TECHNICAL PROGRESS REPORT

Microcosm Studies Prepared with SRS Sediments Augmented with Molasses and Sulfate

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FIU | Applied Research Center
FLORIDA INTERNATIONAL UNIVERSITY

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INTRODUCTION

In 2010, ARCADIS demonstrated the *in situ* injections of a carbohydrate substrate, molasses to create reactive zones for uranium (VI) remediation via the enhanced anaerobic reductive precipitation (EARP) process at the F-Area of the Savannah River Site (SRS). The addition of the molasses substrate solution to groundwater produces anaerobic conditions conducive to uranium reduction and then precipitation as uranium (IV). The SRS soil features very unique environmental conditions due to the naturally low alkalinity. A microcosm study, prepared with sieved SRS sediments, was designed to provide evidence for the capabilities of this remediation technology. The objective of these microcosm experiments was to replicate the anaerobic conditions created as a result of molasses injection performed by ARCADIS at SRS and investigate if any mineralogical changes could occur in the soil. Specifically, the study aims to determine if forms of reduced iron such as siderite and pyrite would be created in the reducing conditions and their potential re-oxidation in sediments when oxidized conditions revert. These experiments will also explain the types of reactions that might occur in the anaerobic aquifer. The experiments conducted last year did not provide any evidence for the formation of siderite and pyrite forms in soil. The low soil pH and low groundwater concentrations of sulfate and bicarbonate to form ferrous carbonate or ferrous sulfide complexes could be major factors for the obtained results. In the current experiments, the media solution was amended with molasses and sulfate to stimulate sulfate-reducing bacteria. The microbial reduction of sulfate produces hydrogen sulfide and releases HCO_3^-, resulting in an increase in alkalinity and pH. It is expected that in anaerobic conditions, sulfate will be reduced to sulfide and bind to ferrous iron in order to create blackish precipitates of pyrite detectable by the XRD analysis. It is also expected that the increase in pH will cause the aqueous phase to become saturated with respect to $FeCO_3$. The experiments will suggest if this technology is a viable option for uranium remediation under SRS conditions.

This progress report presents experimental data collected from the beginning of FIU Year 5 on the sample pH evolution after molasses injection and XRD results to evaluate mineralogical changes that might occur in the anaerobic conditions.

METHODOLOGY

FIU received SRS F-Area sediments collected from a depth of 60-90 feet to be used in the microcosm experiments. To separate the fine and coarse fractions, the sediments were first pulverized using a mortar and pestle and then sieved through a No.80, 180 μm sieve (Figure 1). Sieving was a necessary step to remove larger quartz particles which shield the finer fractions in XRD analysis.

Figure 1. **No. 80, 180 µm sieve.**

For the microcosm experiment, 4 sets of samples were prepared in triplicate for a total of 12 samples. These samples were created in 50-mL polypropylene tubes and were treated using a basal medium solution augmented with sulfate and molasses (Figure 2). The basal medium solution consists of (in g L-1 deionized water): 1.5 $NaHCO_3$, 0.2 NH_4Cl, 0.1 K_2HPO_4 $3H_2O$, 0.055 KH_2PO_4, 0.001 resazurin as a redox indicator, 0.039 Na_2S 9H2O as a sulfur source and reductant, and 0.1 $MgCl_2$ $6H_2O$. In addition, 5 mL L-1 trace metal solution was added. The trace metal solution consists of (in g L-1): 0.005 $FeCl_2$ $4H_2O$, 0.005 $MnCl_2$ $4H_2O$, 0.001 $CoCl_2$ $6H_2O$, 0.0006 H_3BO_3, 0.0001 $ZnCl_2$, 0.0001 $NiCl_2$ $6H_2O$, 0.0001 Na_2MoO_4 $2H_2O$, and 0.002 $CaCl_2$ $2H_2O$. The sulfate used for the augmented samples was from magnesium sulfate anhydrous (MgSO4) and was combined with the basal medium solution to a concentration of 500 ppm. Sets 1 and 4 were inoculated with anaerobic sludge collected from the anaerobic digester of the Miami-Dade South wastewater treatment plant, in order to directly introduce anaerobic bacteria into half of the samples.

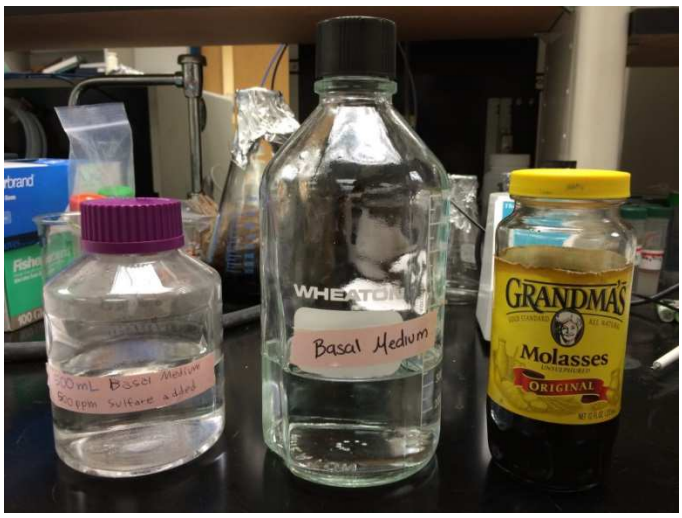

Figure 2. **Basal medium with 500 ppm sulfate, basal medium, and molasses.**

For the Batch 1 samples (Figure 3), Set 1 consisted of 20 mL of soil, 20 mL of basal medium, 500 ppm sulfate, 5-10% by weight molasses, and 5 mL of anaerobic bacteria. Set 2 consisted 20 mL of soil, 20 mL of basal medium, 500 ppm sulfate, and 5-10% by weight molasses. Set 3 consisted of 20 mL of soil, 20 mL of basal medium, and 5-10% by weight molasses. Set 4 consisted of 15 mL of soil, 15 mL of basal medium, 5-10% by weight molasses, and 5 mL of anaerobic bacteria. Set 4 was decreased to 15 mL of soil instead of 20 mL in order to conserve the SRS F-Area sediments for the next batch of microcosm samples. The components of each of the 4 sets from Batch 1 can be found in Table 1.

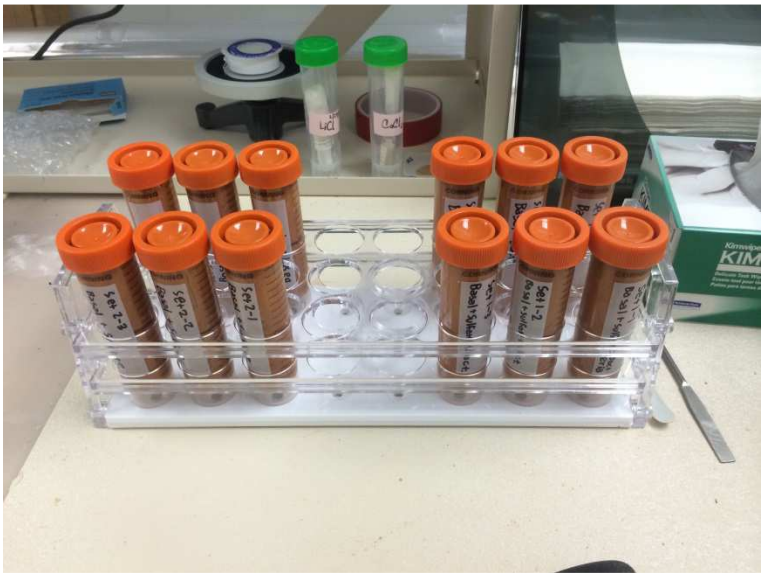

Figure 3. Batch 1 samples.

Table 1. Batch 1 Sample Composition

Batch 1				
Sample Composition	**Set #1**	**Set #2**	**Set #3**	**Set #4**
Soil, mL	20	20	20	15
Basal Medium, mL	20	20	20	15
Sulfate, ppm	500	500	-	-
Molasses, % by weight	5-10%	5-10%	5-10%	5-10%
Anaerobic sludge, mL	0.5	-	-	0.5

It was observed that some of the original 12 samples, which were created and placed in the anaerobic chamber in October 2014, were beginning to dry out. It was decided that a small amount of solution would be added to the samples. At week 6 of the experiment, two solutions were created for this purpose and added to the samples. Solution 1 consisted of 45 mL of basal medium and 7.1 g molasses (5% by weight). This solution was adjusted to a pH of 7.03 before it was added in the amount of 2 mL per sample to the set 3 and set 4 samples. Solution 2 consisted of 45 mL of basal medium augmented with 500 ppm of sulfate and 7.1 g molasses (5% by weight). This solution was adjusted to a pH of 6.99 before it was added in the amount of 2 mL per sample to the set 1 and set 2 samples.

During the monitoring of Batch 1 samples, a sharp decrease in the pH from week 1 to week 2 was noted and an investigation was conducted to determine the cause. It was concluded through an elimination process that the addition of molasses had caused the drop in pH (Table 2). Prior to the molasses addition, the solutions exhibited more basic pH values ranging between 8.7-8.82. These values shifted significantly to below pH 5.0 after the molasses addition. It was noted that the molasses solutions are acidic and, in addition, upon mixing with the solutions, triggers the fermentation process, resulting in a rapid drop in pH.

Table 2. pH Monitoring Data

Measured pH values			
Solution amended with sulfate, basal medium and molasses	**Solution amended with basal medium and molasses**	**Basal medium**	**Solution amended with basal medium and 500 ppm of sulfate**
4.85	4.57	8.7	8.82

In the acidic conditions, carbonic acid is the most prevailing carbonate species, precluding the formation of any significant amount of bicarbonate HCO_3^- and carbonate CO_3^{2-}. In the open system, CO_2 can leave the solution, thus limiting any formation of siderite ($FeCO_3$) solid phases. Due to the acidic conditions within the samples from Batch 1, it was decided that a new set of samples would be created for Batch 2 (Figure 4) using the same basal-molasses solution except that the pH was adjusted to a neutral level before the addition of any sediments. Sample 1 consisted of 12 mL of basal solution augmented with 500 ppm of sulfate, 0.75 grams of molasses (5-10% by weight), 12 mL of SRS F-Area sediments and 0.5 mL of anaerobic bacteria. Sample 2 consisted of 12 mL of basal solution augmented with 500 ppm of sulfate, 0.75 grams of molasses (5-10% by weight), and 12 mL of SRS F-Area sediments. Sample 3 consisted of 12 mL of basal solution, 0.75 grams of molasses (5-10% by weight), and 12 mL of SRS F-Area sediments. Sample 4 consisted of 12 mL of basal solution, 0.75 grams of molasses (5-10% by weight concentration), 12 mL of SRS F-Area sediments, and 0.5 mL of anaerobic bacteria. The components of the Batch 2 samples can be found in Table 3.

To create anaerobic conditions necessary for the experiment, a vinyl anaerobic airlock chamber from COY Lab Products was used (Figure 5). The glove box was vacuumed and purged several times with pure nitrogen gas to establish anaerobic conditions, which were then confirmed by the oxygen gas analyzer. All experimental samples remained in the anaerobic chamber for the duration of the experiment. Oxygen levels were continuously monitored to ensure that no oxygen came into contact with the samples.

Figure 4. Microcosm Batch 2 samples.

Table 3. Batch 2 Samples Composition

Samples composition	Batch 2			
	Set #1	Set #2	Set #3	Set #4
Soil, mL	20	20	20	15
Basal Medium, mL	12	12	12	12
Sulfate, ppm	500	500	-	-
Molasses, % by weight	5-10%	5-10%	5-10%	5-10%
Anaerobic sludge, mL	0.5	-	-	0.5

Figure 5. Anaerobic chamber used for microcosm experiments.

While the samples were in the anaerobic chamber, pH was monitored for both Batch 1 and Bach 2 experiments using a Eutech Instruments 200-Series pH meter and a Fisher Scientific Accumet pH electrode. The pH was measured at different intervals and the data are presented in the results section. Also, the pH measurements can be used to predict the types of reactions that would most likely be occurring.

Before creating any of the samples for the microcosm experiment, XRD analysis was conducted on the original sediments to obtain a reference for comparison. After the samples were created and given time to react in the anaerobic chamber, sub-samples were taken from both of the microcosm experiments to be used for XRD analysis. For Batch 1, a small sub-sample was taken from each of the samples and combined to create a representative sample for each set, with a total of 4 sub-samples. Sub-samples for Batch 1 were taken at week four (4) and week eight (8). For Batch 2, sub-samples were taken directly from each of the tubes for a total of 4 sub-samples. The Batch 2 sub-samples were taken after four (4) weeks in the anaerobic chamber. Each of the sub-samples was placed individually onto a sample-holder (Figure 6) which was then placed into the XRD instrument.

Figure 6. **Amorphous sample holder with sediment to be placed into XRD instrument.**

X-ray diffraction analyses were performed using a Bruker 5000D XRD instrument (Figure 7) set to 35 kV and 40 mA. Diffraction patterns were obtained using a copper Cu Kα radiation source (λ=0.154056 nm) with a tungsten filter. The XRD was programmed to run over a 2-theta (2θ) range from 3° to 70° with a 0.02° step size and 3 second counting per step. After experimental XRD patterns were received, these patterns were analyzed on SigmaPlot software and compared against known XRD patterns for siderite and pyrite.

Figure 7. Bruker 5000D XRD instrument.

RESULTS AND DISCUSSION

Batch 1 Results

In the first microcosm study, there was no visible evidence of bacterial growth in any of the samples. In the samples that were amended with 0.5 mL of anaerobic bacteria, bubbles were observed in week 1 of the experiment but were not present in subsequent weeks. Small white patches of what appears to be fungal growth is present in some of the samples (Figure 8) but it appeared to be a random occurrence as it was not more common in any particular set. All samples including those augmented with sulfate were kept in the anaerobic glove box, making it difficult to detect the odor of possible hydrogen sulfide, an indication of the sulfate bioreduction process. Changes in the sulfate concentrations added to the initial solution and that remaining after keeping the samples under anaerobic conditions will be analyzed further to confirm that changes are due to bacterial activity.

pH evolution

pH measurements suggested that almost all of the samples have followed a similar trend, with a decline in the pH value (Table 4 and Figure 9-Figure 12). This can be attributed to the fermentation process of molasses and the natural acidity of SRS soil used for the microcosm study. It was noted that samples amended with sulfates had slightly higher pH than sulfate-free samples. In addition, there was an increase in the pH of some of the samples from 11/30/2014 to 12/11/2014. This increase in the pH was caused by the addition of a pH-neutral solution to each of the samples to prevent them from drying out. By 12/11/2014, it was observed that the pH again dropped in almost all of the samples.

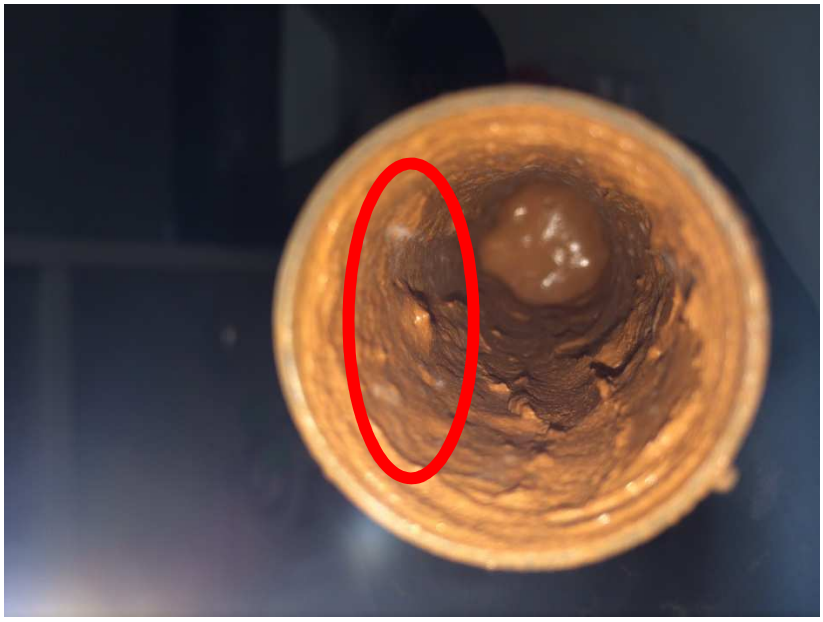

Figure 8. Fungal growth in Batch 1 sample.

Table 4. Batch 1 Samples pH Evolution

Date	Set 1 (Basal Medium, Sulfate, Molasses, Bacteria)			Set 2 (Basal Medium, Sulfate, Molasses)			Set 3 (Basal Medium, Molasses)			Set 4 (Basal Medium, Molasses, Bacteria)		
	pH (1-1)	*pH (1-2)*	*pH (1-3)*	*pH (2-1)*	*pH (2-2)*	*pH (2-3)*	*pH (3-1)*	*pH (3-2)*	*pH (3-3)*	*pH (4-1)*	*pH (4-2)*	*pH (4-3)*
10/13/ 2014	5.95	5.95	5.95	5.95	5.95	5.9	5.55	5.76	5.81	5.95	5.95	5.95
10/21/ 2014	4.81	4.8	4.79	4.91	4.83	4.85	4.77	4.77	4.63	4.86	4.89	4.77
10/30/ 2014	4.82	4.63	4.34	4.85	4.86	4.83	4.86	4.89	4.8	4.93	4.87	4.33
11/30/ 2014	4.74	3.95	3.91	3.89	3.95	4.22	4.26	3.91	4.96	4.11	4.02	4.12
12/11/ 2014	4.73	3.94	3.9	4.01	4.04	4.35	4.39	4.22	5.29	4.37	4.31	4.4
12/18/ 2014	4.87	4.01	3.95	3.87	3.91	4.06	3.91	3.86	4.74	3.94	3.88	3.97

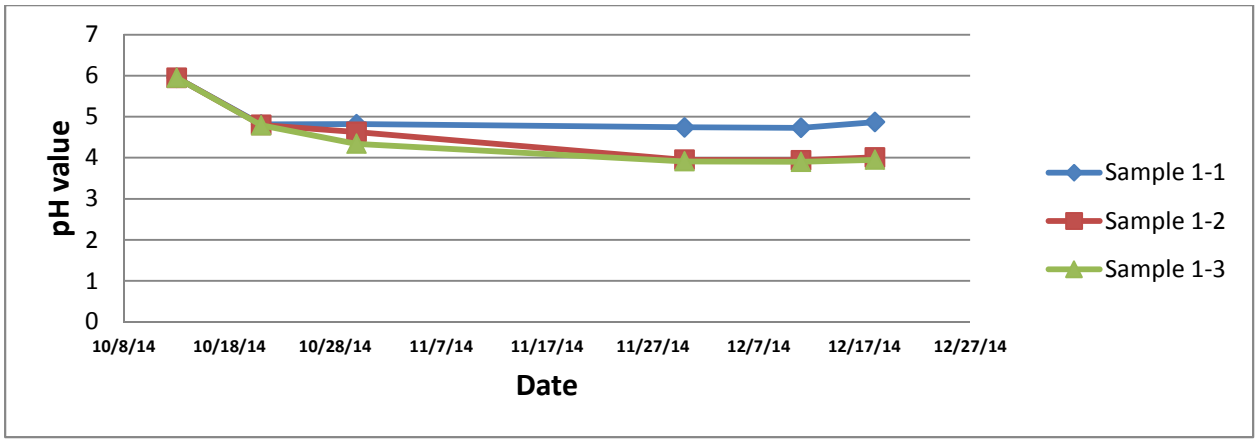

Figure 9. Batch 1, Set 1 pH evolution.

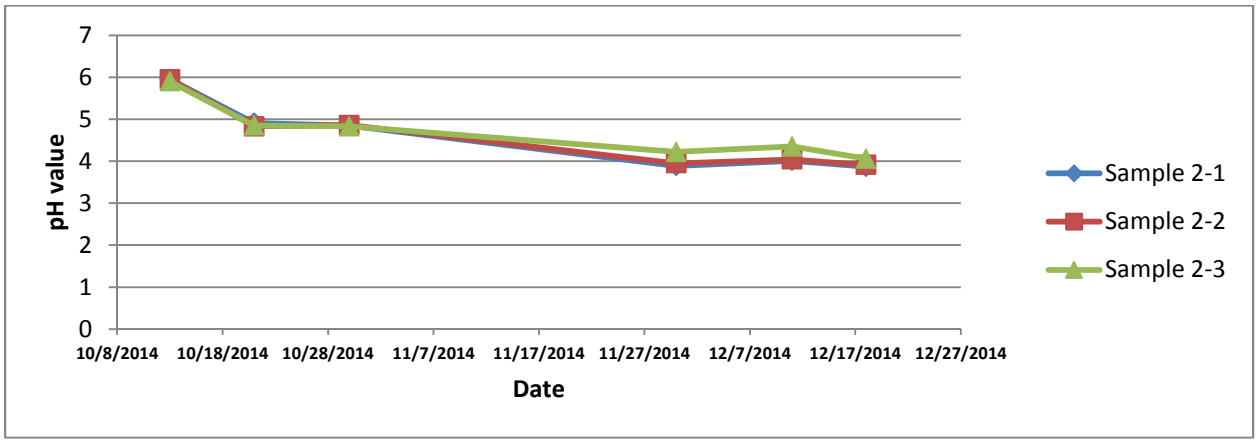

Figure 10. Batch 1, Set 2 pH evolution.

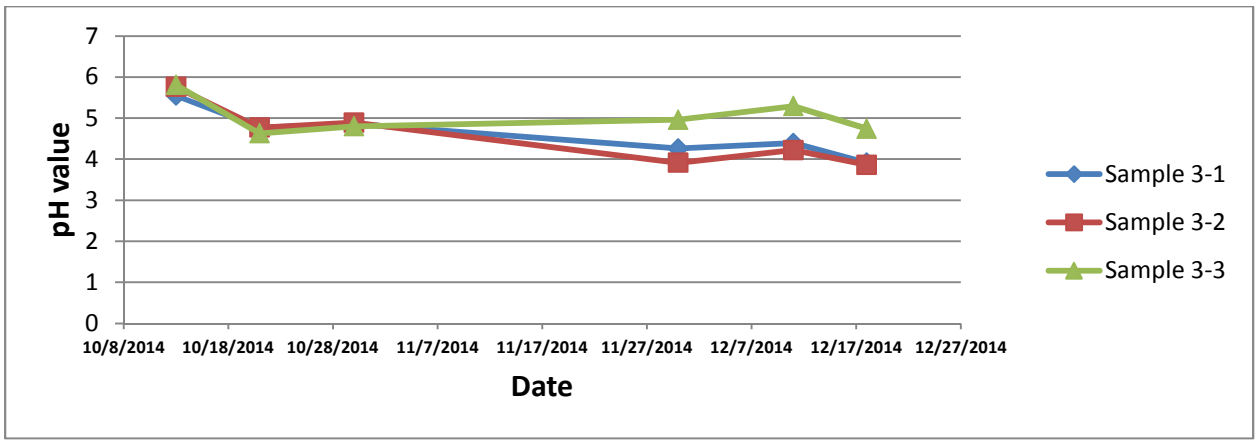

Figure 11. Batch 1, Set 3 pH evolution.

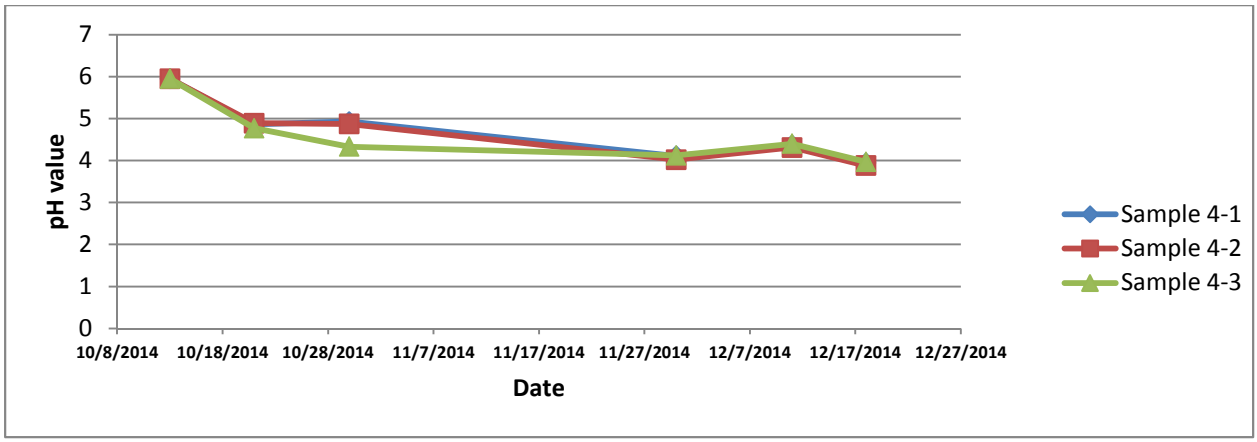

Figure 12. Batch 1, Set 4 pH evolution.

Batch 2 Results

Batch 2 results on pH evolution were found to be similar to Batch 1 and the samples displayed no visible indications of anaerobic bacteria growth. Unlike Batch 1, there was no fungal growth in any of the samples. Any bubble formation in the samples that contained anaerobic bacteria was no longer present past the first week. The sulfate-augmented samples were kept in an anaerobic glove box, preventing the ability to detect any possible hydrogen sulfide odor.

pH evolution

Although Batch 2 was first pH-adjusted to pH 7 before the experiment began, the pH followed the same declining trend (Table 5, Figure 13) as observed in Batch 1. It was concluded that this was the natural condition within the microcosm and that the acidic state was inevitable. Sulfate-amended samples followed the same pH trend as samples without sulfate.

Table 5. Batch 2 Samples pH Evolution

Date	Measured pH values			
	Sample 1: Basal medium, 500 ppm sulfate, molasses, bacteria	Sample 2: Basal medium, 500 ppm sulfate, molasses	Sample 3: Basal medium, molasses	Sample 4: Basal medium, molasses, bacteria
11/24/2014	7	7.02	7	6.99
11/30/2014	4.98	4.92	4.98	5.14
12/11/2014	5.28	5.13	5.23	5.41
12/18/2014	4.71	4.62	4.63	4.74

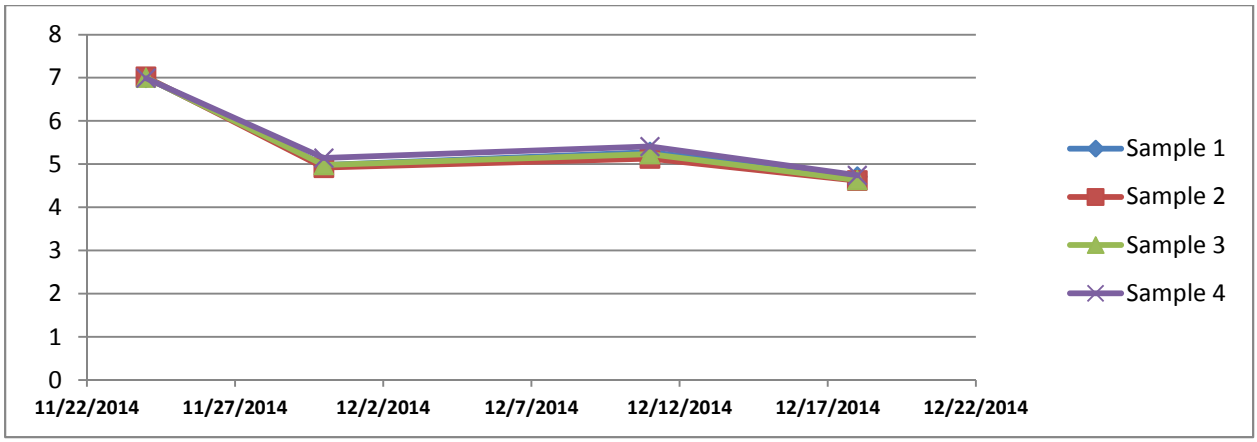

Figure 13. Batch 2 samples pH evolution.

XRD Results

X-ray diffraction (XRD) analyses were conducted on fine clay fractions of previously collected soil samples to obtain a background reading before the beginning of the microcosm study. The results indicated that the sediments contained quartz, kaolinite, montmorillonite, and goethite (Figure 14-Figure 17). This analysis was also the determining factor in deciding the best grain size to sieve the sediments. Since quartz did not shield the other clay and iron-bearing minerals from being observed at 180µm, it was decided that this size sieve would be sufficient. The most prominent peak for quartz was observed at 2 θ 26.65 degrees, montmorillonite at 5.89 degrees, goethite at 21.37 degrees, and kaolinite at 12.37 degrees.

At week 4, a set of 4 sub-samples was obtained from Batch 1. These were analyzed via XRD and revealed no significant matches to siderite or pyrite when considering the angle and intensity of the most prominent peaks (Figure 18-Figure 21). The maximum intensity peaks for siderite occur at 32.49 2-theta value and for pyrite at 28.74 (100%) and 56.75 (84.7%) 2-theta values, respectively. It was noted that sub-sample 4 had a large peak around 70^{o}, which was not seen in the other sub-samples (Figure 21). At week 8, another set of sub-samples was taken, dried and then again analyzed via XRD. The unknown peak observed in sub-sample 4 was no longer present. It was concluded that this peak was not caused by the sediment and was an anomaly. Again there were no matches to siderite or pyrite in the week 8 XRD results (Figure 22-Figure 25). For Batch 2, XRD analysis was conducted at week 4. No matches to siderite or pyrite were observed in any of the Batch 2 sub-samples (Figure 26-Figure 29) except set 3 (Figure 28) where a tiny peak appeared at a 2-theta value of 28.7, which is very close to the maximum intensity peak of pyrite. We will continue monitoring the evolution of this peak in the next sampling events. All samples in both batches displayed nearly identical XRD patterns when compared against the original XRD pattern of the soil before the microcosm experiment began.

Background Sample

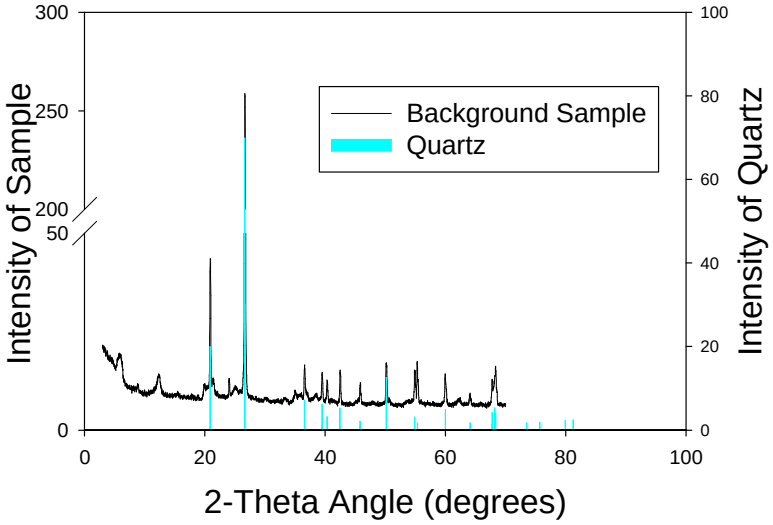

Figure 14. Background sample vs. quartz.

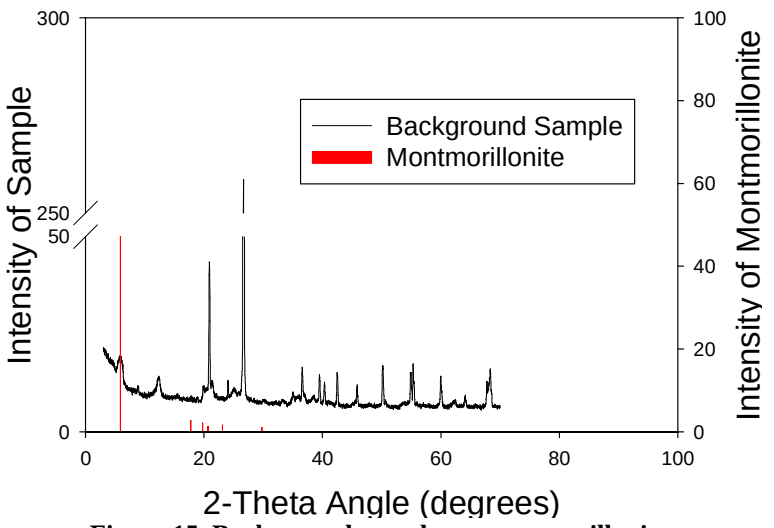

Figure 15. Background sample vs. montmorillonite.

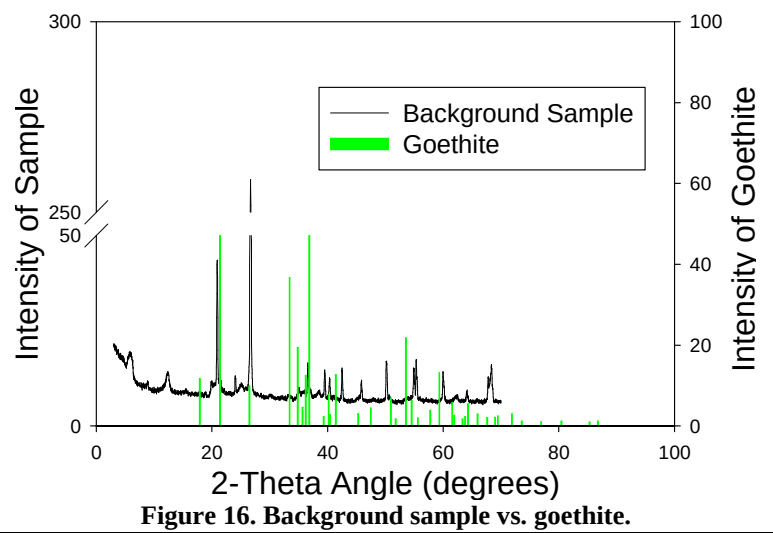

Figure 16. Background sample vs. goethite.

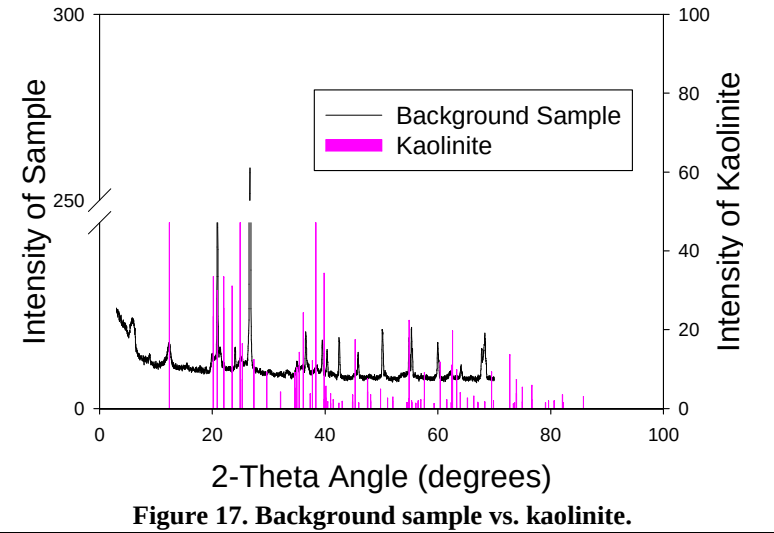

Figure 17. Background sample vs. kaolinite.

Batch 1/ Sub-Sampling 1

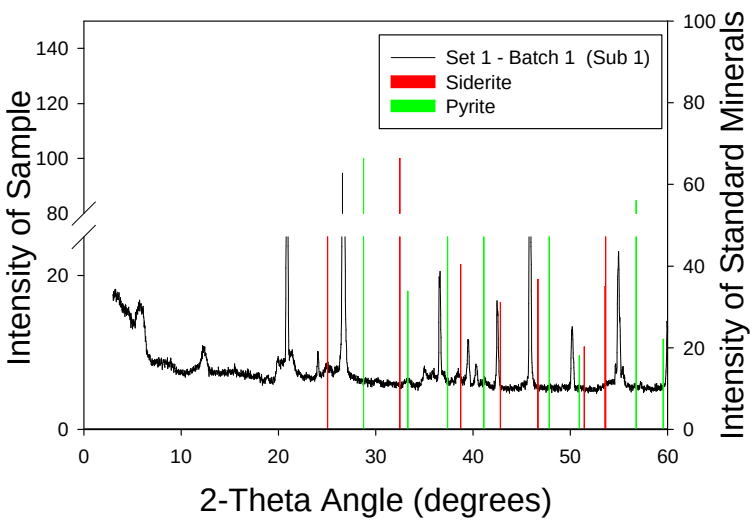

Figure 18. Set 1 vs. siderite and pyrite.

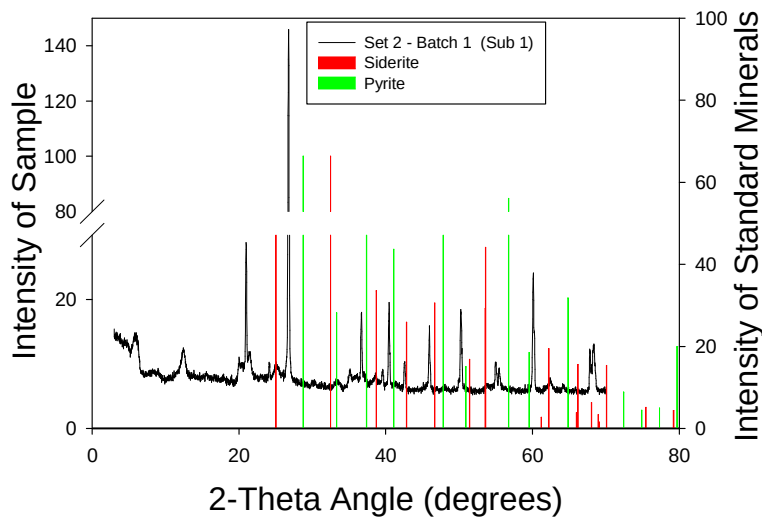

Figure 19. Set 2 vs. siderite and pyrite.

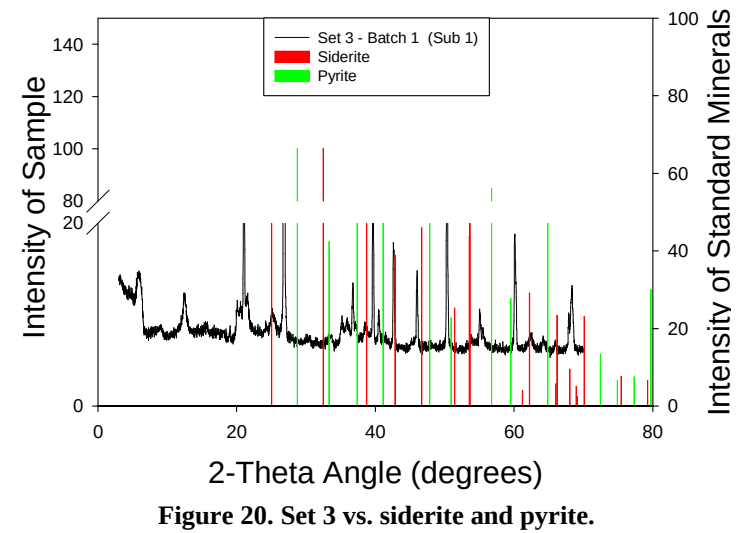

Figure 20. Set 3 vs. siderite and pyrite.

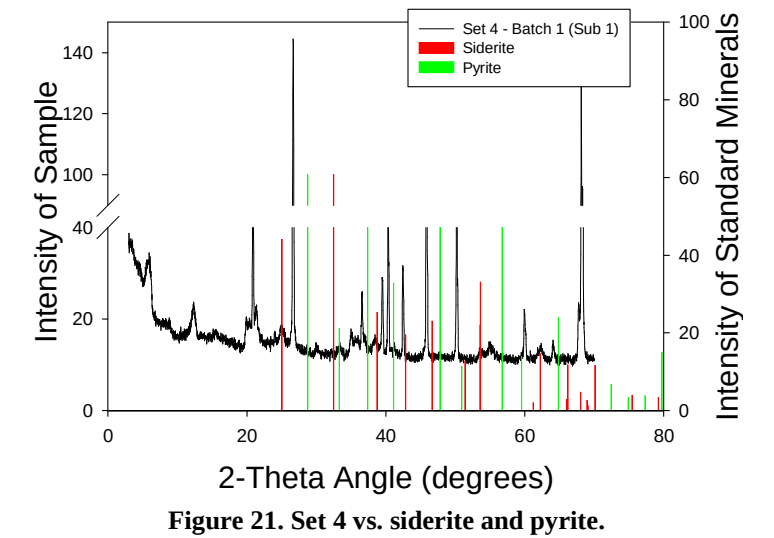

Figure 21. Set 4 vs. siderite and pyrite.

Batch 1/ Sub-Sampling 2

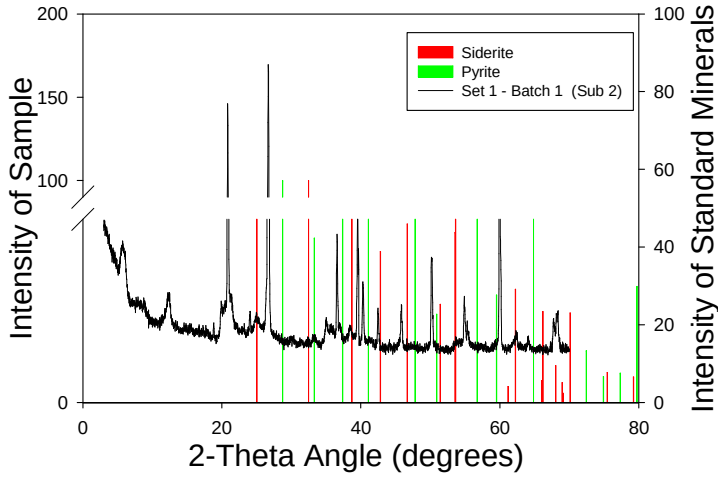

Figure 22. Set 1 vs. siderite and pyrite.

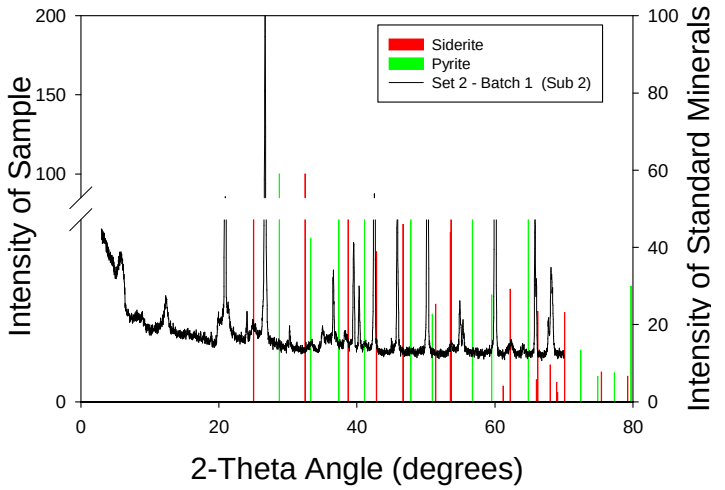

Figure 23. Set 2 vs. siderite and pyrite.

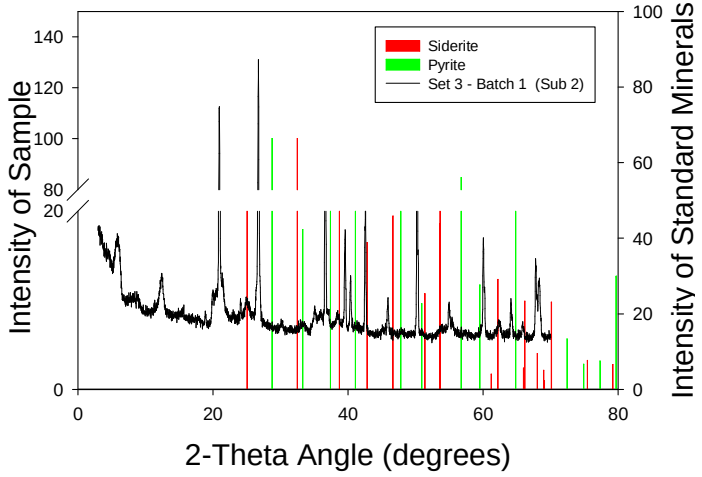

Figure 24. Set 3 vs. siderite and pyrite.

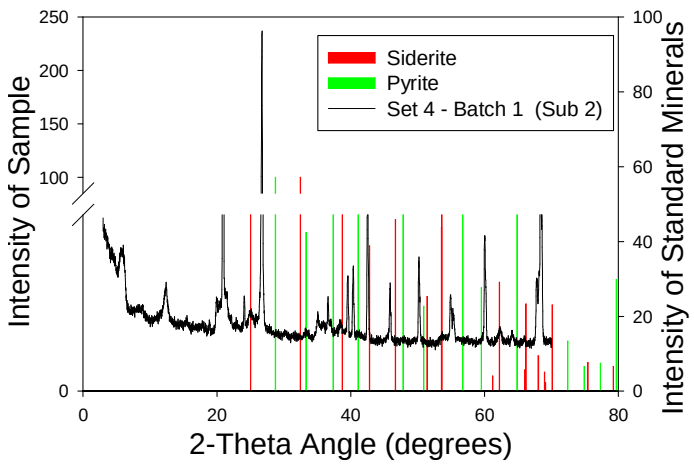

Figure 25. Set 4 vs. siderite and pyrite.

Batch 2/ Sub-Sampling 1

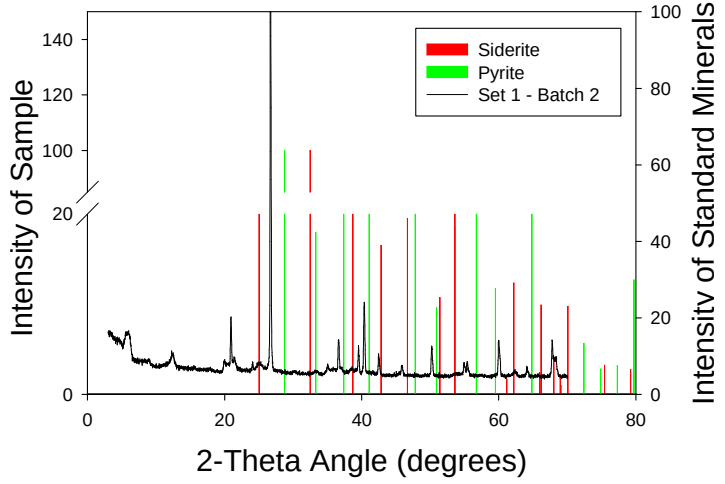

Figure 26. Set 1vs. siderite and pyrite.

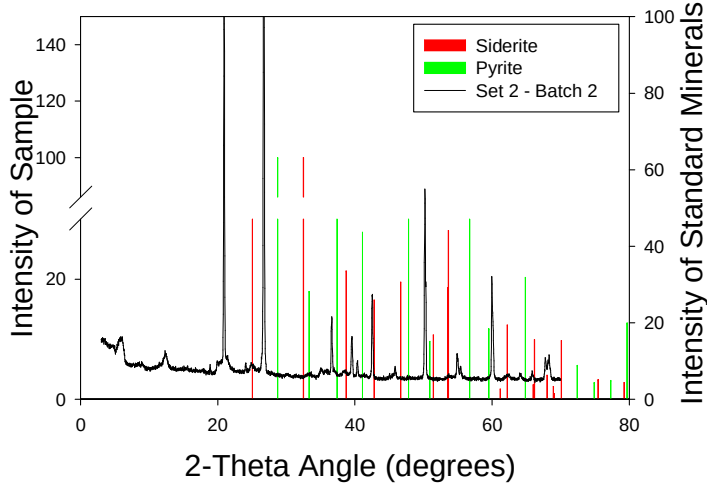

Figure 27. Set 2 vs. siderite and pyrite.

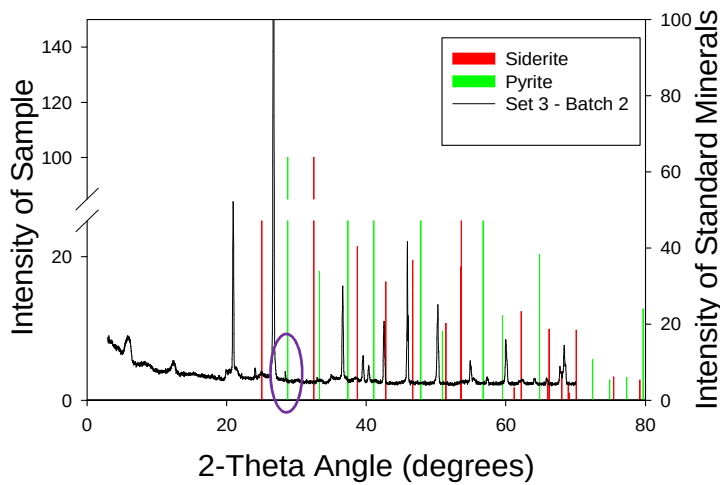

Figure 28. Set 3 vs. siderite and pyrite.

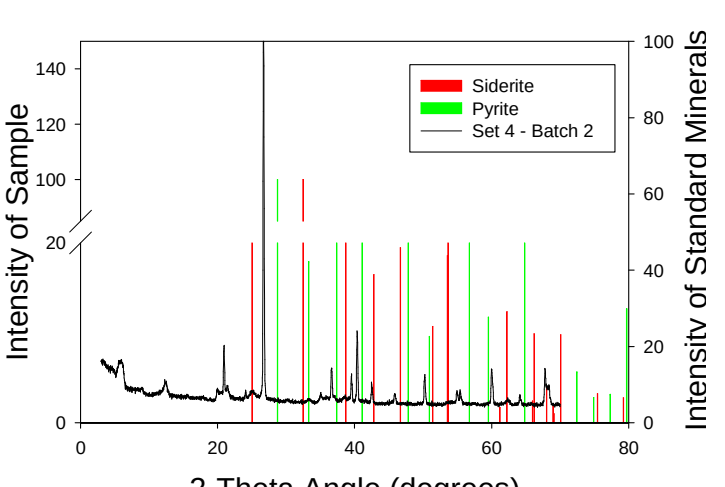

Figure 29. Set 4 vs. siderite and pyrite.

FUTURE WORK

To determine if any changes will occur after a longer period in the anaerobic chamber, another set of sub-samples will be collected from Batch 2 and analyzed via XRD. In addition, another set of pH measurements will be taken for the pH evolution study. Soluble ferrous iron concentration will be measured via ICP-OES in the supernatant solution. In samples amended with sulfate, concentrations will be examined using an ion chromatography analysis. Upon completing these tests, the next step will be to establish what kinds of changes occur when the samples are re-oxygenated. The samples will be taken out of the anaerobic chamber and placed on the bench at atmospheric conditions. After the samples have been given sufficient time to oxygenate, pH levels, sulfate and iron concentrations will be measured again. In addition, another round of XRD analysis will be conducted to observe for mineralogical changes.

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