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: January 24, 2014
to the U.S. Department of Energy
by Dr. Yelena Katsenovich,
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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 NH_3 is a new technology to decrease U(VI) mobility in the VZ.

Project Description- Task 1.1

- The injection of NH_3 causes
  - formation of ammonia hydroxide (NH_4OH)
  - increase in pH
  - dissolution of Si and Al from soil minerals
- The subsequent decrease pf 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 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

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

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Overview of Project Tasks

- **Task 1.1: Sequestering uranium at the Hanford 200 Area by *in situ* subsurface pH manipulation using NH_3 gas**
  - Evaluates the role of major pore water constituents (Si, Al, Ca, Mg, HCO_3 ions) on the U(VI) sequestration
  - studies the solubility of uranium-bearing precipitates created after NH_3 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_3-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 contaminates.**

- **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 +/- $1*10^{-4}$ g was used to weigh all sample cups with lids.**

- **Pressure transducer is in the 0-30 psia 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 Rault'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 ~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_3-CO_3 ions**
  - **Solution compositions of known solute ratios are being formulated in crucibles**

Task 1.1 - Characterization of uranium bearing precipitates produced by the 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 [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**
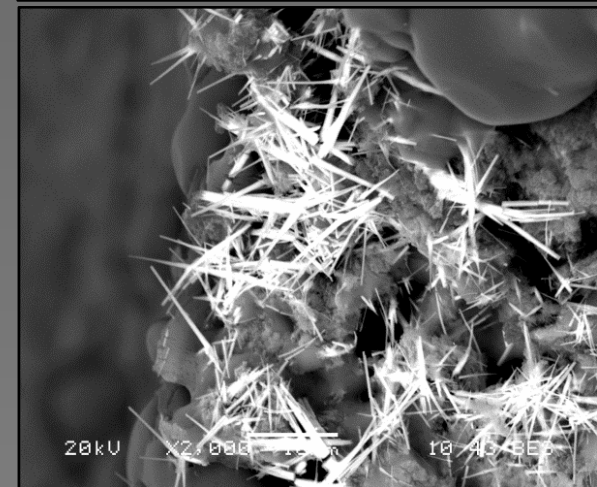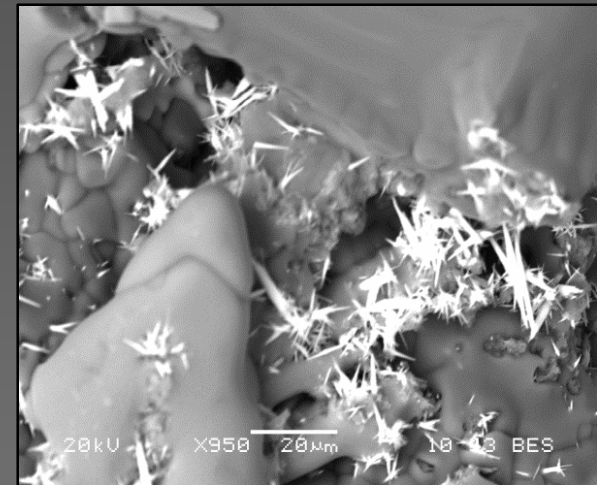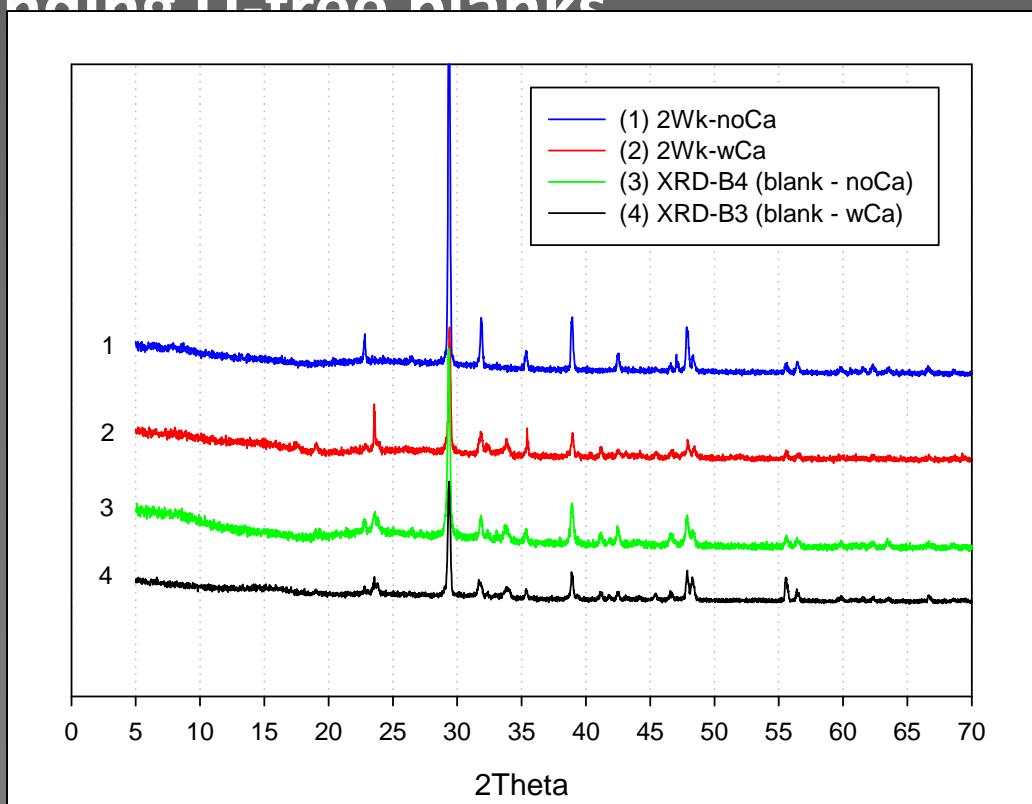
Task 1.1 - Results

- **Micro-Raman image and spectra for samples prepared with (B) and without (T) calcium.**
  - Bands at 800 cm^{-1} & 1000 cm^{-1} are consistent with reported values for the str... $(HCO_3)^{2-}$ and $(CO_3)^{2-}$...
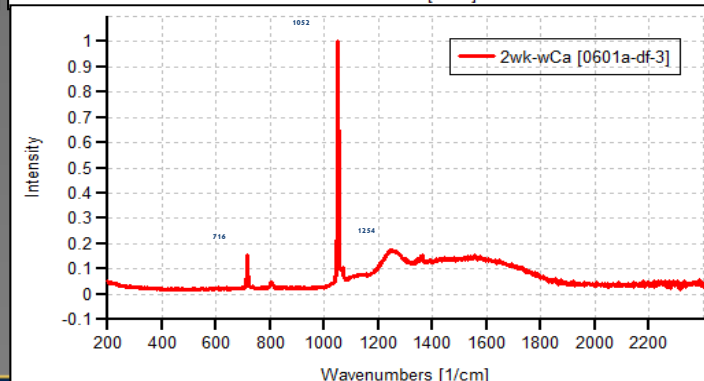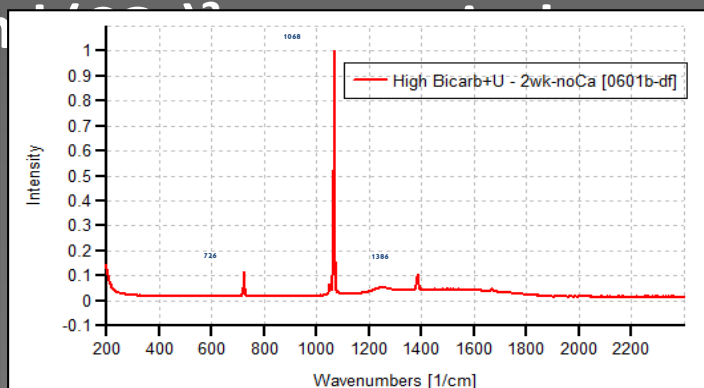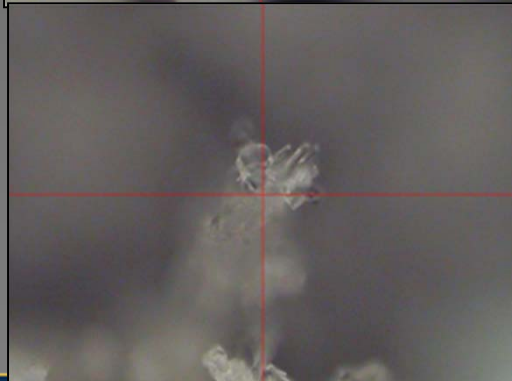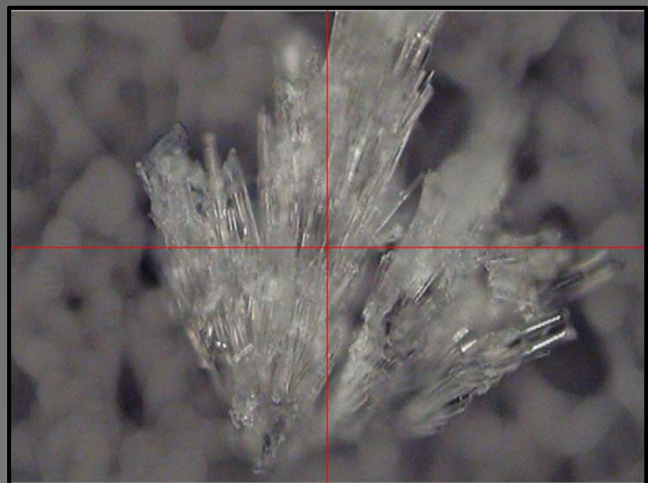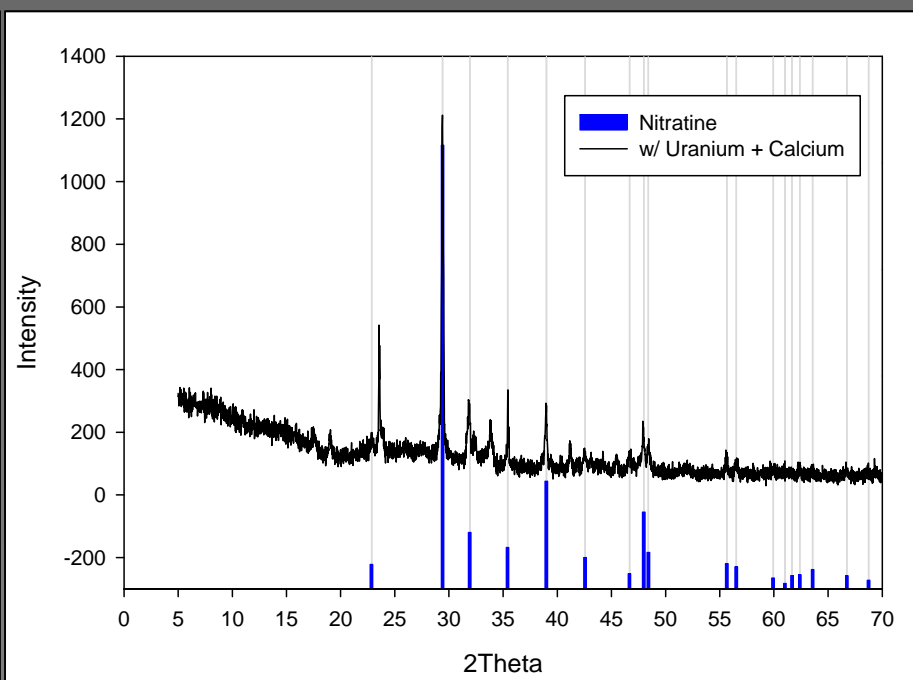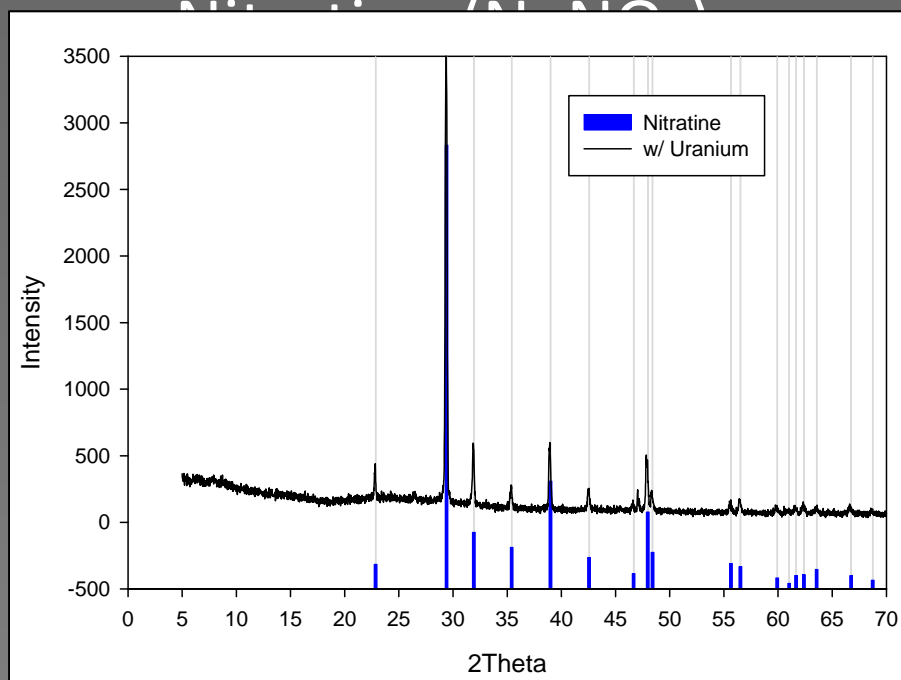
Task 1.1 - Results

- **Comparison of XRD patterns for samples prepared with and without calcium including their corresponding U-free blanks**
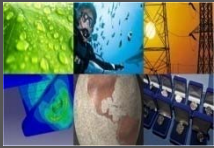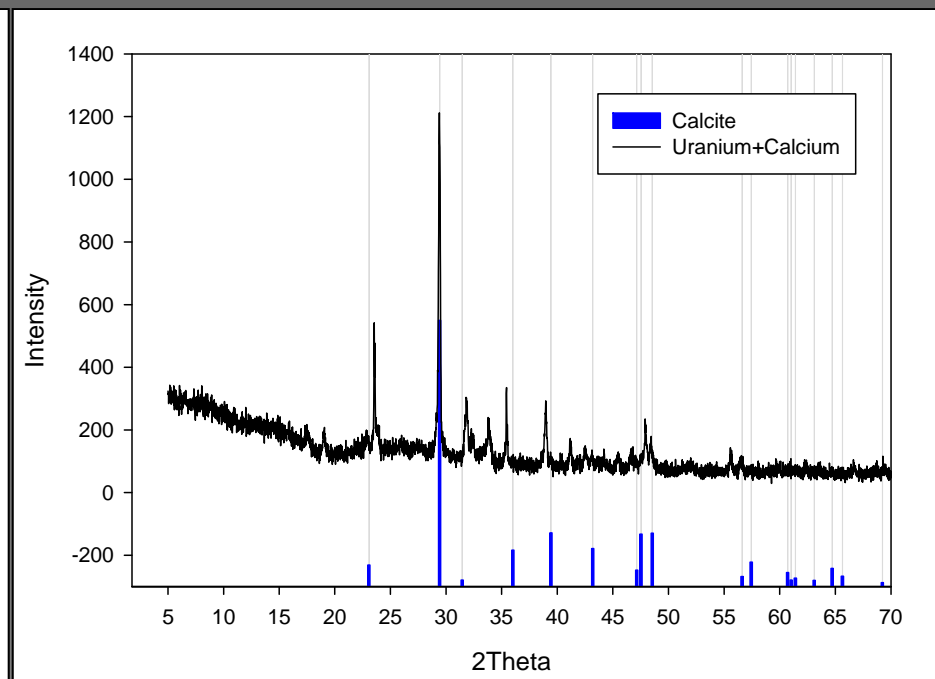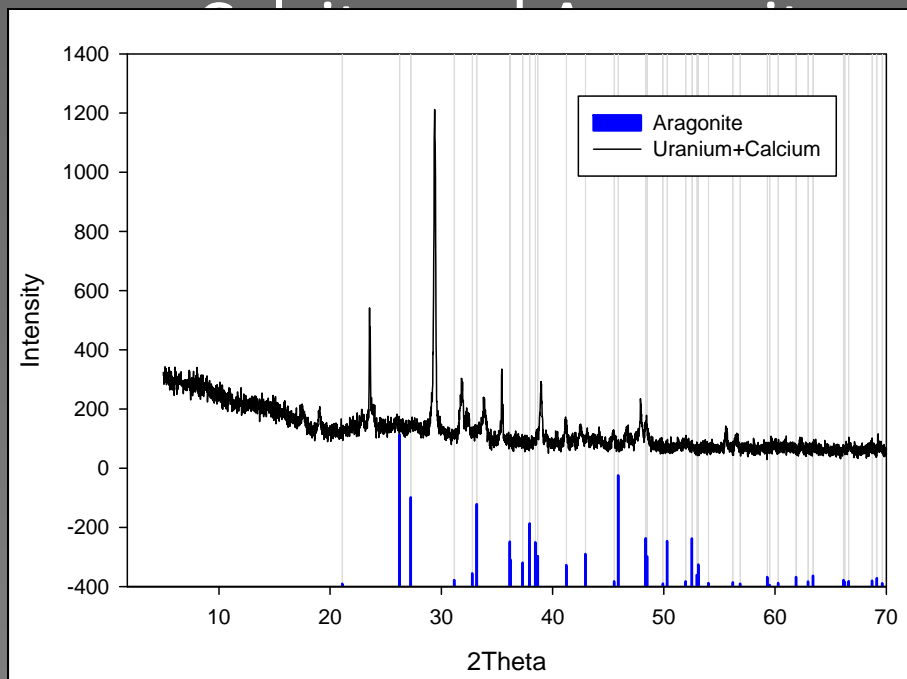
Task 1.1 - Results

- *Match!* analytical software analysis showed a consistent and significant match with only one mineral for all data sets
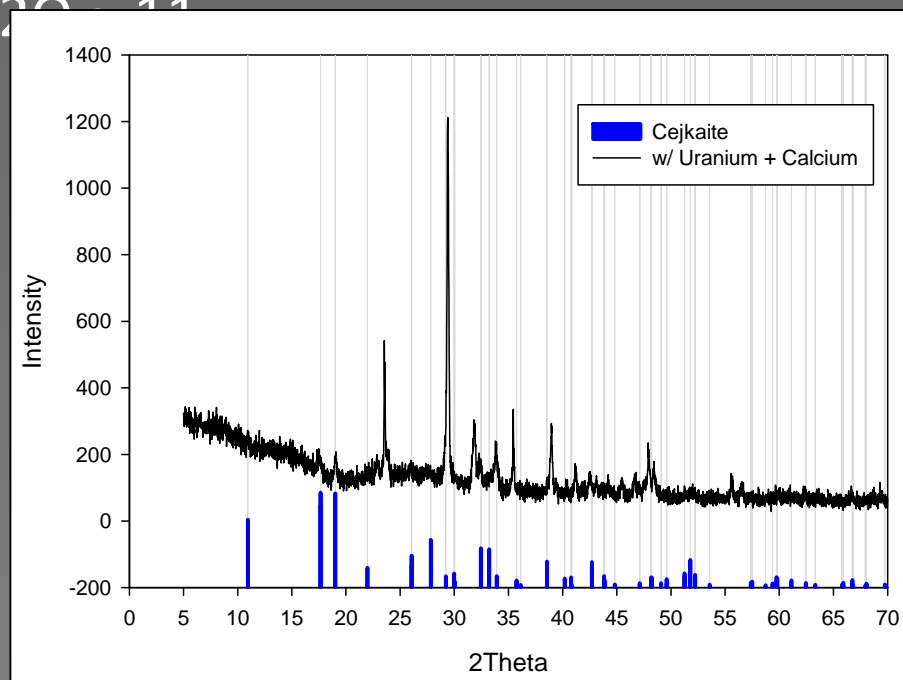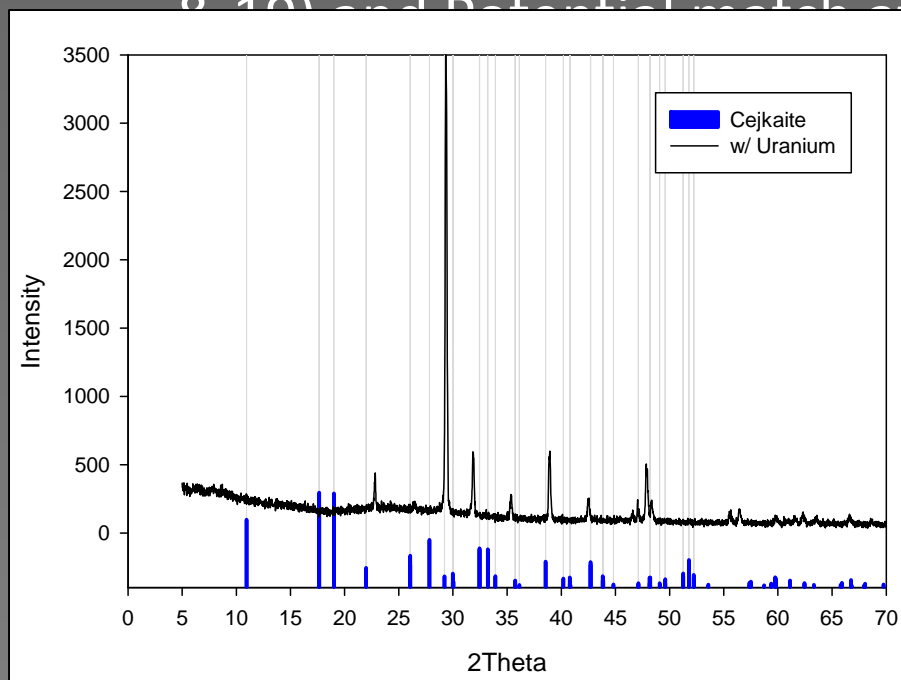
Task 1.1 - Results

- 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 (2Θ ≈ 17.5

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
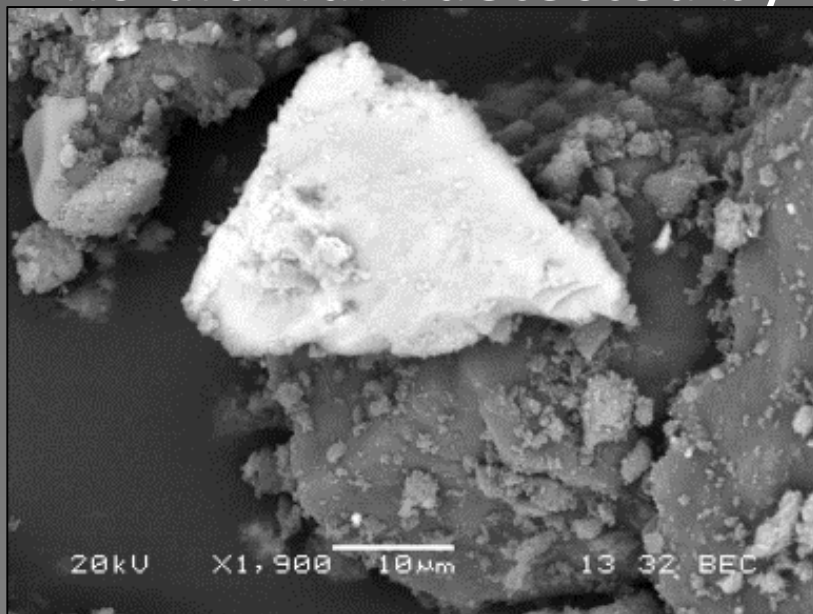
Task 1.1- Summary

- **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 ($NaNO_3$) 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 [$Na_4(UO_2)(CO_3)_3$]
- **Further analysis to characterize the U-bearing phases are ongoing**
  - Elevated uranium content
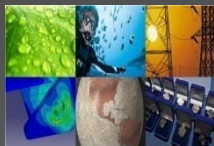 - Evaluating methods of reducing nitratine formation

Task 1.2 - Investigation on microbial meta-autunite interactions: the effect of 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 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 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 μL 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.**
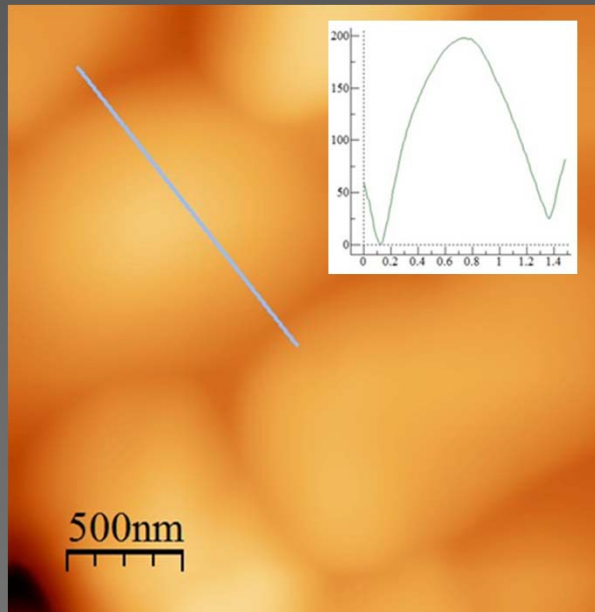
Task 1.2 - AFM results, G968 strain
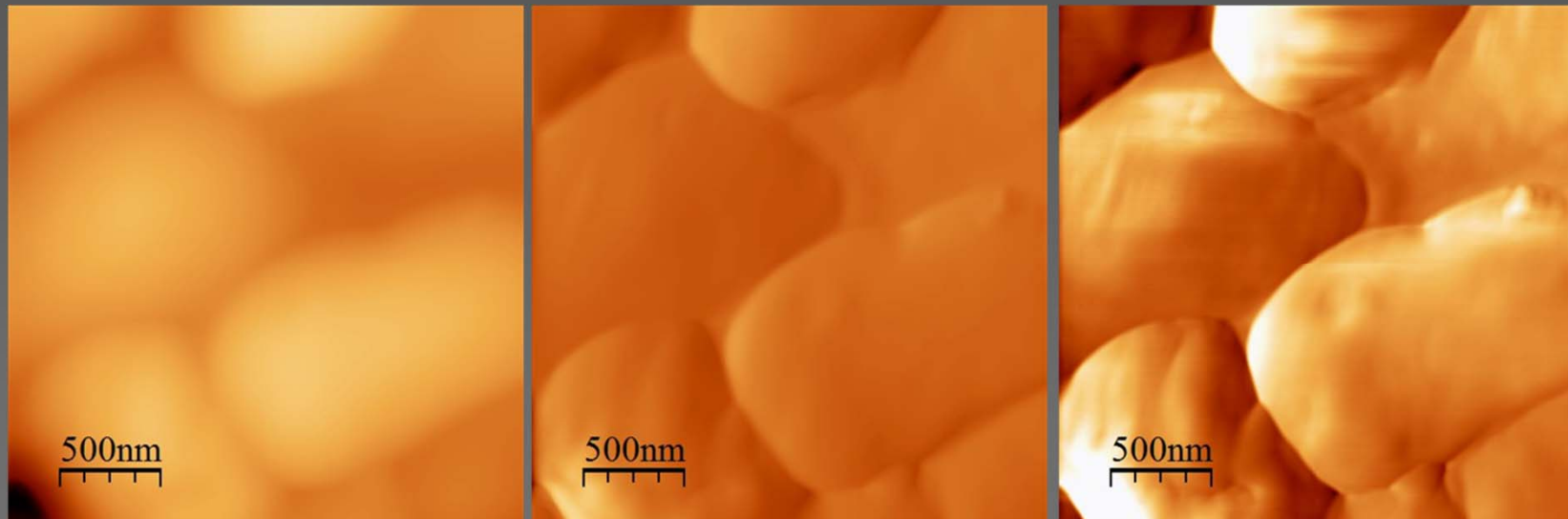
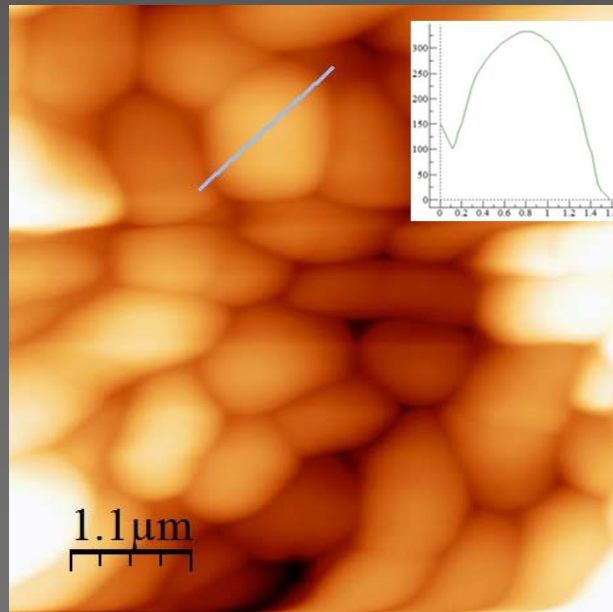- **G968 control sample (scan size 2.5 x 2.5 μm²) illustrating the smooth bacterial surface.**

- **The topographic image on the left, deflection image in the middle and friction image on the right.**
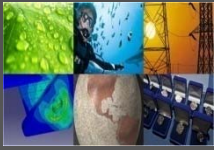
Task 1.2 – AFM results, G968 control samples

Y-198nm
X-1.3um

Y-350nm
X-1.5um
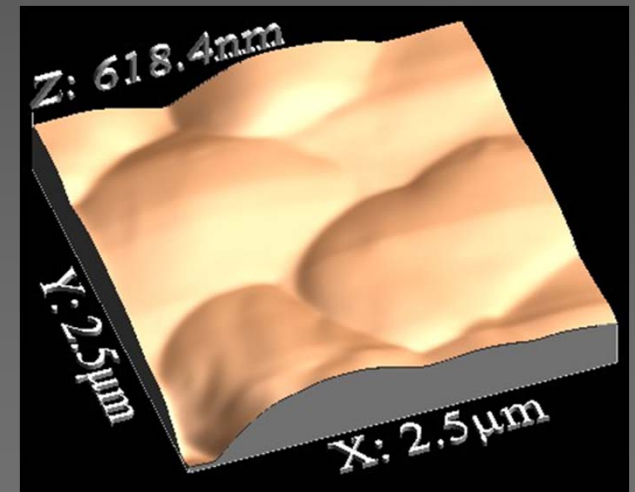
Task 1.2 – AFM results, 10 ppm of U(VI), no HCO$_3$

X- 1.2 um
Y- 205 nm
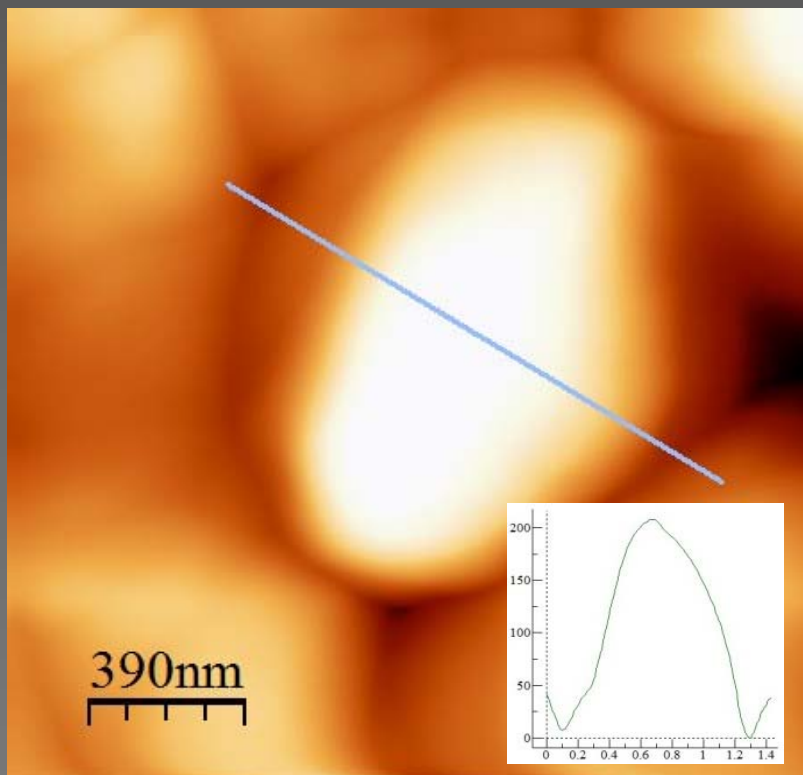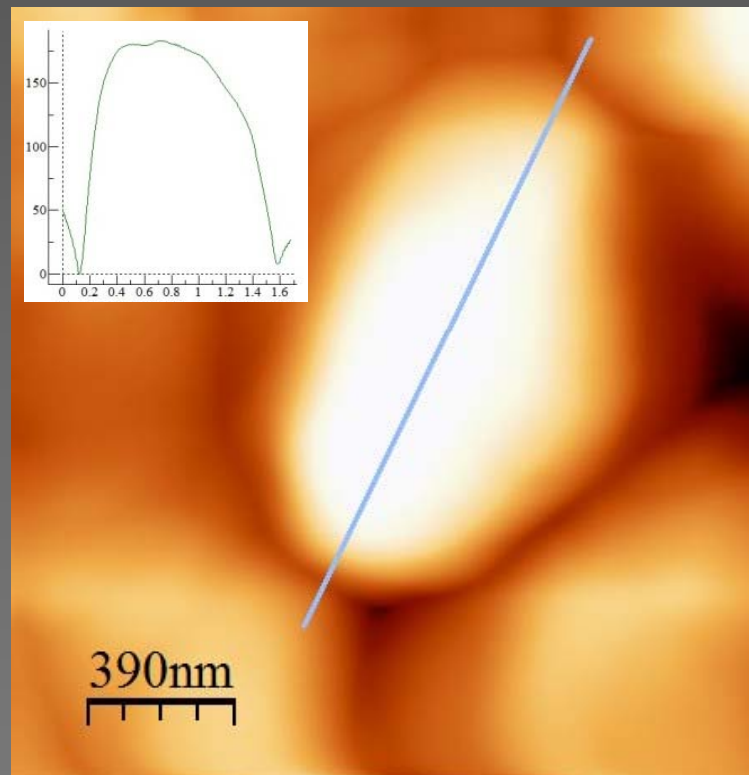
X- 1.5 um
Y- 175 nm

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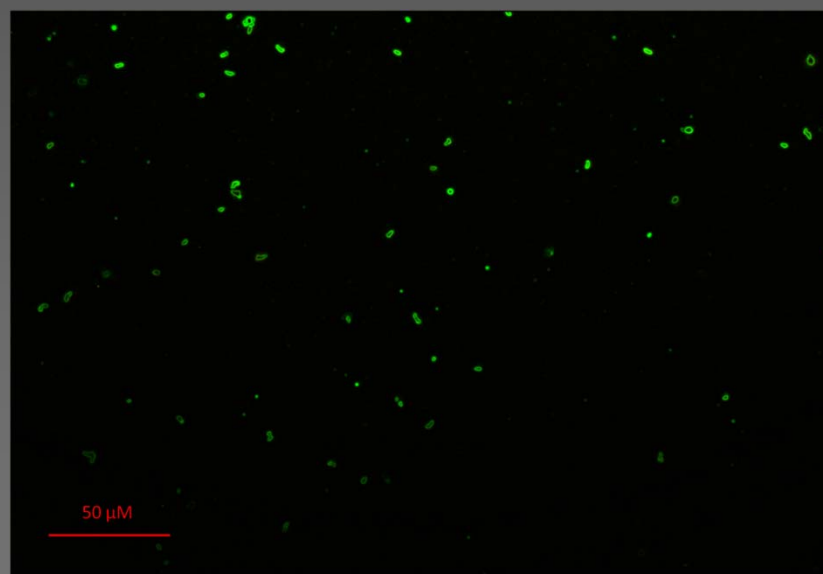
Live/ Dead assay of sample containing 5 ppm of U(VI) with 5 mM bicarbonate.

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

50 μM

50 μM
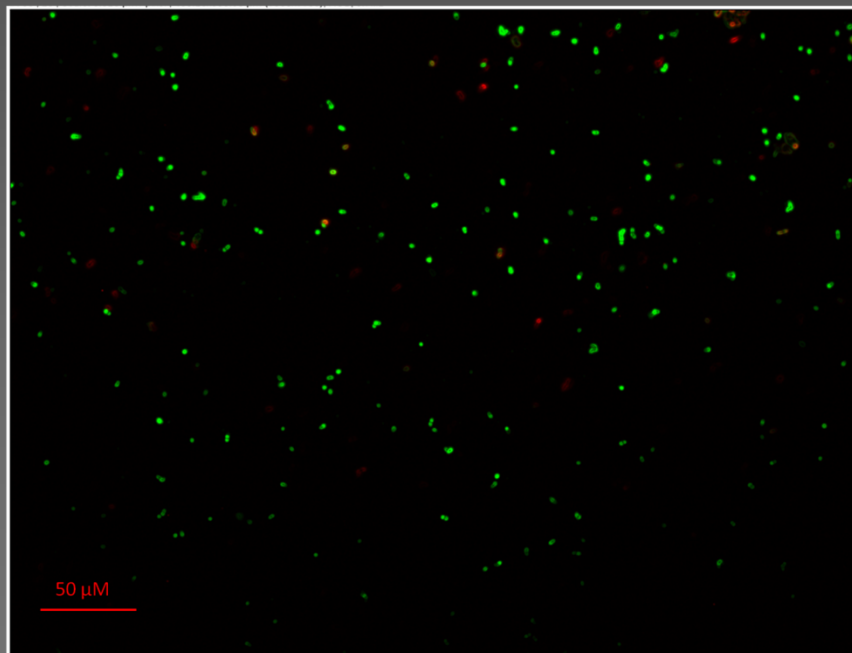
Live/ Dead assay of sample containing 10 ppm of U(VI) with no bicarbonate.
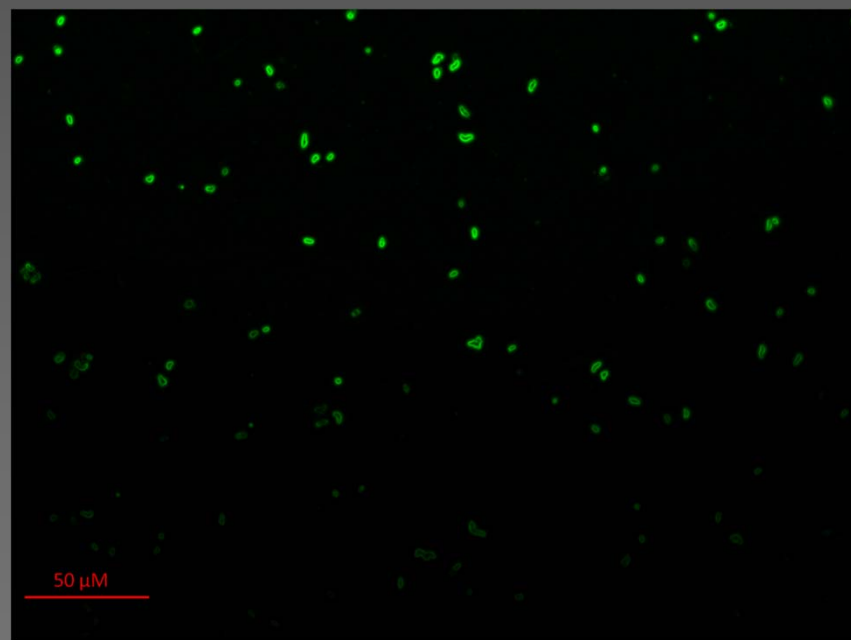
Live/ Dead assay of sample containing 10 ppm of U(VI) with 5 mM bicarbonate.

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 investe if contaminates 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 humid 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®**
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 millon/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$_2$ (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± 8.81% at pH 4 was observed for batch #5.**

- **The next highest U(VI) removal of 78.1 ±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±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**

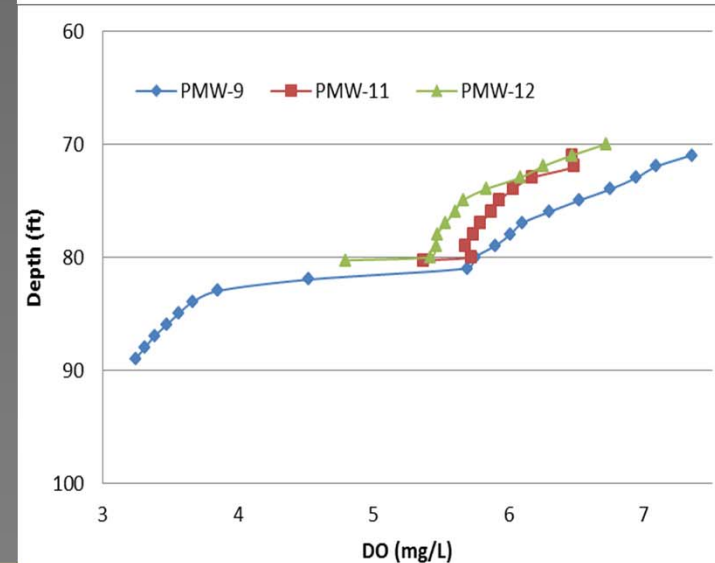- **Designed re-oxidation period with three environments**
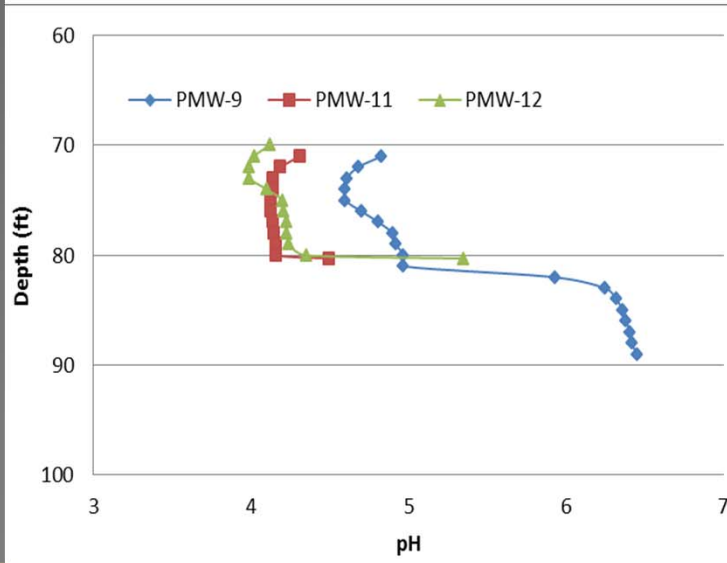
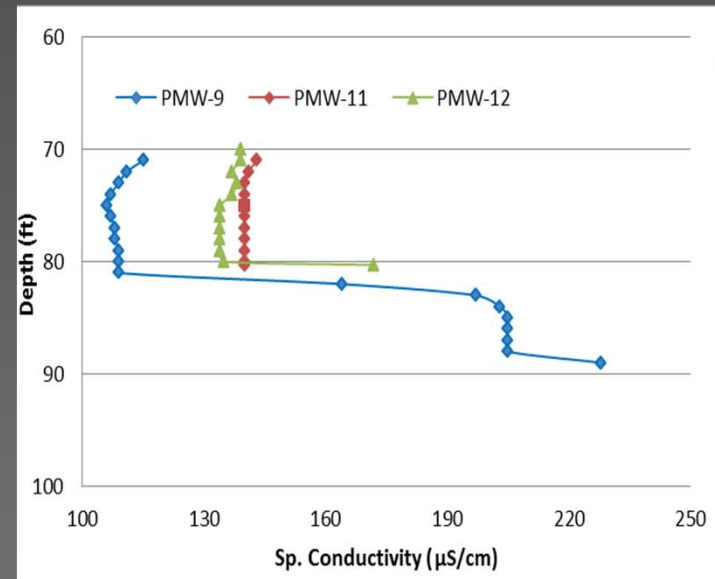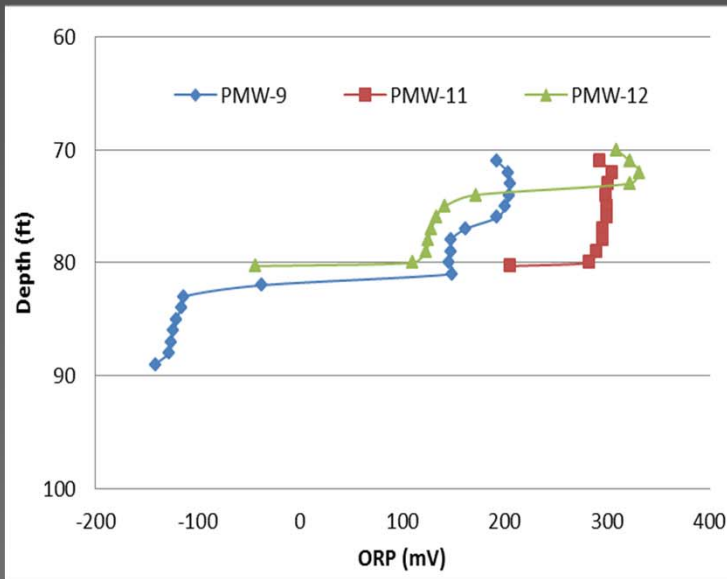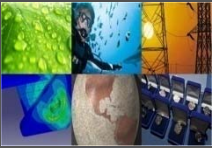- **Completed XRD Analysis**

Task 2.2 - Depth Profile Analysis
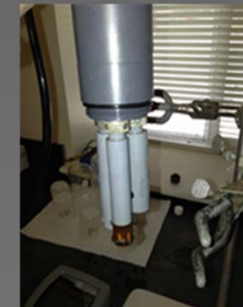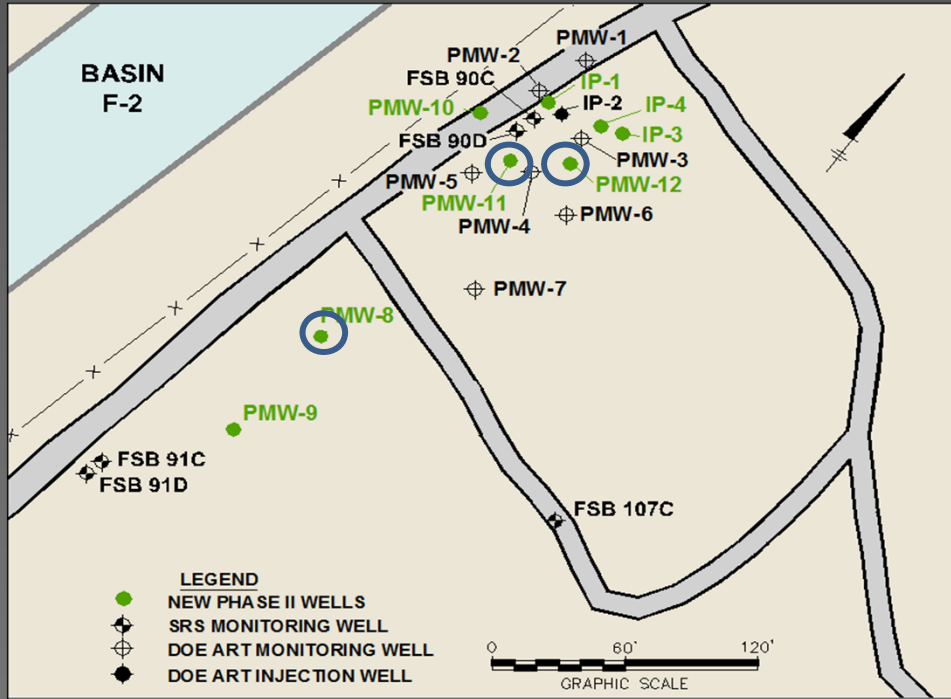
- Contamination source: radiological waste
  - Nuclear reactors
  - Support facilities
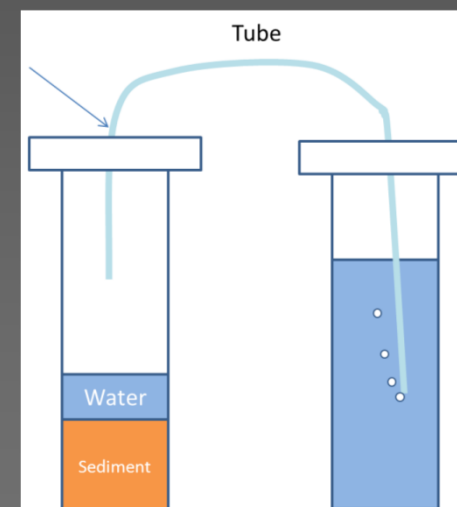
Task 2.2 - Depth Profile Analysis

Task 2.2 - Previous Experiments

Microcosm set up

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

Tube

Water

Sediment

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**
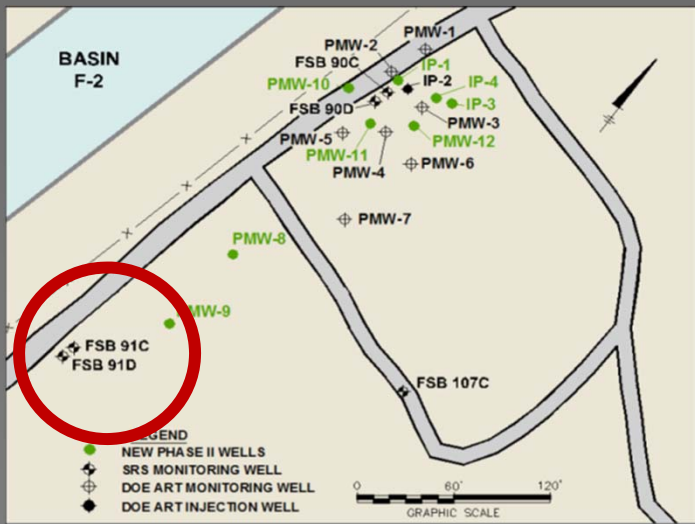- **Bacteria were not present in the soil**

Samples after 4 Weeks

Task 2.2 - Sample Source

Core samples from FSB 91C

Task 2.2 - Experimental Approach

Anaerobic Chamber → Prevents O_2 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 $NaHCO_3$
0.2 g NH_4Cl
0.1 g $K_2HPO_4 \cdot 3H_2O$
0.055 g KH_2PO_4
0.001 g resazurin as a redox indicator
0.039 g/L $Na_2S \cdot 9H_2O$ as a sulfur source and reductant
0.1 g $MgCl_2 \cdot 6H_2O$

Trace metal solutions recipe (per liter)
0.005 g $FeCl_2 \cdot 4H_2O$
0.005 g $MnCl_2 \cdot 4H_2O$
0.001 g $CoCl_2 \cdot 6H_2O$
0.0006 g H_3BO_3
0.0001 g $ZnCl_2$
0.0001 g $NiCl_2 \cdot 6H_2O$
0.0001 g $Na_2MoO_4 \cdot 2H_2O$
0.002 g $CaCl_2 \cdot 2H_2O$

Task 2.2 - Experimental Approach

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

Diluted Molasses Solution

Sediment
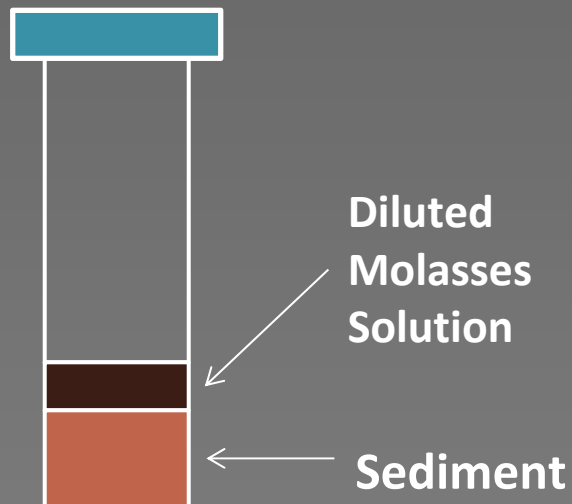
Samples placed in the anaerobic chamber

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 µL 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

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

- **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**

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**
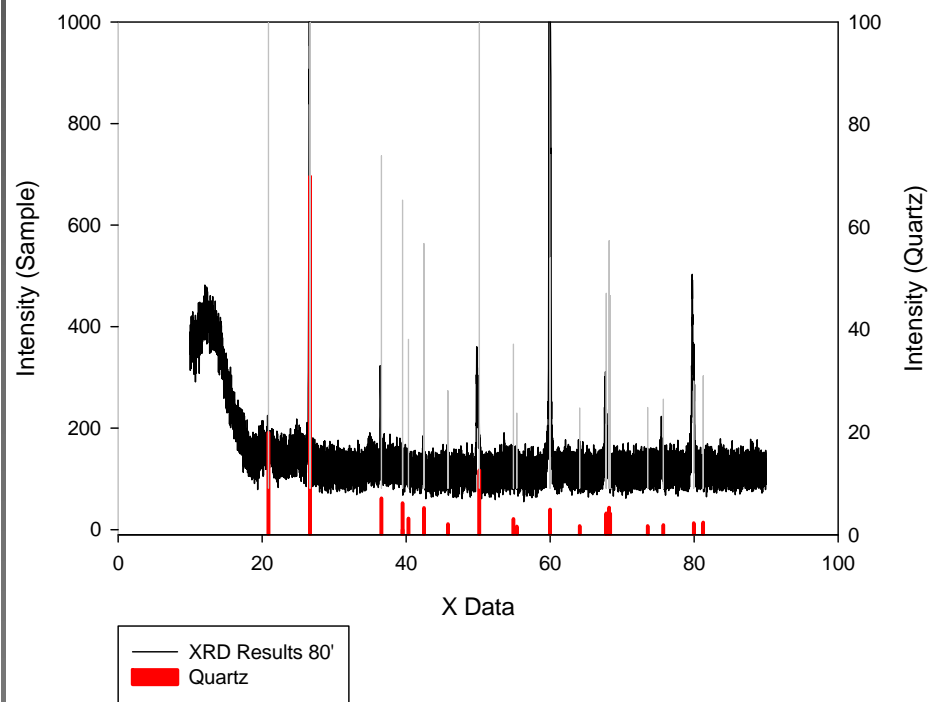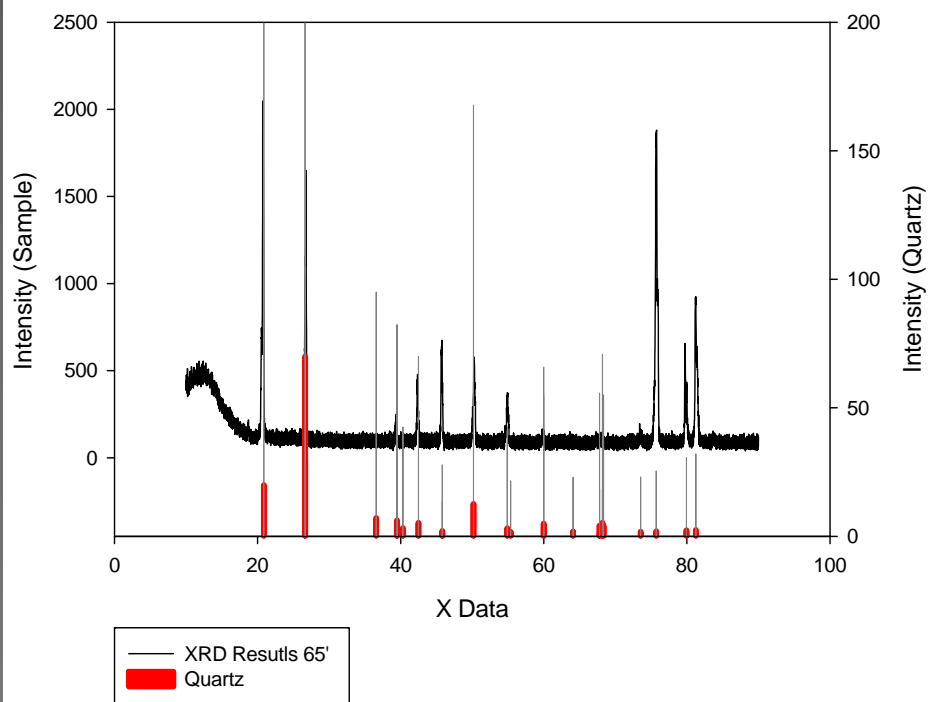 - **Work bench at atmospheric Oxygen levels**

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
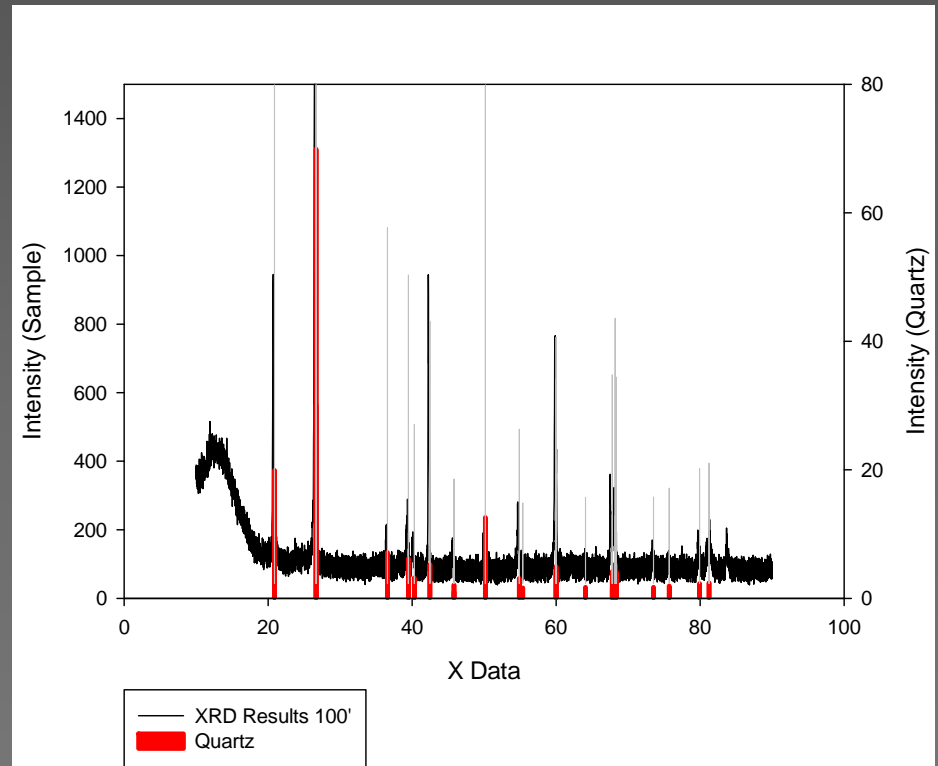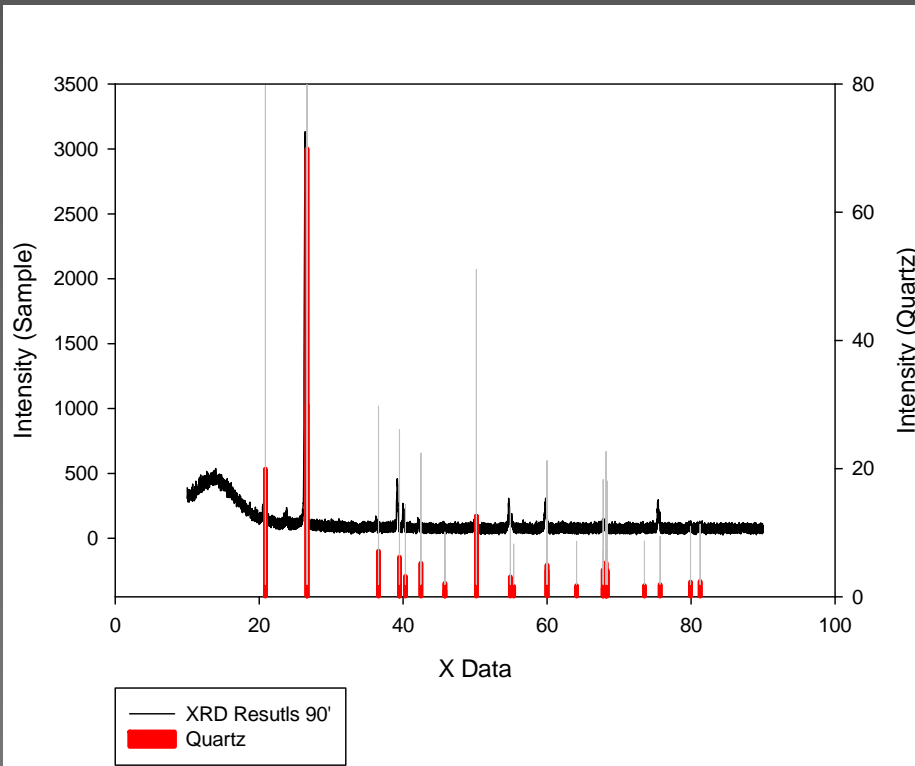 - Supernatant is filtered (0.45 μm)

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**