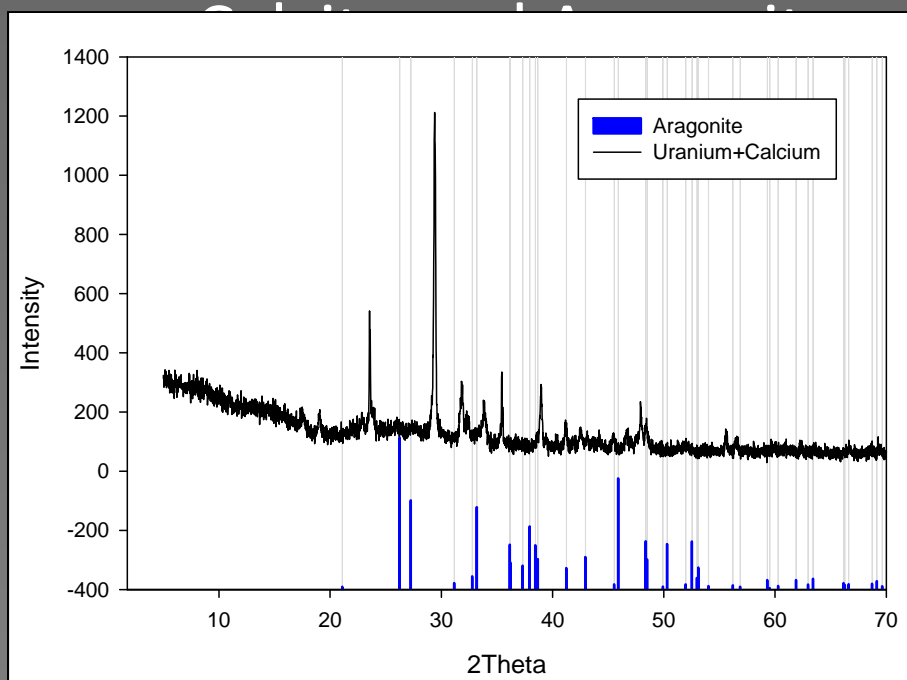
- *Match!* analytical software analysis showed a consistent and significant match with only one mineral for all data sets





## Task 1.1 - Results

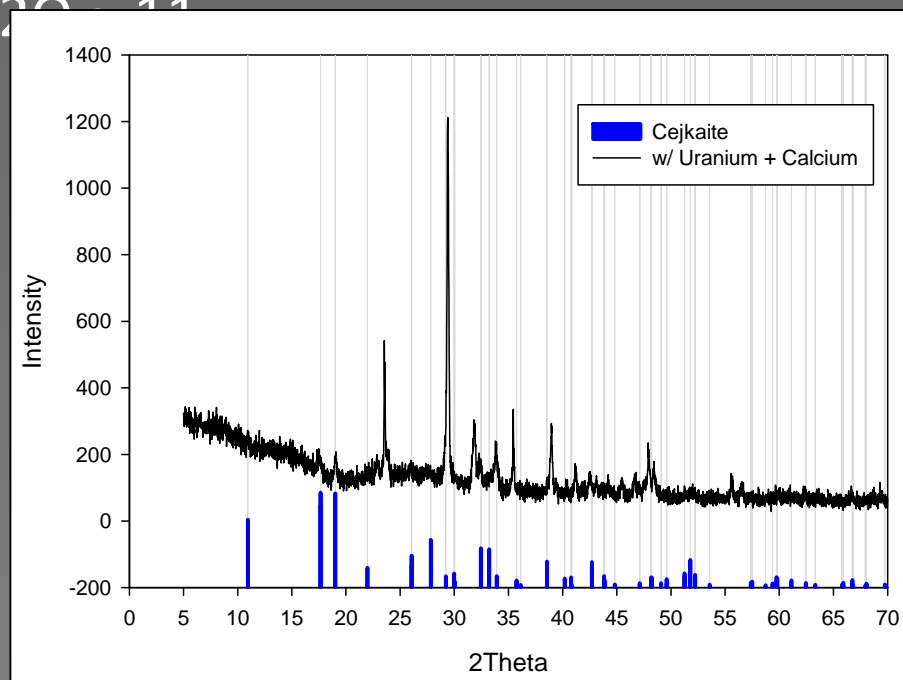
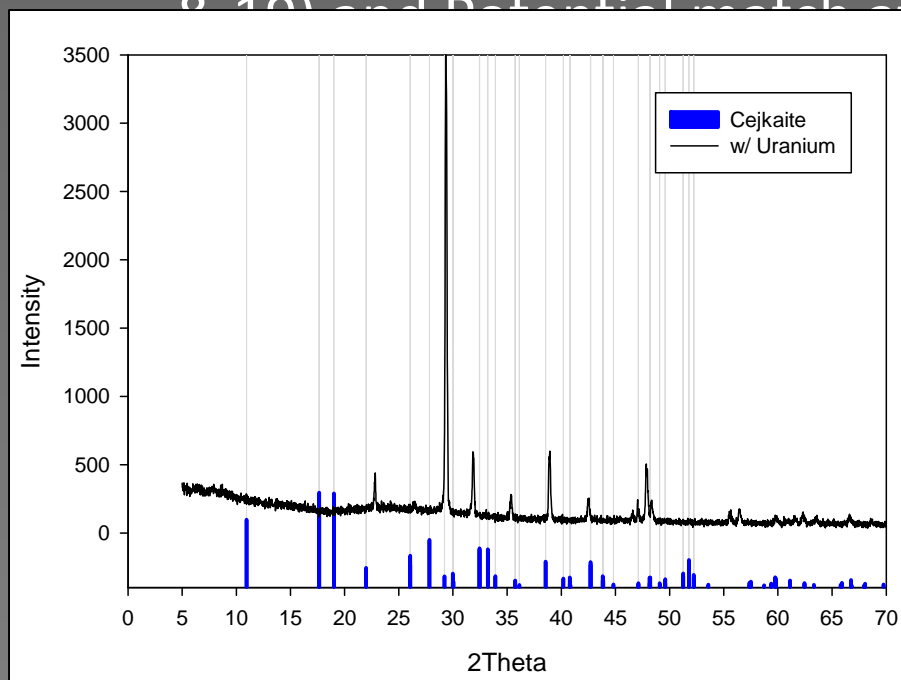
- Performed manual comparisons to calcium-based minerals predicted by Visual Minteq speciation software in concurrent studies





## Task 1.1 - Results

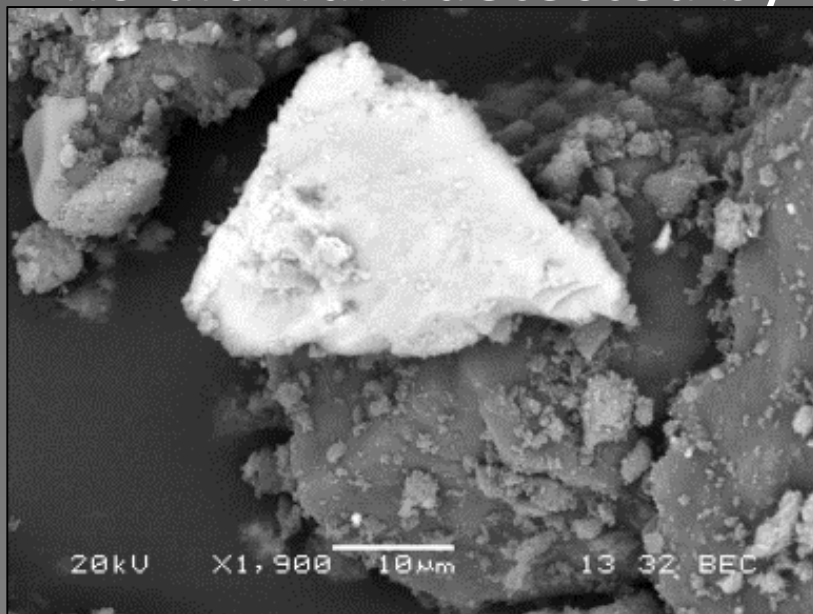
- Compared reference XRD patterns with those produced by uranium samples
  - Cejkaite for samples with and without calcium
  - Significant matches for the most prominent peaks ( $2\theta \approx 17.5$  and  $2\theta \approx 11$ )





## Task 1.1 - Results

- SEM images showing the uranium-rich areas of the pulverized calcium-containing sample after XRD analysis
  - no uranium detected by EDS in calcium-free sample





## Task 1.1- Summary

- The SEM analysis of the precipitates enables the spotting and confirmation of the sites with elevated uranium content
- Raman microscope analysis anticipated being able to distinguish and preferentially analyze the uranium containing crystal-shaped structures that were spotted in SEM
  - Analysis of the general area showed peaks consistent with a uranyl-carbonate compound





## Task 1.1- Summary

- Powder XRD analysis confirmed crystallinity
  - Overwhelming presence of nitratine ( $\text{NaNO}_3$ ) in all samples
  - No predicted or library searched diffraction patterns for uranyl compounds in the majority of samples
  - Speciation predictions suggested calcium uranyl carbonate forms but XRD pattern was more consistent with the sodium based Cejkaite [ $\text{Na}_4(\text{UO}_2)(\text{CO}_3)_3$ ]
- Further analysis to characterize the U-bearing phases are ongoing
  - Elevated uranium content
  - Evaluating methods of reducing nitratine formation



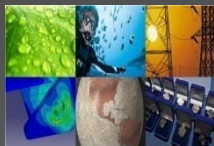
## Task 1.2 - Investigation on microbial meta-autunite interactions: the effect of $\text{HCO}_3^-$

- In 2005, the Hanford Site initiated a study to identify and evaluate remedial strategies to sequester uranium contamination in the subsurface.
- The injection of a polyphosphate amendment shown to significantly reduce uranium in groundwater plumes.
- Polyphosphate injection into contaminated groundwater resulted in the formation of uranyl phosphate solid phases 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.



## Task 1.2 - Investigation on microbial meta-autunite interactions: the effect of $\text{HCO}_3^-$

- Autunite, as a phosphorus-containing mineral, can attract bacteria to liberate phosphorus, causing U release back into the environment.
- 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 autunite minerals in oxidized conditions pertaining to the Hanford Site and to study the effect of *Arthrobacter oxydans* SMCC G968 strain on the U (VI) release from autunite



## Task 1.2 - Objectives

- Inspect the bacterial surface in the presence of bicarbonate and uranium in the solution
  - atomic-force microscopy (AFM)
- Live/Dead Viability Assay to supplement AFM analysis
- Quantitatively illustrate how bacterial cells are affected when exposed to uranium
  - in the presence of varying concentrations of  $\text{HCO}_3^-$  ions



## Task 1.2 - Methodology

- **Bacterial cells growth**
  - 5% PYTG liquid culture media
  - two days
- **Log 7 cells/mL of the bacterial stock solution were incorporated with uranyl nitrate and synthetic groundwater (SGW) media to create individual samples for analysis.**



## Task 1.2 - Methodology

- Analyzed samples included:

Concentration of Uranyl Nitrate (ppm)	Concentration of Bicarbonate (mM)
0	0
5	0
5	5
10	0
10	5





## Task 1.2 - Methodology

- **Samples for viability assessment were similar to samples used for AFM imaging.**
  - Grown in synthetic groundwater media and amended with varying concentrations of bicarbonate ions
- **3  $\mu\text{L}$  of the dye mixture was added for each mL of the bacterial suspension.**
  - equal parts of SYTO9 and propidium iodide

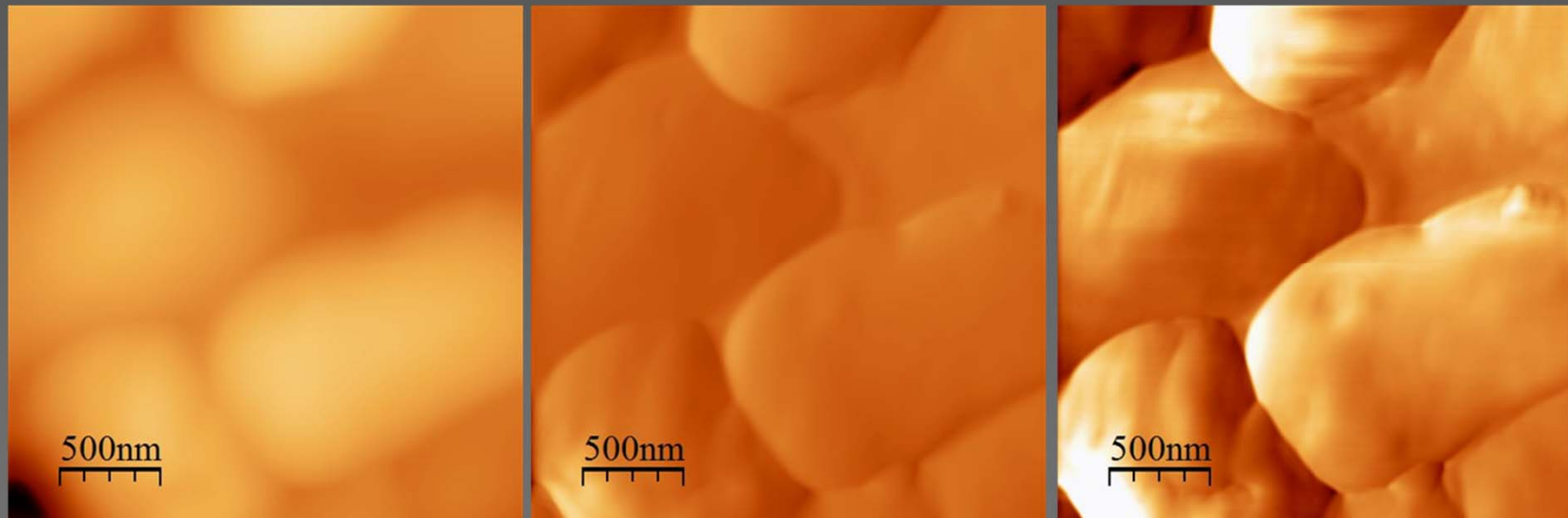


## Task 1.2 - Methodology

- 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 fluorescence microscope.



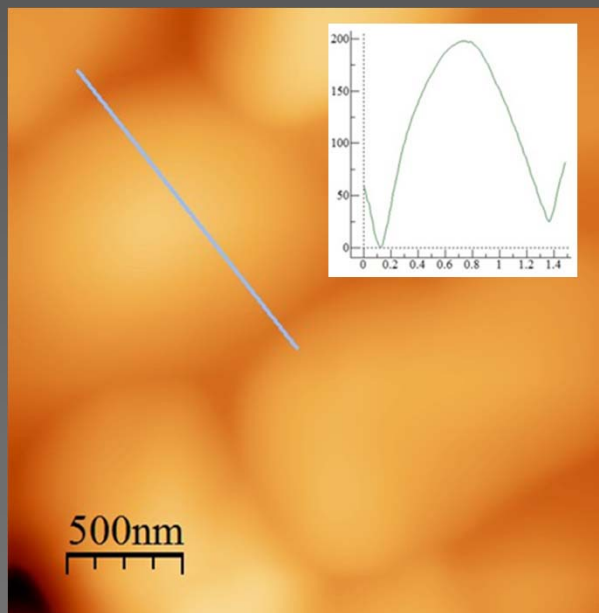
## Task 1.2 - AFM results, G968 strain



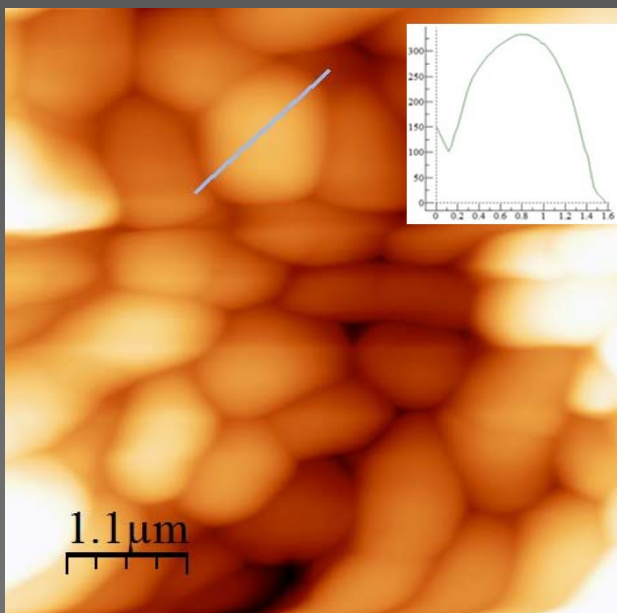
- G968 control sample (**scan size  $2.5 \times 2.5 \mu\text{m}^2$** ) illustrating the smooth bacterial surface.
- The topographic image on the left, deflection image in the middle and friction image on the right.



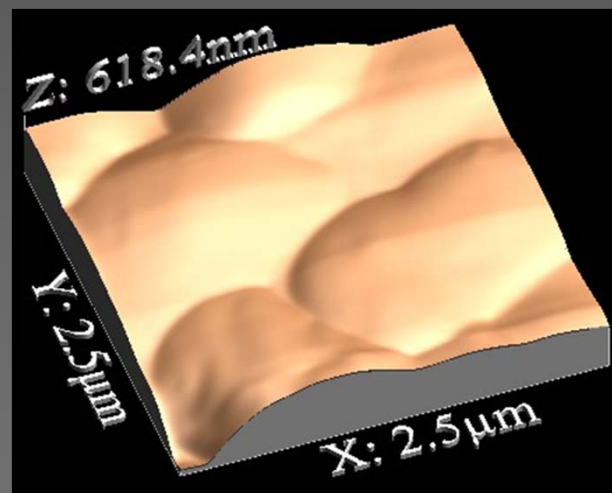
## Task 1.2 - AFM results, G968 control samples



Y-198nm  
X-1.3μm

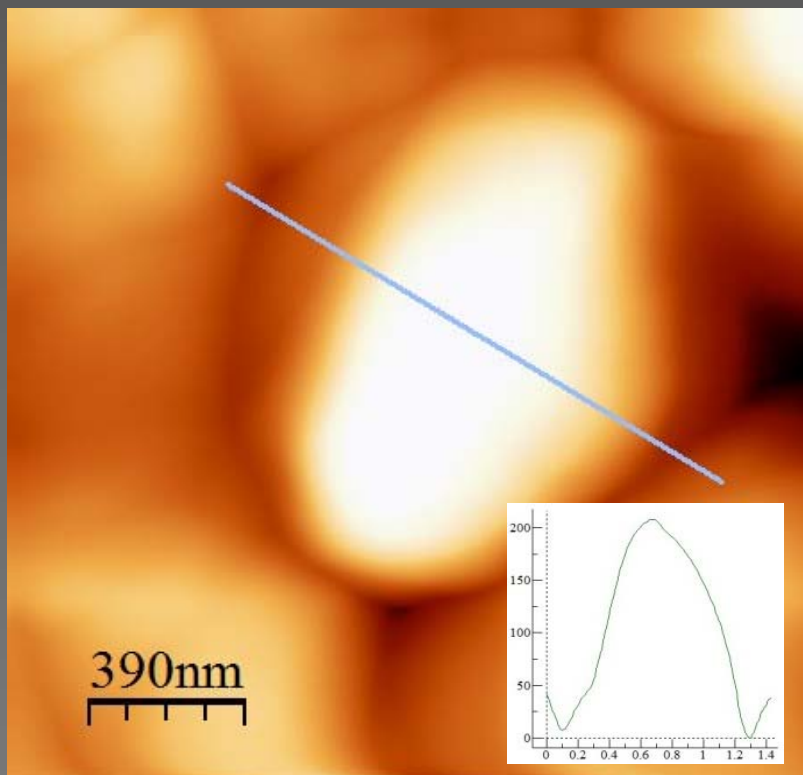


Y-350nm  
X-1.5μm

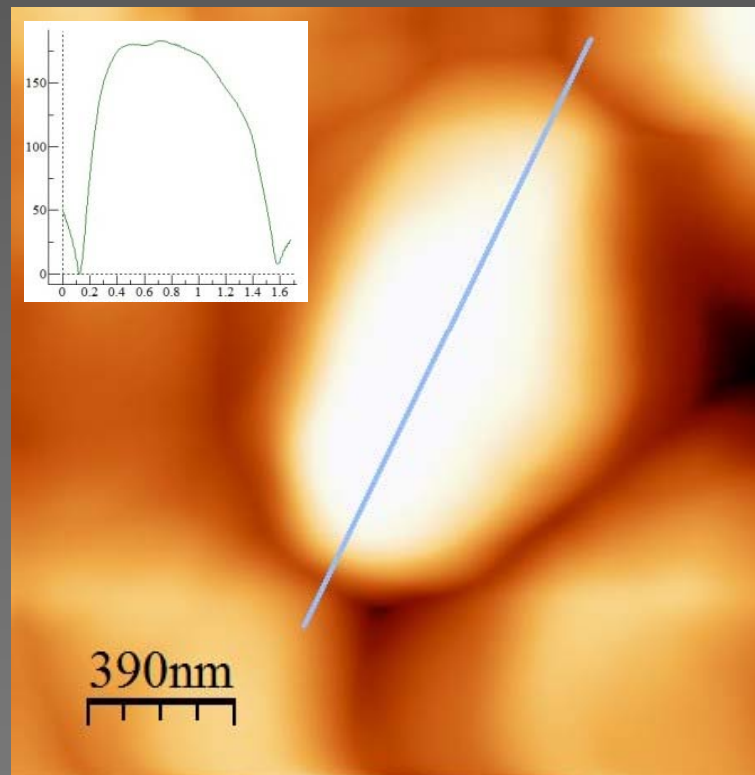




## Task 1.2 - AFM results, 10 ppm of U(VI), no HCO<sub>3</sub>



X- 1.2  $\mu\text{m}$   
Y- 205 nm



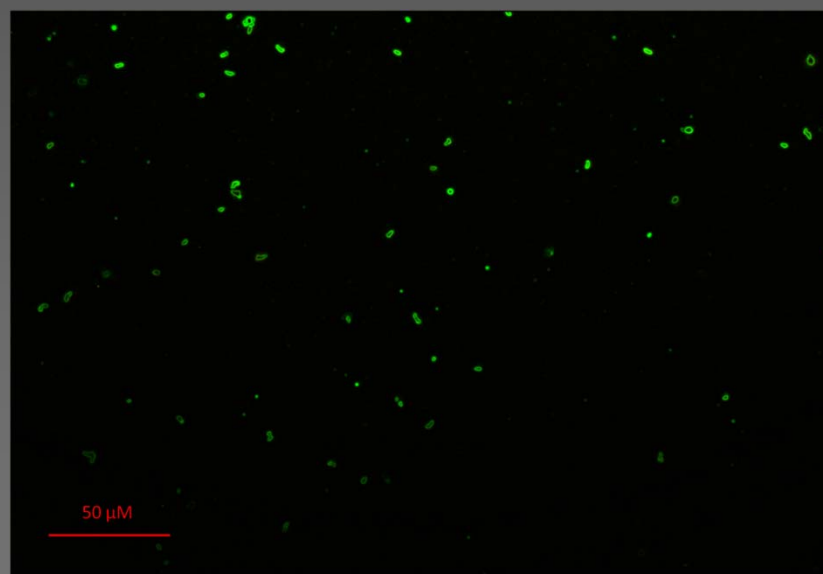
X- 1.5  $\mu\text{m}$   
Y- 175 nm



## Task 1.2 - Cell Viability via Live/Dead BacLight Bacterial Viability Kit



Live/ Dead assay of sample containing 5 ppm of U(VI) with no bicarbonate.

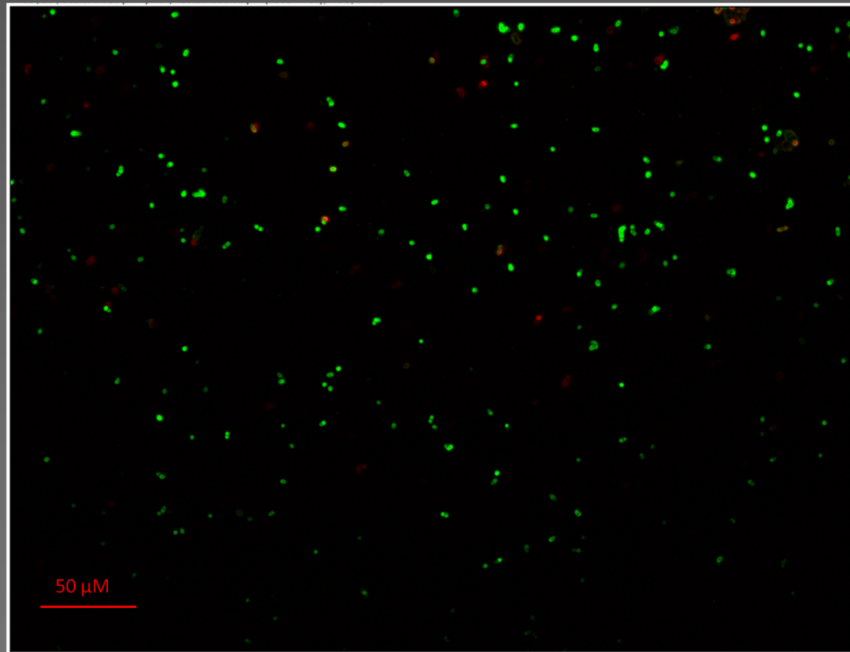


Live/ Dead assay of sample containing 5 ppm of U(VI) with 5 mM bicarbonate.

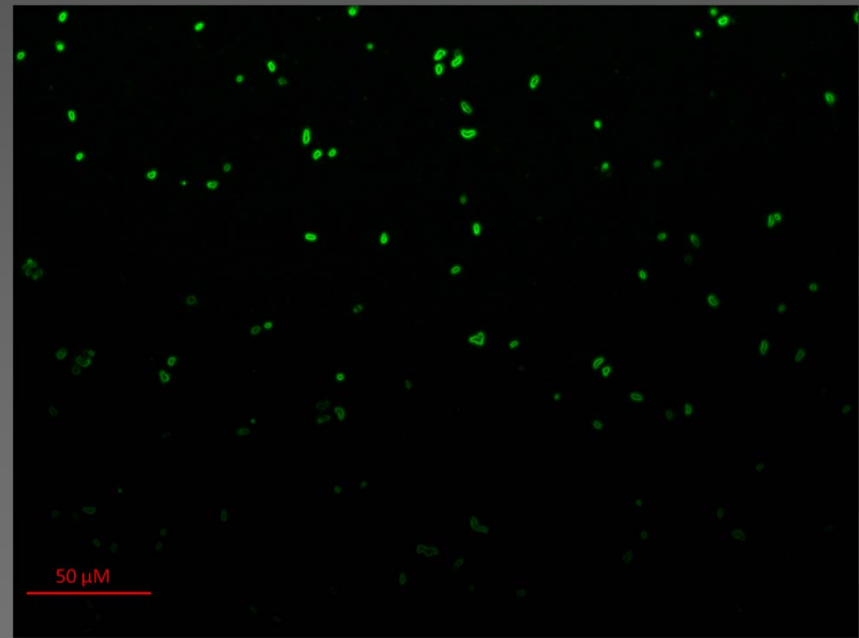




# Task 1.2 - Cell Viability via Live/Dead BacLight Bacterial Viability Kit



Live/ Dead assay of sample containing 10 ppm of U(VI) with no bicarbonate.



Live/ Dead assay of sample containing 10 ppm of U(VI) with 5 mM bicarbonate.



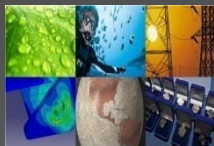
## Task1.2 - Accomplishments

- **AFM Imaging**
  - Images, roughness analyses, adhesion forces, and profile plots
- **Viability of cells**
  - Via live/dead assay
  - Via plates



## Task 1.2 - Future work

- Complete processing of images for roughness, adhesion forces, and profile plots.
- Complete viability assessment of G968 cells via Live/Dead assay.
- Prepare MS thesis on these results and manuscript for publication.



## Major Accomplishments

- Two manuscripts were published in peer-reviewed journals:
  - Gudavalli, R.P.; Katsenovich, Y.; Wellman, D.; Lagos, L.; and Tansel, B.; 2013. Quantification of kinetic rate law parameters for the dissolution of sodium meta-autunite as a function of aqueous bicarbonate concentration, *Environmental Chemistry* 10(6),p. 475-485.  
<http://dx.doi.org/10.1071/EN13117>
  - Gudavalli, R.P.; Katsenovich, Y.; Wellman, D.; Idarraga, M.; Lagos, L.; and Tansel, B.; 2013. Comparison of the kinetic rate law parameters for the dissolution of natural and synthetic autunite in the presence of bicarbonate



## Major Accomplishments

- **Manuscript in preparation for a peer-reviewed journal:**
  - Gudavalli, R.P., Katsenovich, Y., Wellman, D., Lagos, L., Effect of high concentrations of bicarbonate on the dissolution of calcium meta-autunite.
- **Submitted two proceeding papers to WM2014. Both papers were accepted for oral presentations.**
- **4 student posters to be prepared for the WM2014 conference.**
- **Completed FIU Year-End Report on time according to schedule.**



## Project Description- Task 2

- The study investigates the hypothesis that some uranium in the SRS F/H area treatment zone is bound to colloidal silica.
- Studies if any synergy exists between silica and humic acid (HC)
  - a major component of soil organic matter and an important ligand affecting the mobility behavior of radionuclides in the environment.
- A microcosm study to investigate if contaminants remain sequestered after the ARCADIS demonstration of *in situ* molasses addition for U(VI) remediation via the Enhanced Anaerobic Reductive Precipitation (EARP) process at the SRS F-Area.
  - mineralogical changes during reduction and re-oxidation processes to verify the viability of the technology.





# Overview of Project Tasks

- **Task 2.1 (new): FIU's support for groundwater remediation at SRS F/H Area** - This task investigates
  - Whether a base solution of dissolved silica can be used to replace the carbonate base previously used to increase the pH of the treatment zone
  - Any synergy exists between humic acid (HA) and colloidal silica that can influence the behavior of U(VI)
- **Task 2.2 (new): Monitoring of U(VI) bioreduction after ARCADIS demonstration at F-Area**
  - Investigates if phases of reduced iron (siderite and pyrite) would arise in the reducing zone.
  - Evaluates soil samples for re-oxidation.



# Staff and Students

**Project Manager:**

**Leonel Lagos, Ph.D., PMP<sup>®</sup>**

**Faculty/Staff:**

**Dr. Yelena Katsenovich**

**Dr. Ravi Gudavalli**

**Students/DOE Fellows:**

**Valentina Padilla**

**Joel McGill**

**Hansell Gonzalez**

**Christian Pino**

**PNNL Collaborator:**

**Dr. Miles Denham**

**Dr. Brian Looney**



## Task 2.1 - Background

- The Savannah River Site (SRS) was one of the major DOE industrial complex responsible for plutonium production
- The F/H Area Seepage Basins received ~ 1.8 billion gal of acidic waste; **Contaminated plume pH: 3 - 5.5**
- The acidic nature of the basin waste solutions caused the mobilization of metals and radionuclides
- F-Area groundwater contamination:
  - **tritium, uranium-238, iodine-129, strontium-90, curium-244, americium-241, technetium-99, cadmium and aluminum.**



## Task 2.1 - Background

- The pump-and-treat water treatment unit built in 1997 became less effective.
  - **Issues: costly \$1 million/month; produced solid rad. waste**
- A base injection system using a sodium hydroxide solution directly into the F-Area groundwater started in 2004, replacing the expensive pump-and-treat unit.



## Task 2.1 - Objective

- Investigate if dissolved sodium silicate solutions have enough alkalinity to replace the carbonate base used to correct the acidic nature of the contaminated sediments.
- Any synergistic interactions between U(VI) ions, HA and Si:
  - Study the influence of humic acid (HA) and Si on the sorption of U(VI) onto F/H Area sediments.
- Study the effects of environmental variables such as pH, concentrations of HA and Si on the removal behavior of U(VI) in the multi-component batch systems.



## Task 2.1 - Progress

- Initiated investigation to find possible synergy between humic acid and colloidal silica on U(VI).
- Created an experimental matrix involving colloidal silica, humic acid, and naturally present sediment.
- Determined the desired concentrations and ratios of each of constituents to simulate the U(VI) sorption process.





## Task 2.1 - Experimental Approach

- Systems to analyze with pH adjusted in the range 3 - 8.

Batch #	Silica (Si), mM	Humic Acid (HA), ppm	Uranium [U(VI)], ppm	Sediments
1	3.5	-	0.5	-
2	3.5	10	0.5	-
3	-	10	0.5	-
4	3.5	-	0.5	Yes
5	3.5	10	0.5	Yes
6	-	10	0.5	Yes
7	-	-	0.5	Yes



## Task 2.1 - Experimental approach

- Prepared soil mixture by combining soil samples from various depths ranging from 60-105 ft.
  - 10 grams of soil from each depth was used to prepare 60 gram soil mixture
- Sample 4, 5, 6, and 7 each contained 800 mg.
  - soil/solution ratio is 1:20
- Amended test solutions with 0.5 ppm U(VI).
- Adjusted pH using 1% HCl or 0.1M NaOH.
- Each sample was prepared in triplicate.



## Task 2.1- Experimental approach

- Samples were placed on the shaking platform for 24 hours (Chen & Wang 2006), followed by centrifuging at 2700 rpm & 22°C for 30 mins.
- Samples with sediments were filtered using 0.45µm syringe filter.
- Calibrated Optima ICP and KPA instruments to run samples for analysis.



# Task 2.1 - Experimental Set Up





## Task 2.1 - Sample Preparation

pH 4 Adjusted Set	SiO <sub>2</sub> (ml)	Humic Acid (ml)	Sediments (mg)	Uranium U(VI) (ml)	Acid (ml)	Base (ml)	Water (ml)	Initial pH	Total Volume (ml)
Batch #1	4.48			0.02	0.015	0.045	35.440	4.00	40
Batch #2	4.48	4.00		0.02	0.005	0.000	31.495	4.03	40
Batch #3		4.00		0.02	0.000	0.005	35.975	4.00	40
Batch #4	4.48		800.00	0.02	0.000	0.020	35.480	3.98	40
Batch #5	4.48	4.00	800.00	0.02	0.020	0.000	31.480	4.04	40
Batch #6		4.00	800.00	0.02	0.000	0.000	35.980	4.02	40
Batch #7			800.00	0.02	0.000	0.010	39.970	4.02	40
Total	125.44	112	22400	0.56	0.060	0.225	881.44	4.01	1120



## Task 2.1- pH4 Results

Sample-Description	U(VI) Avg Removal, %	Std. Dev	Si Avg Removal, %	Std. Dev	Fe, ppm
Batch 1	34.36	23.13	94.09	0.92	No soil
Batch 2	22.33	1.65	62.76	13.17	No soil
Batch 3	15.18	3.42	No Si		No soil
Batch 4	57.71	7.50	79.19	2.27	0.1-0.2
Batch 5	87.36	8.81	83.37	5.58	0.2-1.3
Batch 6	78.10	1.34	No Si		0.2-0.6
Batch 7	45.83	3.02	No Si		0.1-0.3



## Task 2.1- pH4 Conclusions

- The max U(VI) removal of  $87.36 \pm 8.81\%$  at pH 4 was observed for batch #5.
- The next highest U(VI) removal of  $78.1 \pm 1.34\%$  was observed for batch #6 (no colloidal silica).
- Colloidal Si gave higher removal without HA.
- In combination with sediments, Si showed lower results on U removal than HA.





## Task 2.1 - pH5 Results

Sample Description	U(VI) Avg Removal, %	Std. Dev	Si Avg Removal, %	Std. Dev	Fe, ppm
Batch 1/ Filtered	87.92/ 92.8	1.98/0.8	94.09	0.92	No soil
Batch 2	29.65	19.55	62.76	13.17	No soil
Batch 3	9.29	5.43	No Si		No soil
Batch 4	88.66	0.39	79.19	2.27	0.01-0.02
Batch 5	81.16	3.7	83.37	5.58	0.09-0.11
Batch 6	86.05	2.47	No Si		0.19-0.24
Batch 7	88.81	3.34	No Si		0.1



## Task 2.1 - pH5 Conclusions

- Sediment bearing samples with Si showed higher U removal ( $92\pm 0.8$ ).
- HA slightly reduced U adsorption.
- Need to repeat several samples after completion of KPA retrofit.



## Task 2.1 - Moving Forward

- Retest pH 5 samples with KPA that gave errors.
- Almost completed preparation of pH 6 test group; shaking centrifugation and filtering is underway; soon to complete data analysis via KPA & ICP.
- Begin pH 7 preparation and testing.
- Create graphical representation of U (VI) removal data of various batches as a function of pH.



## Task 2.1 - Future Work

- Determine which batch combination has the highest uranium removal capabilities and at which pH.
- Determine at which pH values HA and colloidal Si possess the most notable adsorption properties.
- Discuss experimental results with SRS contacts.
- Explore the effect of higher HA concentration.

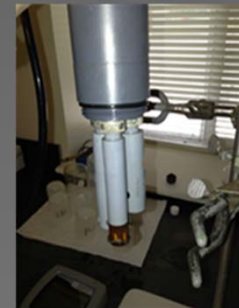
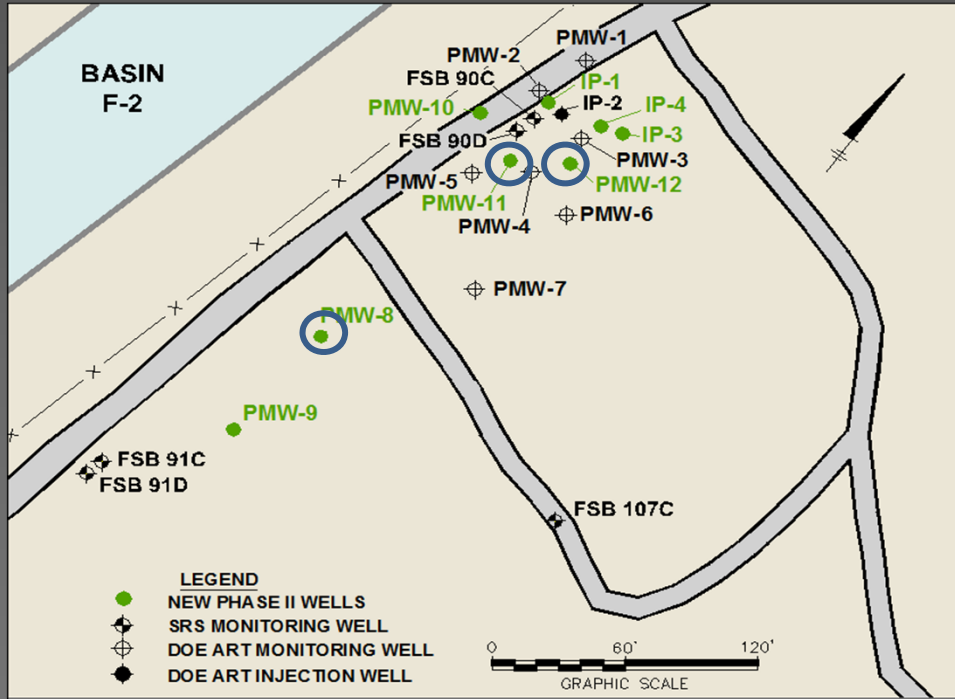


## Task 2.2 - Progress

- Design Microcosm experiment that mimics Enhance Anaerobic Reductive Precipitation (EARP) implementation technology performed at Savannah River Site by ARCADIS
- Completed two batches of samples
- Designed re-oxidation period with three environments
- Completed XRD Analysis

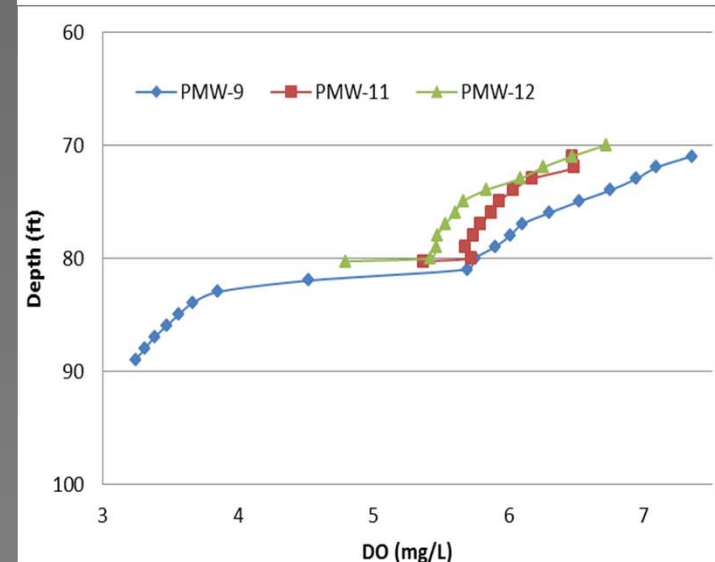
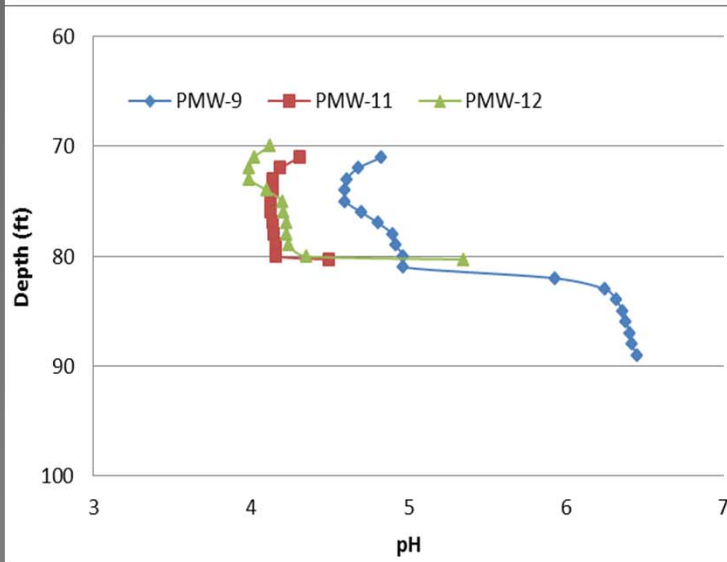
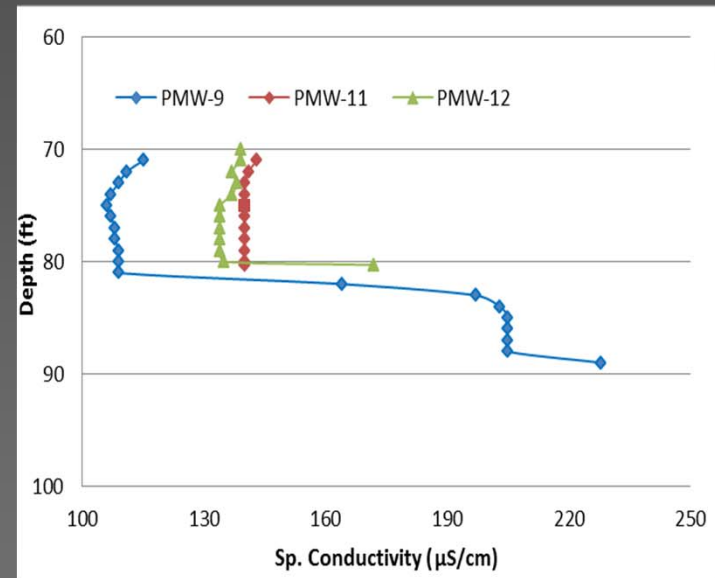
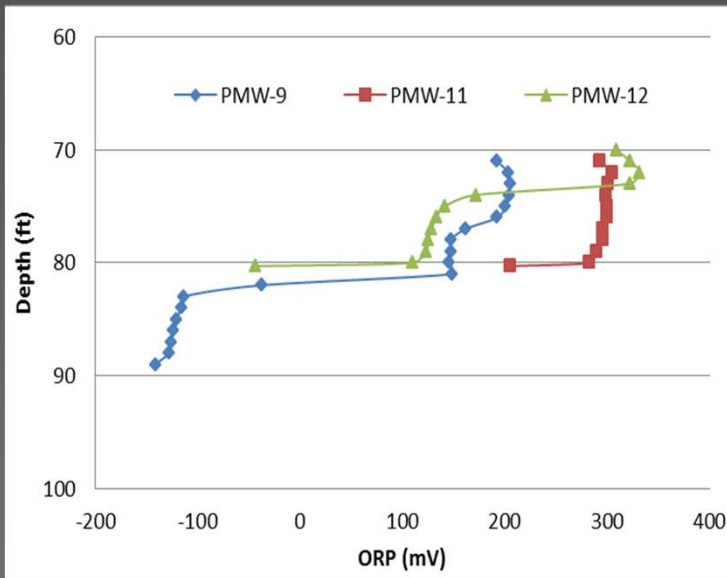
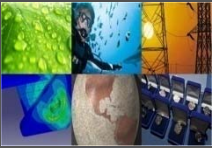


# Task 2.2 - Depth Profile Analysis



- Contamination source: radiological waste
  - Nuclear reactors
  - Support facilities

# Task 2.2 - Depth Profile Analysis





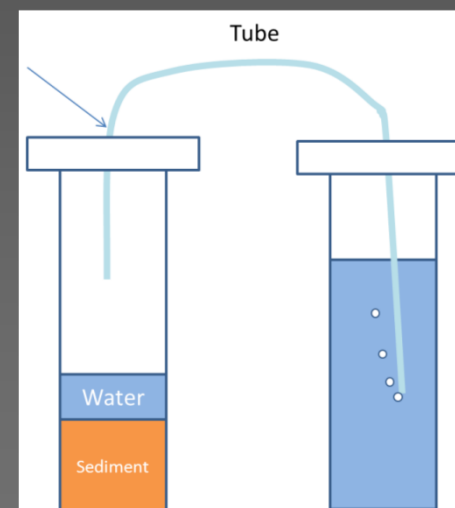


## Task 2.2 - Previous Experiments

- 20 mL of sediment
- 10 mL solution mixture
- DI water
- 0.014 g of  $\text{NaNO}_3$  (equivalent to 200 mg/L)
- 7 g of molasses (equivalent to 20% by weight of the solution).



Microcosm set up



Wrap tape around the tube to prevent any possible air exchange



## Task 2.2 - Previous Experiments

Unsuccessful experiment → Why?

- Slow rate of bacterial growth
- Air inside the tube
- System was not completely sealed off
- Bacteria were not present in the soil



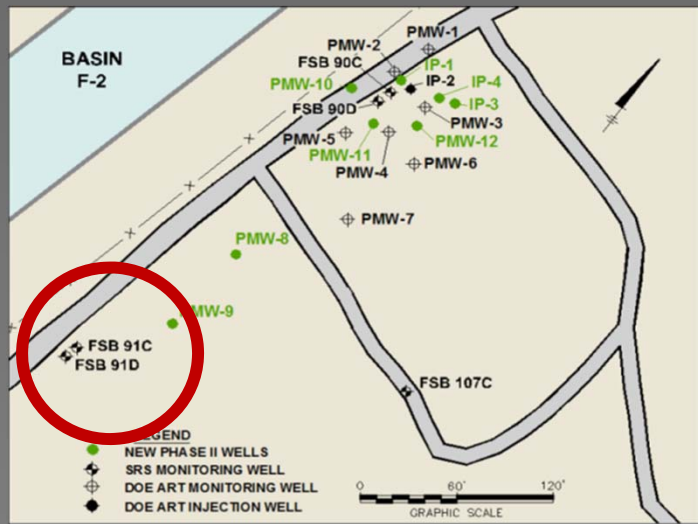
Samples after 4 Weeks



# Task 2.2 - Sample Source



Core samples from FSB 91C





## Task 2.2 - Experimental Approach

Anaerobic Chamber → Prevents O<sub>2</sub> from entering the system







## Task 2.2 - Experimental Approach

- To enhance bacterial growth, basal medium was added to the solution

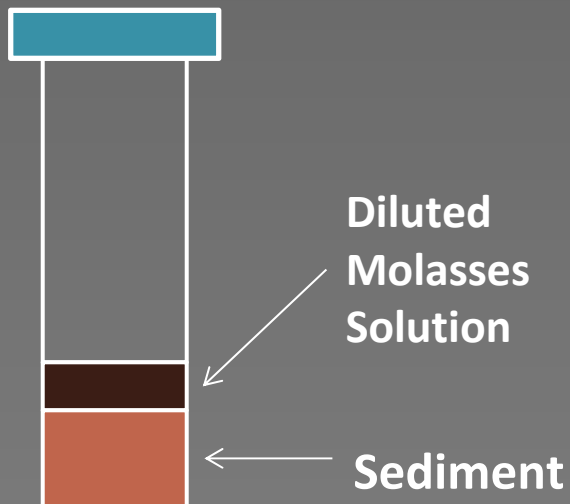
The basal medium (per liter)
1.5 g $\text{NaHCO}_3$
0.2 g $\text{NH}_4\text{Cl}$
0.1 g $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$
0.055 g $\text{KH}_2\text{PO}_4$
0.001 g resazurin as a redox indicator
0.039 g/L $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ as a sulfur source and reductant
0.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$

Trace metal solutions recipe (per liter)
0.005 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$
0.005 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$
0.001 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
0.0006 g $\text{H}_3\text{BO}_3$
0.0001 g $\text{ZnCl}_2$
0.0001 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$
0.0001 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$
0.002 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$



## Task 2.2 - Experimental Approach

- **Composition of the sample:**
  - Sediment to fill 10-mL volume
  - 10 mL of diluted molasses solution



Samples placed in the anaerobic chamber



## Task 2.2 - Results

- **Batch 1**
  - 1 set with duplicates (12 samples)
  - Descending trend in pH for all samples but one (95')

pH Measurements							
	Sample	65'	80'	90'	95'	100'	105'
Week 1	1	5.53	5.21	4.65	4.29	4.89	4.54
	2	5.67	5.08	4.75	4.28	5.15	4.40
Week 6	1	4.28	4.26	4.10	4.63	4.38	4.36

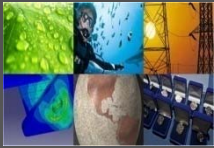




## Task 2.2 - Results

- **Batch 2: 1 set with duplicates (12 samples)**
- **Modifications:**
  - Amended with 100  $\mu$ L bacteria (from anaerobic digester)
  - Each sample was supplemented with additional 0.5 mL of molasses a week after initial addition

pH Measurements							
	Sample	65'	80'	90'	95'	100'	105'
Initial	1	6.27	4.33	6.59	7.12	5.17	6.53
	2	6.68	3.71	6.42	7.01	6.28	7.01
Week 1	1	3.79	3.81	4.09	4.2	3.89	4.22
	2	3.86	4.27	4.09	4.29	3.93	4.25
Week 6	1	3.75	3.90	3.81	3.80	4.07	3.85



## Task 2.2 - Control Samples

- **Composition of the sample:**
  - Sediment to fill 10-mL volume
  - 10 mL of diluted basal medium (no molasses)
- pH of basal Medium = 8.55
- Opposite to Batch sample results
  - Ascending instead of Descending trend in pH
- Contradicts hypothesis that the pH of the samples was dropping due to the reaction of the solution with the acidic soil
- Supports that pH is increasing due to the **production of organic acids via the molasses**

pH Measurements		
Depth	Initial	After 2 weeks
65'	6.89	7.85
80'	5.76	5.65
90'	6.74	8.06
95'	7.52	8.56
100'	6.43	7.88
105'	7.13	8.46



## Task 2.2 - Fungi Growth



Example of fungi growth on some of the samples.



## Task 2.2 - Re Oxidation Period

- After six weeks samples will be sacrificed
- Three types of environments:
  - Anaerobic chamber with no Oxygen
  - Small chamber containing 2000 ppm Oxygen
  - Work bench at atmospheric Oxygen levels

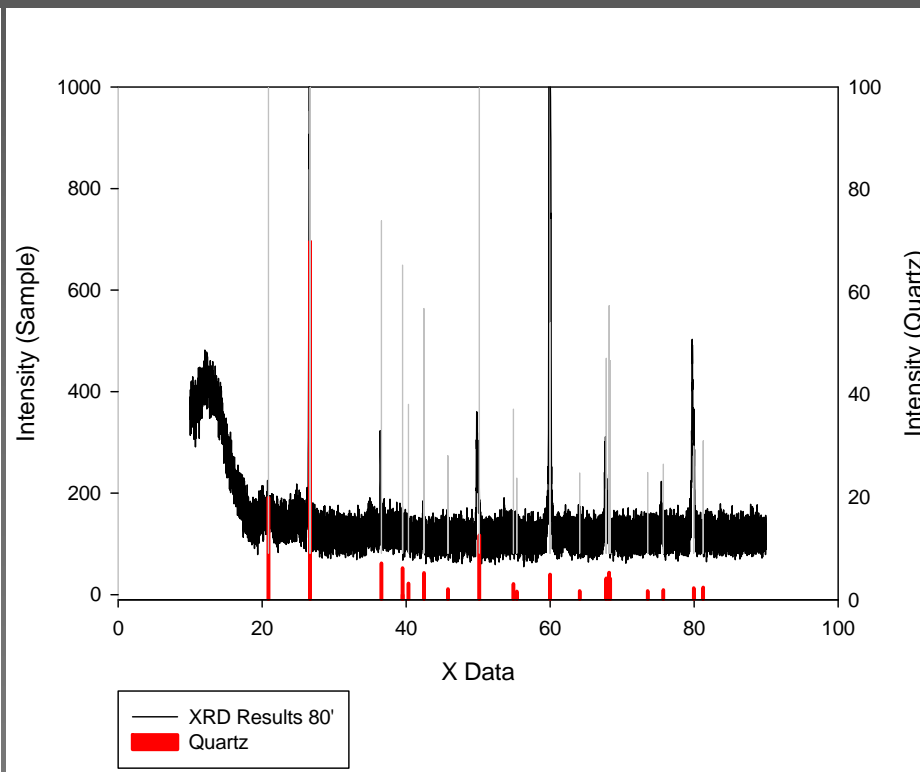
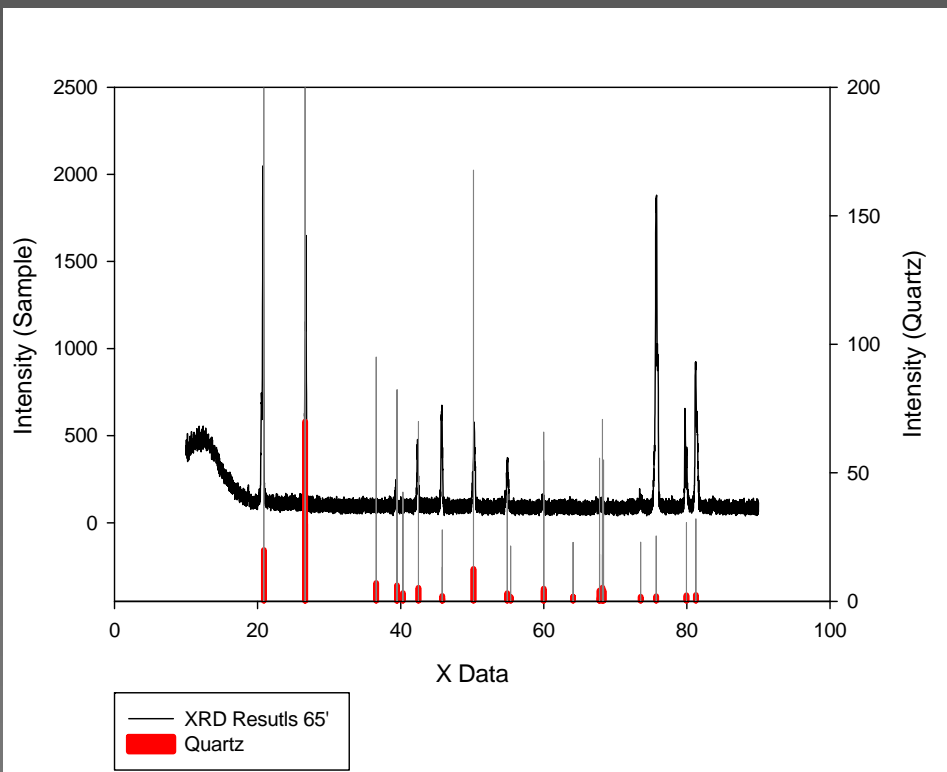


## Task 2.2- Analytical Methods

- Solid Phase → XRD
  - Samples are dried in their respective oxygenated environments (3 weeks)
  - Transported in sealed containers
- Liquid Phase → ICP
  - Liquid is centrifuged
  - Supernatant is filtered (0.45  $\mu\text{m}$ )

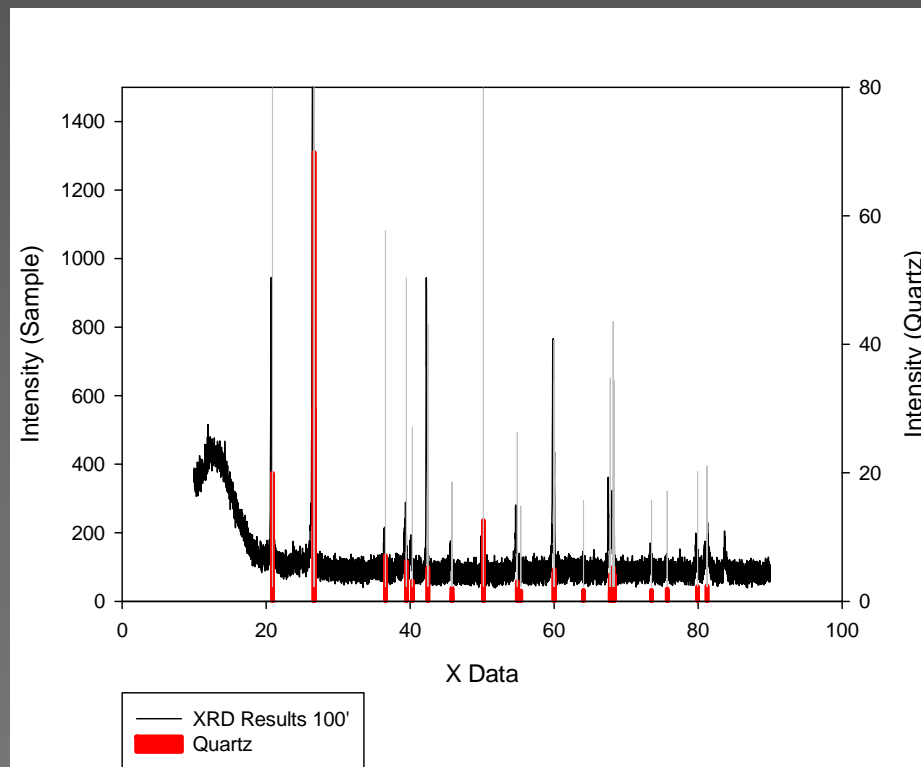
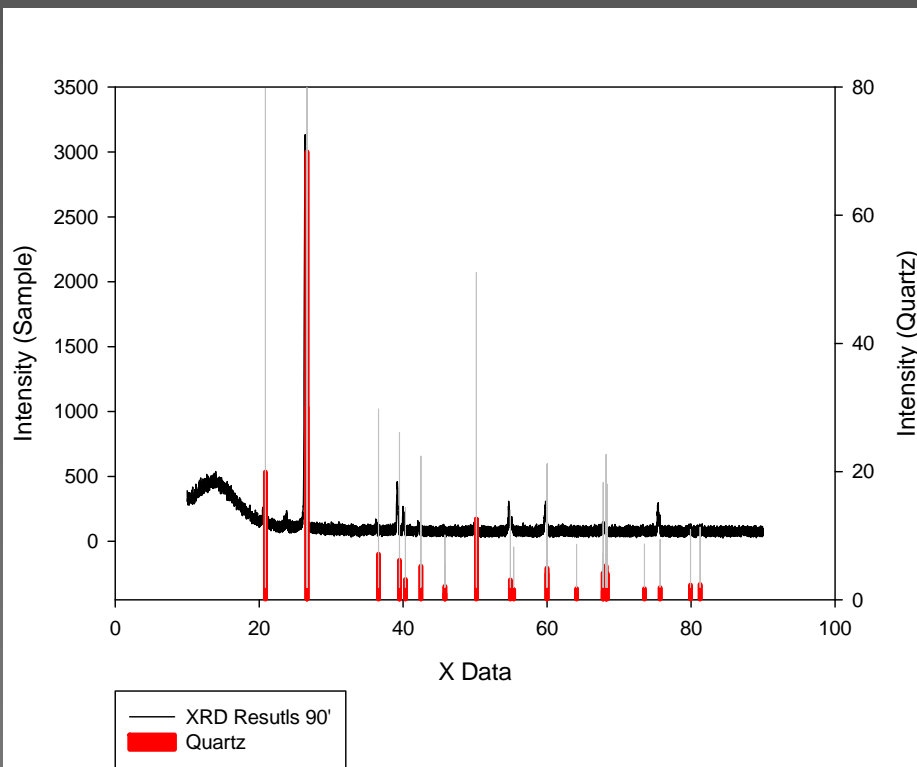


# Task 2.2-XRD Analysis (Original Samples)





## Task 2.2-XRD Analysis (Original Samples)



- Most likely match for the results is quartz; 80% of the peaks match and line up at most depths.
- The intensity ratios are sometimes off, could be because those peaks belong to some other mineral also present in the sample but in smaller quantities.





## Task 2.2 – Future Work

- Perform XRD analysis for remaining samples
- Continue with identifications of minerals and analysis of XRD Results
- Receive mineral trap diffusion samples and start analyzing them via SEM/ED and XRD



# Major Accomplishments

- 2 student posters to be prepared for the WM2014 conference.
- Hansell Gonzalez has been accepted to the Ph.D. program. Two students are currently performing master level research based on the project's tasks.



# Acknowledgments

- Funding for this research was provided by US DOE Contract: DE- EM0000598