Carbon and iron additions to stimulate in-pit sulfate reduction and removal

Dr. Nathan Johnson and Dr. Xianben Zhu

University of Minnesota Duluth
Department of Civil Engineering

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Research Report

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Abstract

Large volumes of water containing elevated concentrations of sulfate and other dissolved solids are present in abandoned mine pits on the Mesabi Iron Range. The release of water with elevated sulfate and, to a lesser extent hardness, is an environmental concern owing to its potential effects on wild rice, mercury, and phosphorus. Using conventional technology for treatment of mine pit waters is a challenge owing to the large volumes of water present and discharge patterns which can be driven by natural hydrologic processes. Biological sulfate reduction is used in both engineered and natural sulfate treatment systems in a process whereby carbon provides the fuel to drive the transformation of sulfate to sulfide, and iron or another metal provides a means to remove sulfide from water.

The primary objective of this study was to determine whether the artificial addition of iron and carbon could be used to stimulate biological sulfate reduction and remove high sulfate and hardness from mine pit waters on the Mesabi Iron Range. Towards this end, short term, batch laboratory studies tested the effectiveness of different carbon and iron sources at both room temperature and 4^{o}C under mixed and unmixed conditions. The effectiveness of carbon and iron sources was evaluated based on the rate at which sulfate was removed and the ability of added iron to keep hydrogen sulfide, a toxin to the sulfate reduction process, concentrations low. The ability to simultaneously remove hardness through precipitation with carbonate was also evaluated.

Of the carbon sources tested, ethanol was the most effective in driving biological sulfate reduction. While >90% sulfate reduction was observed after only 3 weeks in well-mixed ethanol-amended waters at room temperature, the reaction occurred 10-20 times slower at 4^{o}C. Iron materials were not added in great excess; however, of the two iron sources tested, iron carbonate was most effective at keeping hydrogen sulfide concentrations low. No combinations of iron and carbon amendments were able to remove hardness effectively. Due to the high cost and non-local source of commercial iron carbonate, future investigations should further consider the ability of locally available minerals to effectively provide iron for in-situ sulfate reduction and removal processes.

1. Background

Waters influenced by mining on the Mesabi Iron Range are of environmental concern due to the presence of elevated concentrations of sulfate and hardness (major divalent cations are calcium and magnesium). As pits dewatered during past mining operations fill with water, some accumulate high levels of these ions. If these pits fill completely, they have the potential to discharge into local waterways and present a risk to ecosystem health.

Sulfate reduction to sulfide is a natural, biologically mediated process which has the potential to remove sulfate from water. If metals are present to precipitate reduced sulfur into insoluble metal sulfides, the resulting solids can be removed by gravity or filtration (EPA 2008a). In both natural and engineered treatment settings, the addition of a carbon source to facilitate this biological process is used to treat waters for metals and sulfate (EPA 2008b, Jong and Perry 2003). In many cases, sulfate is accompanied by low pH and must be neutralized prior to treatment with biological sulfate reduction. The high pH of waters on the Mesabi Iron Range keeps most metals out of solution, and therefore a source of iron or another metal is necessary to complete the sulfate reduction and removal process. Though iron is present in vast quantities on the Mesabi Iron Range, most accessibly in the form of stockpiled (oxidized, ferric, Fe^{3+}), a reduced form of iron (reduced, ferrous, Fe^{2+}) is necessary to bind with and remove sulfide. The major cations of concern, such as calcium and magnesium, form carbonate minerals in nature and could, theoretically, form under conditions occurring in mine water pits. The formation of these minerals, however, is kinetically limited and removal in time scales conducive to treatment is challenging.

2. Objectives

The primary goal of this study was to determine whether the addition of iron and carbon can be used to effectively remove high sulfate and hardness from mine waters on the Mesabi Iron Range. The specific objectives include:

a. Compare the effectiveness of commercially available carbon sources in facilitating sulfate reduction process and quantify rates of sulfate reduction at relevant temperatures.

b. Determine the effectiveness of different methods of iron addition (oxidized or reduced) for scavenging sulfide (produced as a result of sulfate reduction) under both well-mixed and unmixed conditions at cold (4C) and warm (25C) temperatures.

c. Determine whether high CO_2 contained in waters following iron and carbon additions could remove hardness (Ca & Mg).

3. Methods

Overall Experimental Design

A preliminary set of experiments were performed under well-mixed, room temperature conditions to first test (a) which carbon source was most effective in reducing sulfate and (b) whether a commercially available reduced iron source (siderite, ferrous iron carbonate) could provide iron to remove sulfide. A follow up set of experiments utilized the most effective carbon source (ethanol) to test (a) different iron sources (reduced iron carbonate vs. oxidized taconite tailings), (b) the effect of temperature, and (c) the effects of well-mixed vs. stagnant experimental conditions.

Sources of Materials

Sediment collected from Lake Manganika (located south of Virginia, MN) was used to inoculate experiments with sulfate reducing bacteria (SRB). Previous studies have shown evidence of vigorous sulfate reduction in this lake's sediments (Berndt and Bavin, 2011). Water used for the experiment was collected from Second Creek (located east of Aurora, MN) which is fed by the overflow from a high-sulfate (~500-1000mg/L) and high-hardness (50-70mg/L Ca2+, 175-275mg/L Mg2+) mining pit. Candidate carbon sources including pure ethanol, biosolids provided by Western Lake Superior Sanitary District (WLSSD), and commercially available molasses were tested in a preliminary experiment. Commercially available siderite ($FeCO_3$, ~60% purity, Eastern Minerals) and taconite tailings (Minntac) were used as iron sources. The sources and basic properties of the above materials are summarized in Table 3. 1.

Table 3.1 The sources and basic properties of the materials used in this study.

Material	Source	Key properties
Sediment	Lake Maganika peripheral sediment (not from deep area)	Water content: approximate 99.5%

Material	Source	Key properties
Water sample	Second Creek	SO_4^{2-} content: ~1000mg/L Ca2+: ~50-60mg/L Mg2+: ~250-300mg/L Alk: ~350-450mg/L (as CaCO3)
Ethanol	Purchased	Purity: 100%
Biosolids	Western Lake Superior Sanitary District (WLSSD)	Carbon content: ~35%
Molasses	Commercially available	Carbon content: ~16g/L
Siderite (ferrous iron carbonate)	Purchased (Eastern Minerals)	$FeCO_3$ content: ~60%
Taconite tailings (ferric and ferrous Fe silicates, carbonates and oxide mix)	Taconite tailings (Minntac)	Iron content ~12%

Experimental design

Preliminary experimental design

A preliminary set of batch experiments was conducted in well-mixed, 125ml serum bottles with varying amounts of carbon (ethanol, biosolids, and molasses) and iron (siderite) added to high-sulfate/hardness water. Bottles were stored under well-mixed, or occasionally mixed (daily) conditions at 25C and sacrificed for the analysis of sulfate, pH, sulfide, and ferrous iron at times between 0 and 4 weeks. For all bottles, Lake Manganika sediment was added to approximately 0.625 grams (dry weight) of per L of water. Treatments 1-3 (Table 3.2) used ethanol as carbon source at a carbon content of 60mmol/L (Fauville et al. 2004) and various iron loadings in the form of siderite (Table 3.2).

Treatments 4-6 using biosolids as a carbon source at various carbon contents of approximately 250 mmol/L, 500mmol/L, and 750 mmol/L of carbon. The total Fe^{2+} loading was 10 mmol/L. Treatment 7 contained molasses at a carbon content of approximately 100 mmol/L and Fe^{2+} mass equal to 10 mmol/L. Table 3.2 summarizes the experiments that were conducted during the second phase of the study. In addition to the various iron and carbon additions, a mixture of only sediment and water was used as a blank for this study. All experiments were performed in triplicate.

Table 3.2 Summary of reactants and conditions for preliminary experiments.

Treatment number	**Carbon source**	**Carbon content, mmol/L**	**Fe^{2+} concentration, mmol/L**	**Temperature, °C**	**Reaction time, weeks**
1	Ethanol	60	3	25	0, 1, 2, 3, 4
2	Ethanol	60	10	25	0, 1, 2, 3, 4
3	Ethanol	60	20	25	0, 1, 2, 3, 4
4	Biosolids	250	10	25	0, 1, 2, 3, 4
5	Biosolids	500	10	25	0, 1, 2, 3, 4
6	Biosolids	750	10	25	0, 1, 2, 3, 4
7	Molasses	100	10	25	0, 1

Secondary experimental design

After establishing a successful carbon source in the preliminary experiments, a second set of experiments used only ethanol as a carbon source, but (a) introduced taconite tailings as a source of iron to combine with and remove reduced sulfide from the system. (b) examined the effect of temperature by conducting parallel experiments at 25 and 4C, and (c) investigated the effect of no or minimal mixing. These experiments were conducted in two batch physical systems.

Treatments 1-3 (Table 3.3) for the secondary experiments were again carried out in 125mL serum bottles with taconite and ethanol. Treatments 1 and 2 both contain taconite tailings, but different in the incubation temperature. Treatment 3 was also performed at 4C, but contained siderite as an iron source. Treatments 4-6 were identical in content to treatments 1-3, but were carried out in 3" diameter, 12" tall columns without active mixing. Treatment 7 was performed in a column, but only mixed occasionally (<few hours).

Tables 3.3 and 3.4 summarize the treatments for the secondary study. Several bottle blanks (lacking carbon, iron, or sediment amendment) were also included.

Table 3.3 Summary of the experimental treatments for secondary experiment

Treatment number	**Bottle/ column**	**Mixing/Non-mixing**	**Temperature, °C**	**Fe^{2+} source, Siderite/Tailings**
1	Bottle	Mixing	25	Tailings
2	Bottle	Mixing	4	Tailings
3	Bottle	Mixing	4	Siderite
4	Column	Occasional mixing	25	Tailings
5	Column	Non-mixing	25	Tailings
6	Column	Non-mixing	4	Tailings
7	Column	Non-mixing	4	Siderite

Table 3.4 Summary of blanks for secondary experiment. All blanks were in 125mL serum bottles

Blank number	**Mixture type**	**Fe^{2+} source, Siderite/Tilling**
1	DI water	None
2	Water sample	None
3	Water sample +sediment	None
4	Water sample +sediment + ethanol	None
5	Water sample +ethanol	None
6	Water sample	Tailings
7	Water sample	Siderite

Experimental procedures

Preliminary experimental procedures

To initiate experiments with the required inoculum (sediment), carbon source (ethanol/ biosolids/ molasses), and iron mass (siderite), the following procedure was used. First, 202.5 g (wet, 99.5% water content) of sediment was added to 1.62 L water sample and mixed in a 2500ml beaker to create 0.625 g/L sediment (Dry) content. While maintaining stirring, 90mL of this mixture was transported into replicate 125mL amber bottles under a nitrogen atmosphere. Next, 0.157mL of ethanol was injected in

the bottles, creating 60 mmol/L carbon. For samples using biosolids and molasses as carbon source, the carbon sources were mixed with sediment and water sample before they were transferred into 100mL amber bottles.

Iron carbonate (siderite) was suspended in DI water and, while maintaining stirring, the appropriate volume of siderite solution was pipetted into the amber bottles to reach target Fe^{2+} mass (volume times assumed suspended concentration) (Table 3.2). Approximately 10% of the Fe needed for complete removal of sulfide was added initially, and the remaining 90% was added after 12 days. After filling with the sediment mixture, carbon source, and Fe^{2+} source, bottles were sealed with 1cm thick butyl rubber stoppers (Bellco glass) and aluminum crimp caps to maintain strictly anaerobic conditions and placed on a continuous shaker table. Figure 3.1 depicts the experimental setup process. Replicate bottles were created for each treatment so that each bottle was sampled only once before being discarded.

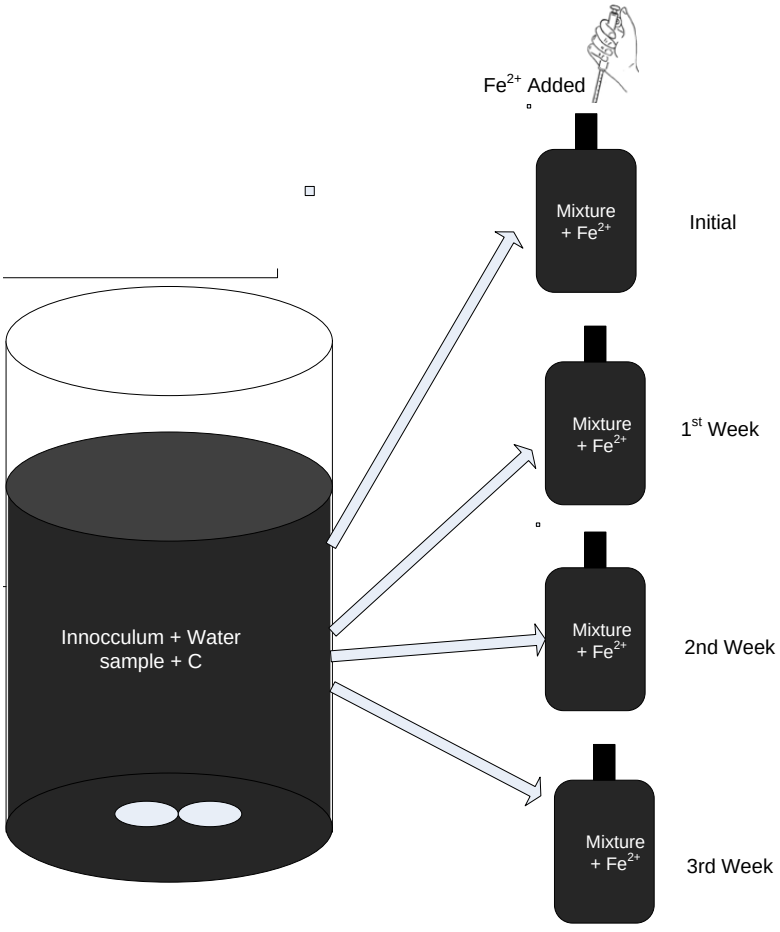

Figure 3.1 Schematic of experiment setup for bottles.

Secondary experimental procedures

Bottles were prepared as described for the preliminary experiment and the contents of each bottle are summarized in Table 3.5.

Table 3.5 Contents of each (80mL) bottle for secondary experiment.

Iron added	0.82	mmol
$FeCO_3$	157.82	mg
Tailings	544.22	mg
Ethanol	0.54	mmol
	0.03	mL
Sediment	8	mL wet
	0.04	g dry

Columns for secondary experiments were fabricated by capping both ends of a 3" diameter, 12" high clear polycarbonate column. Sampling ports were inserted by drilling holes and inserting butyl rubber stoppers along the side of the column located at 2.5 inches, 5.75 inches, and 9 inches from the bottom. A sampling port was also set in the bottom cap. One set of replicate columns (treatment 7) were periodically mixed (~3x per week) and only a single sampling port was inserted in the middle of these columns. Columns contained 1.375L of water and to this was added 10% wet content sediment in order to obtain 0.5g/L solids. Ethanol and iron supplement was added directly to each of 12 columns (Treatments 4-7). Triplicate columns were stored at either 25 or 4C. The contents of columns are shown in Table 3.6.

Table 3.6 Contents of each (1375mL) column for secondary experiment.

Iron added	10.63	mmol
$FeCO_3$	2056	mg
Tailings	7088	mg
Ethanol	7.1	mmol
	0.41	mL
Sediment	104	mL wet

	0.52	g dry

Sample collection and analytical

At the appropriate time, bottles were opened under anaerobic conditions and a portion of the fluid was poured into a 50mL plastic centrifuge tube, centrifuged at 7000mpr for 30 minutes if necessary, and filtered using 0.45 um disposable polyethersulfone cartridge filter. Measurements carried out for all treatments include pH, HS^-, Fe^{2+}, and SO_4^{2-}. Oxidation reduction potential was also measured for some samples. The pH probe was either directly inserted into the sample bottles soon after opening or in filtered samples. Little difference was observed in pH after filtering suggesting that CO_2 and H_2S gasses are not escaping quickly (15-30min) after samples are collected. Once a bottle was opened and sample was collected from it, it was discarded or re-sealed and frozen.

To sample columns, each column was carefully transported into the anaerobic glove bag with minimal mixing, and hypodermic needles were used to extract a subsample of water from each port into a polypropylene syringe barrel for processing and analysis. Although bottles were maintained sealed (and pressurized by produced gasses), it was observed that the columns did not maintain a perfect seal. No attempt was made to characterize the pressure within the column, but bubbles were occasionally observed emanating from the bottom which confounded the expected "unmixed" conditions. After the first several sampling points, it became apparent that large differences were not seen between the bottom, middle, and high sampling ports so a single sample was collected for later sampling events. Importantly, however, the columns did remain unmixed between sampling events which should have greatly reduced the proximity of solids (sediment, siderite, taconite) to the bulk fluid. Water extracted from the columns was treated in the same manner as samples from bottles as described above.

Sulfate was measured by a standard Hach turbidometric method (detection limit ~10mg/L) for the preliminary experiments and by ion chromatography (Dionex 1100 with A22 column, detection limit ~0.08mg/L) for the secondary experiment. In both cases, sulfate was measured following acidification to pH 2.5 with HCl, purging of dissolved gasses to remove sulfide and avoid oxidation to sulfate, and dilution with MilliQ water. Samples for total dissolved sulfide (sum of H2S and HS-) were mixed 1:1 with sulfide antioxidant buffer (Eaton et al. 2005) and measured with an ion specific electrode with a detection limit of 5uM. Ferrous iron was measured using a standard phenanthroline method (Phillip and Loveley, 1987. pH was measured with an electrode calibrated immediately prior to sampling. ORP was

measured using a platinum electrode with a silver-sulfide reference electrode (-197mV relative to Standard Hydrogen Electrode). Cations were measured by ICP on a subset of samples and acidified sample (~0.2% HCl). Carbonate concentration was determined by a standard alkalinity titration adjusted for the quantity of bisulfide (Eaton et al. 2005).

4. Results

Results from preliminary experiments

Results from the preliminary set of experiments are shown in Figure 4.1-Figure 4.3. Both ethanol and biosolids effectively reduced sulfate within 4 weeks in the 25C bottles during the preliminary experiment (Figure 4.1). As a soluble material, ethanol appeared to be a preferable carbon source since sulfate dropped more quickly in bottles amended ethanol than biosolids. In bottles amended with molasses, sulfate appeared to increase and this was accompanied by a sharp decrease in pH to <5 (data not shown). This behavior may have been due to the specific commercial source of molasses, but due to the effective reductions observed with ethanol, further experiments utilizing molasses were not performed. It is important to note that sulfate was also reduced in bottles containing only sediment and water with no iron addition (Figure 4.1).

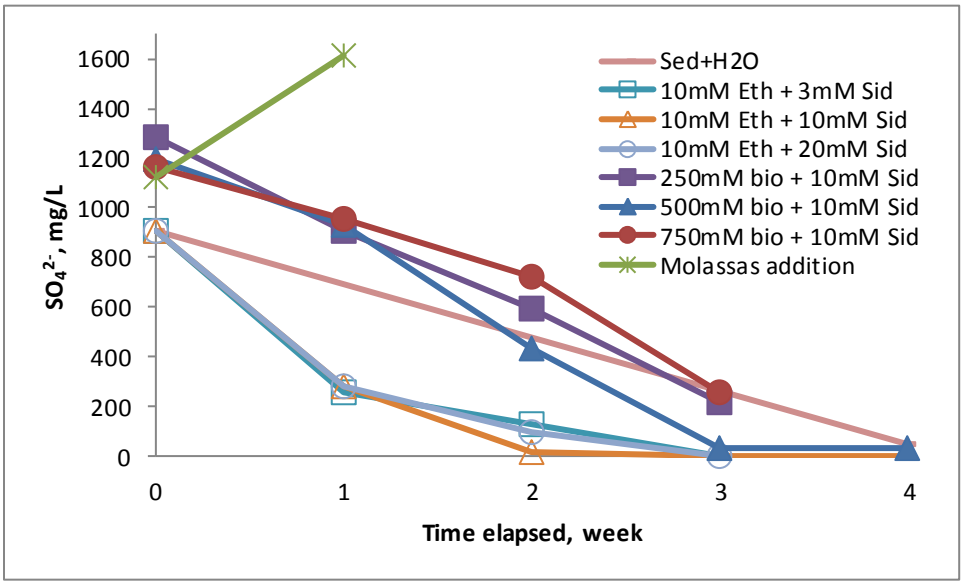

Figure 4.1 Change of sulfate concentration with time in preliminary experiments.

Sulfide concentrations (total dissolved sulfide, sum of H2S and HS-) rose quickly in the bottles amended with ethanol after the first week due to a low mass of ferrous iron (Figure 4.2). After 12 days, bottles

were opened under an anoxic atmosphere and additional ferrous iron was added to bring up to 3, 10, 20mM iron. Bottles amended with biosolids contained 10mM iron from the beginning of experiments. Sulfide dropped considerably following this iron addition in the ethanol bottles, but remained between 0.5 and 1.5mM. Sufide in bottles amended with biosolids rose slowly to eventual concentrations between 0.05 and 0.75mM. One biosolids amended bottle sampled at 4 weeks showed a large increase in sulfide to over 2.5mM. Bottles without iron addition showed relatively low sulfide (~0.5mM after 4 weeks).

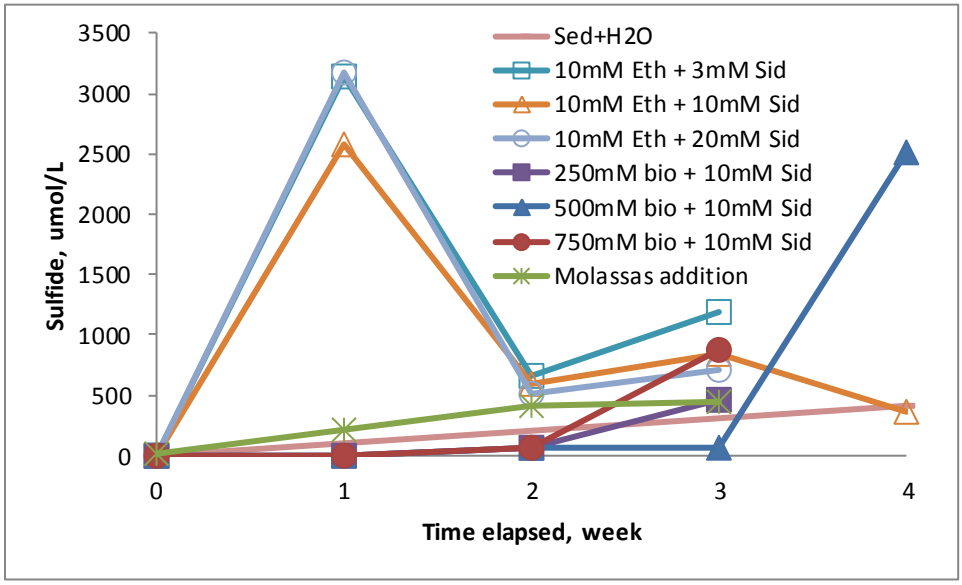

Figure 4.2 Change of sulfide concentration with time in preliminary experiments

Ferrous iron concentrations dropped over time in all bottles, but remained well above detection limits until 3 weeks (Figure 4.3). With the exception of the 750mM biosolids amendment, all treatments began between 0.1-0.2mM dissolved ferrous iron and droped to near zero over the three weeks of the experiment. The reasons for high ferrous iron in the highest biosolid amendment is unknown and the pH of this amendment was very similar to other biosolids amendments.

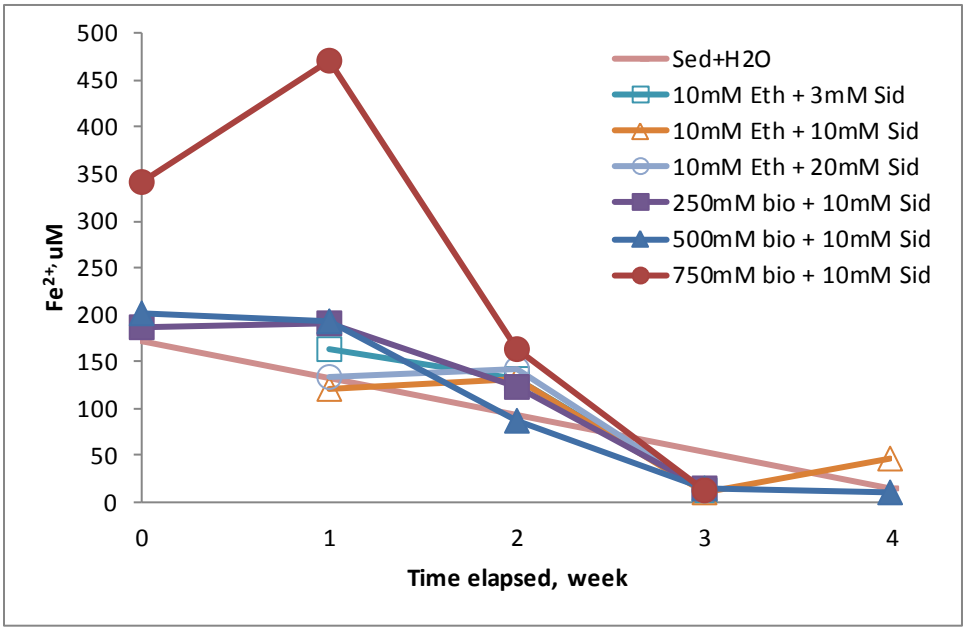

Figure 4.3 Change of Ferrous iron concentration with time in preliminary experiments.

Measurements for pH and ORP were made in water samples from both the preliminary and secondary experiments, but may have been compromised. pH was not consistently measured immediately after opening bottles and the loss of CO2 from samples almost certainly artificially raised the measured pH for many samples. Similarly, ORP was sometimes measured a full day after the sample had been opened and dissolution of H2 from the glove box atmosphere (~2% H2) may have artificially lowered the measured ORP. Because these measurements may have been compromised, results are reported in the appendix (Figures S1 and S2) and should be interpreted with caution.

For the first set of experiments, the sulfate reduction rate (SRR) was calculated in two different ways. The first was simply the rate of loss of sulfate in the bottles in units of mmol/L/day. The second method assumed SRR to be a first order function of the sulfate present:

$$r_{SO4}\left[\frac{mol}{L-day}\right] = -k\left[\frac{1}{day}\right]C_{SO4}\left[\frac{mol}{L}\right]$$

Equation 4-1 First order rate of sulfate reduction

In this case, the reaction of sulfate in the batch bottle system would be given by:

$C_t = C_0 e^{-kt}$ Equation 4-2 Assumed concentration dependence on time in bottles

and the reaction rate, k [1/day] can be found by plotting $\ln\left(\frac{C_t}{C_0}\right)$ versus t. Since all amendments for biosolids and all amendments for ethanol behaved similarly, the average value of sulfate for each carbon source at 0 weeks, 1 week, and 2 weeks was used to find the sulfate reduction rate. The preliminary set of experiments at 25C (using ethanol, biosolids, and siderite) resulted in reaction rates of 0.61mM/day (0.173 [1/day]) for ethanol and 0.47mM/day (0.052 [1/day]) for biosolids as shown in Figure 4.1 and Figure 4.4.

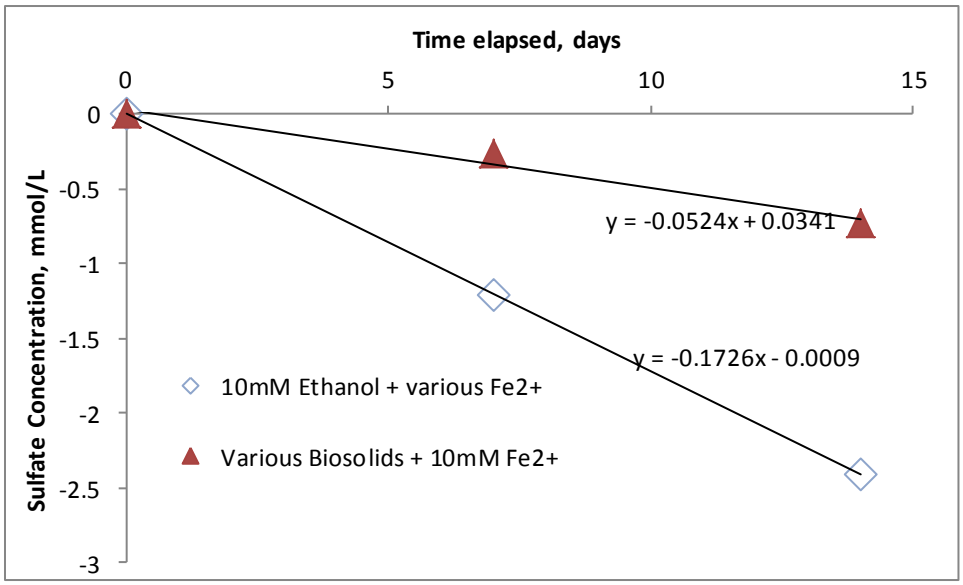

Figure 4.4 Average sulfate reduction rates observed for ethanol and biosolids at 25C.

Results from secondary experiments

In the second set of experiments, sulfate reduction again occurred rapidly over the first two weeks in well-mixed 25C experiments using taconite as an iron source (

Figure 4.5). However, complete reduction was not observed as it was in the siderite-amended preliminary experiments. At 4C, sulfate reduction occurred with both siderite and taconite as an iron source, and no significant difference was seen over the 9 week incubation.

Sulfide concentrations built up rapidly in the well-mixed 25C incubation using taconite as an iron source (

Figure 4.6). Concentrations in excess of 2mM were observed after only one week and, though a lower value was observed at 14 days, the concentration was consistently >3.5mM at 21 days. Sulfide inhibition of sulfate reducing bacterial activity has been observed at sulfide concentrations between 3-5mM (Maillacheruvueta et al. 1993). At 4C, little sulfide was observed after 14 days, but began to build up in the well-mixed bottles after 45 days (Figure 4.6). Bottles containing siderite had appreciably less sulfide than those containing taconite at 45 and 63 days.

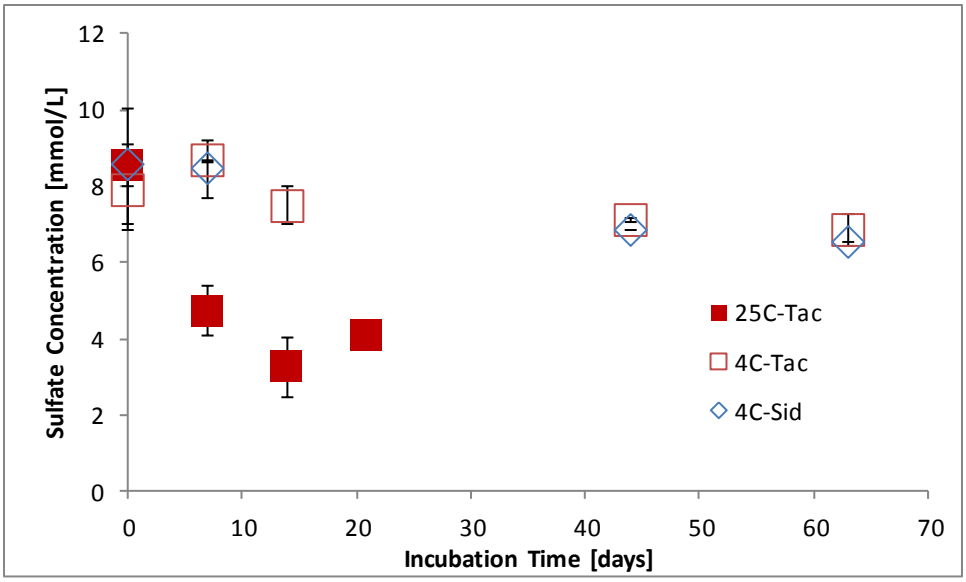

Figure 4.5 Sulfate concentrations in secondary well-mixed experiments

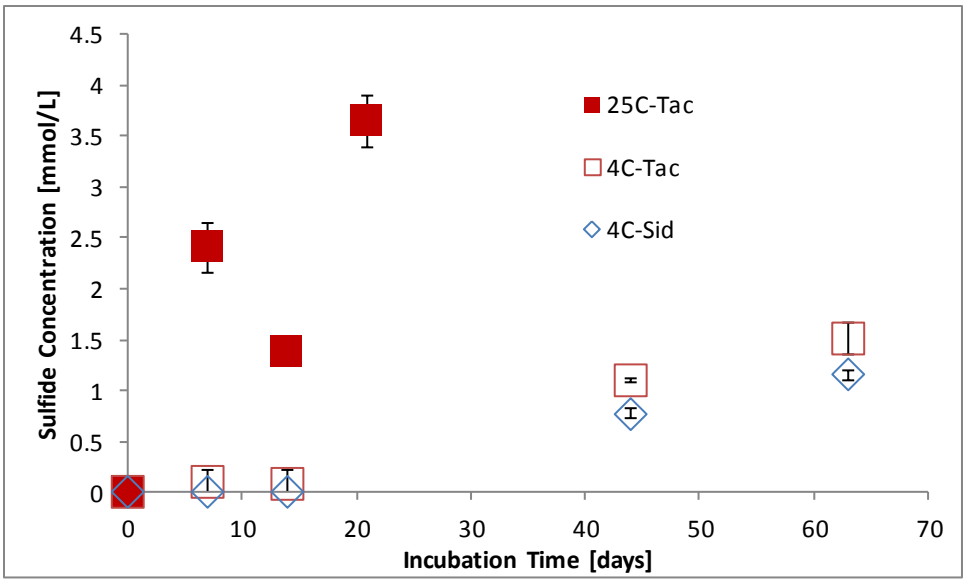

Figure 4.6 Sulfide concentrations in secondary well-mixed experiments

Iron concentrations remained low in well-mixed experiments amended with taconite and siderite (

Figure 4.7) during the secondary experiment. Although some low Fe concentrations were detected, they were all near the method reporting limit of 8umol/L. These low iron concentrations are to be expected given the very high sulfide observed, circumneutral pH (6.5-8), and likely formation of insoluble iron-sulfide solid phases.

Sulfate reduction rate (Figure 4.8), quantified again by plotting the ln of C/C_0 vs. time for the well-mixed secondary experiments, resulted in a rate of 0.38mM/day (0.07/day) for the 25C experiments and 0.035mM/day (0.0046/day) and 0.022M/day (0.0029/day) for incubations at 4C containing siderite and taconite respectively. Since sulfate reduction appeared to slow after 14 days, only concentrations from

0, 7, and 14 days were used to calculate SRR at 25C.

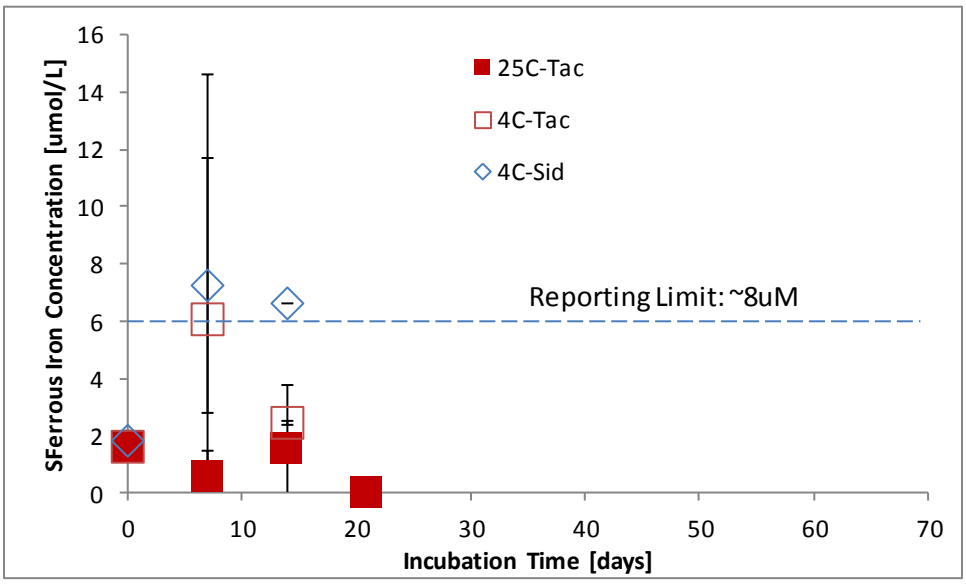

Figure 4.7 Ferrous iron concentrations in secondary well-mixed experiments.

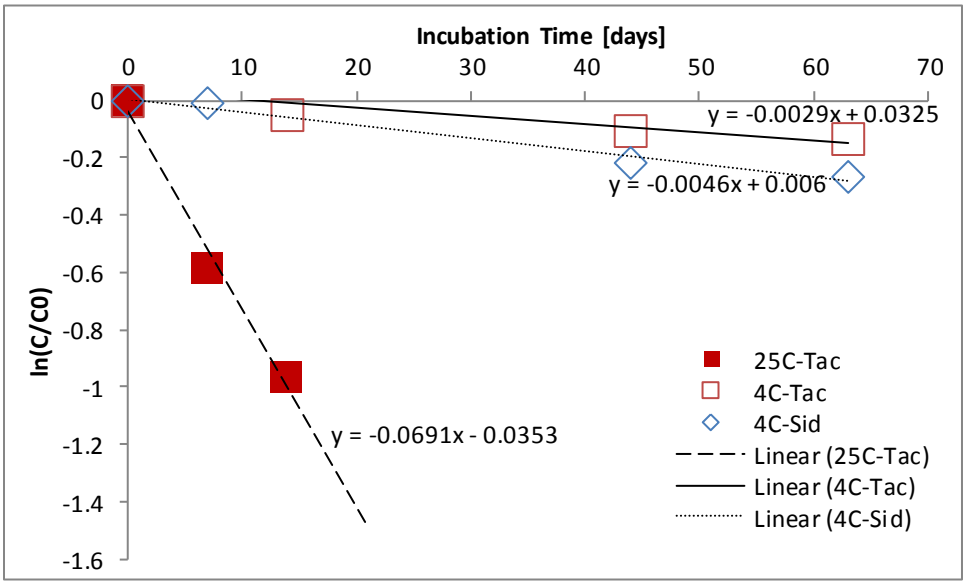

Figure 4.8 Sulfate reduction rates for secondary well-mixed experiments.

For column (unmixed) experiments, sulfate was again reduced much more quickly at 25C than at 4C (Figure 4.9), while sulfide built up to 3-4mM in 25C column waters and remained low (<1mM) at 4C after 8 weeks (Figure 4.10). Although some difference was observed between occasionally mixed system and the unmixed 25C column experiments, both showed rapid reduction of sulfate suggesting that bacteria

performing sulfate reduction have colonized the water and are not only in the solid-phase inoculum. Similar to well-mixed system, columns amended with siderite rather than taconite had less porewater sulfide.

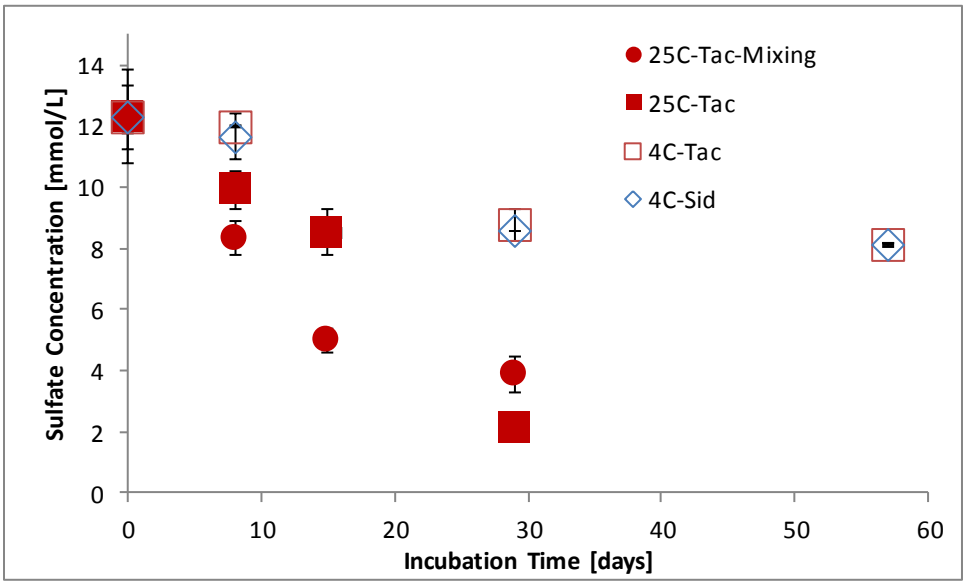

Figure 4.9 Sulfate concentrations in secondary column experiments

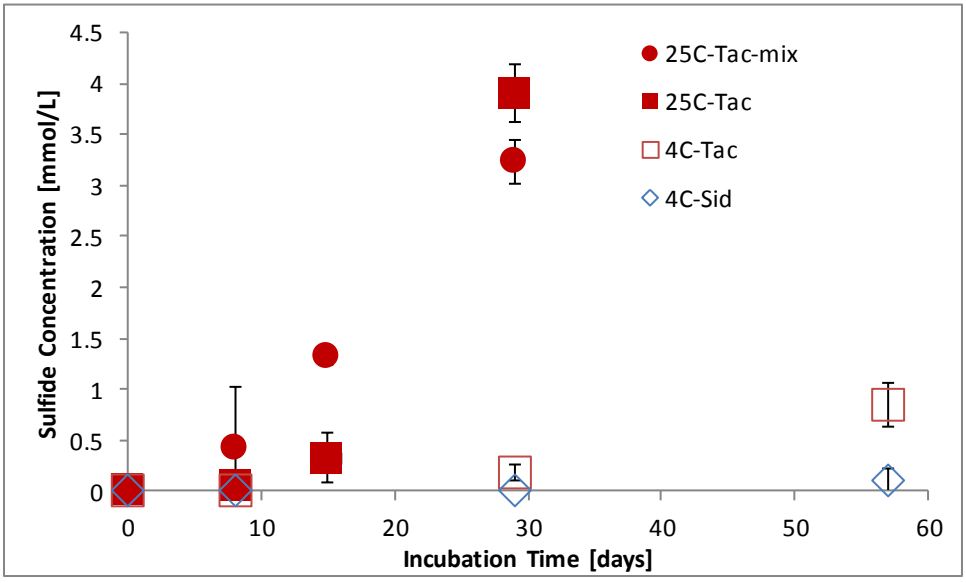

Figure 4.10 Sulfide concentrations in secondary column experiments

The only ferrous iron concentration measured consistently above the reporting limit for column experiments was in the siderite amended column at 4C (Figure 4.11) after 14 days. The parallel column

with taconite showed much higher sulfide concentrations at 28 and 56 days and dissolved iron concentrations below the method reporting limit.

Sulfate reduction rates measured in the column studies were 0.49mM/day (0.06/day) for the occasionally mixed 25C column, 0.35mM/day (0.025/day) for the unmixed 25C column, and 0.078mM/day (0.0078/day) for the 4C columns amended with taconite and siderite.

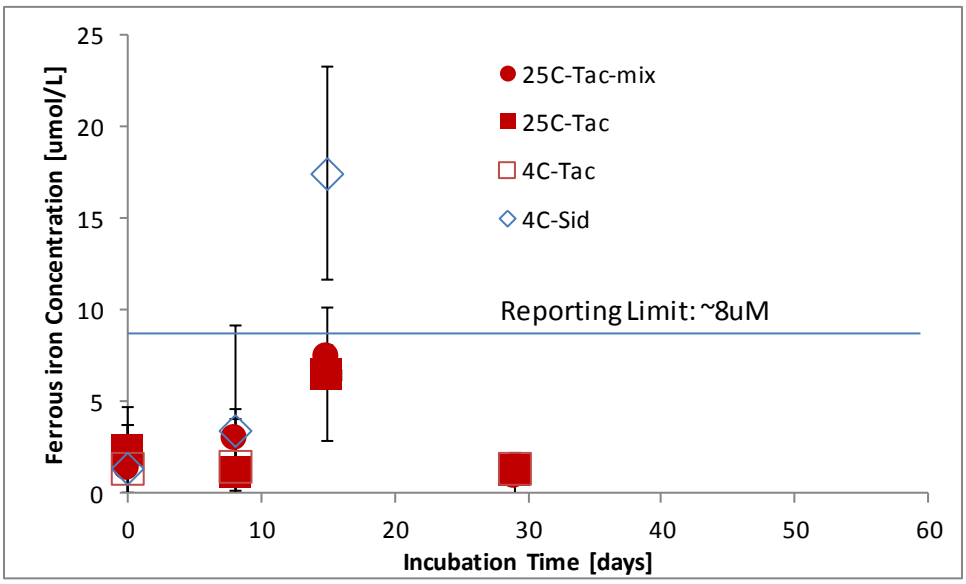

Figure 4.11 Ferrous iron concentrations in secondary column experiments.

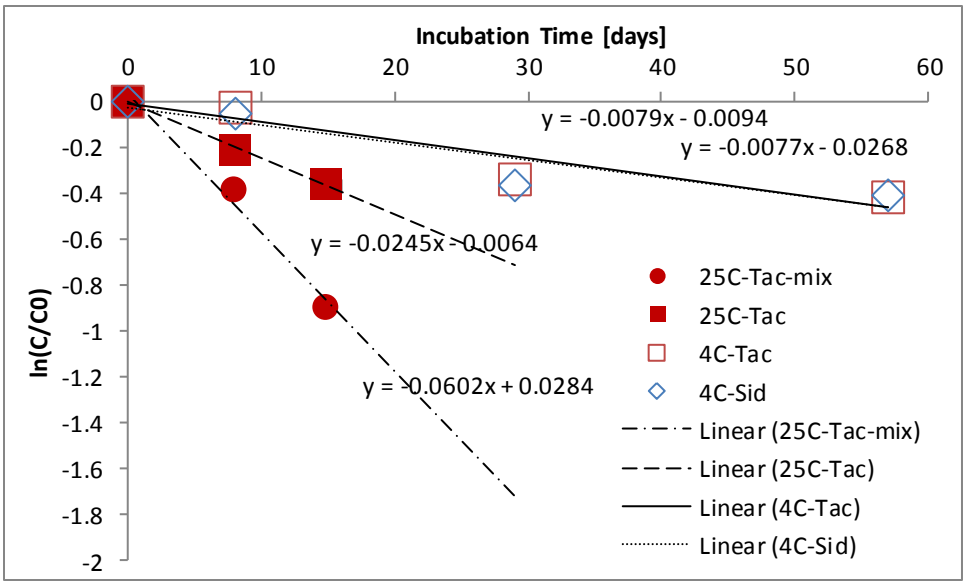

Figure 4.12 Sulfate reduction rates for secondary column experiments.

An attempt was made to quantify vertical structure of water chemistry in small-scale column experiments, however very little difference was seen between samples collected at the bottom, middle, and top of columns (

Figure 4.13).

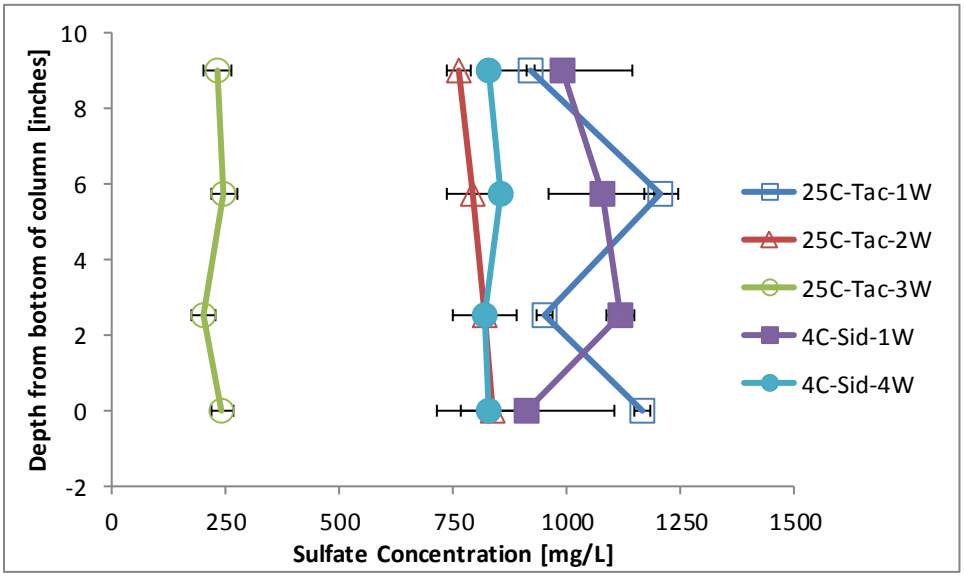

Figure 4.13 Vertical measurements for sulfate in columns at several time points.

The capacity for the sulfate/iron amended systems to remove hardness (Ca^{2+} & Mg^{2+}) was also investigated by making measurements in selected experiments to quantify both total carbonate and a suite of cations. Carbonate in several samples collected near the end of the secondary experiment was estimated as the non-sulfide alkalinity and determined by a standard HCl titration. Measured values (expressed as mg/L $CaCO_3$) are shown in Figure 4.14. All values are higher than that observed in the original source water (350-450 mg/L $CaCO_3$). A significant difference was observed between 25C bottles amended with 10mM ethanol and those with only sediment and water after 3 weeks. However, little difference in carbonate concentration was observed between the 4C samples amended with siderite and those amended with taconite. This suggests that the major source of carbonate to the water was biological and not from dissolution of iron carbonate.

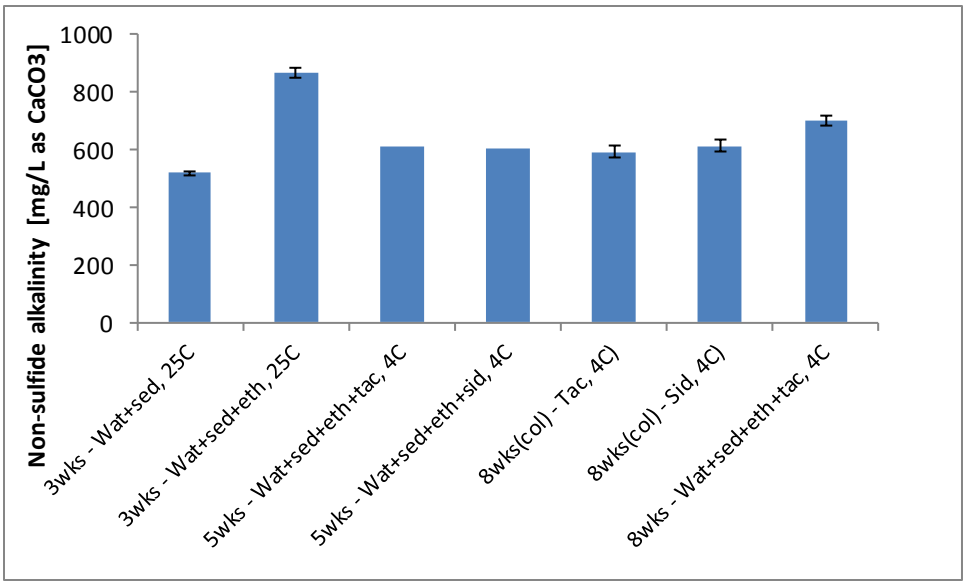

Figure 4.14 Non-sulfide alkalinity measured as a proxy for carbonate in bottles and columns.

Major and minor cations at one and four week time points are shown in Figure 4.15 for 25C bottles (a-c), 25C columns amended with taconite (d-f), and 4C columns amended with taconite and siderite (g-i). Little change is observed in major cations (Ca^{2+} and Mg^{2+}) indicating that even though a large amount of carbonate was present in the experiments, little reduction in Ca or Mg carbonates was observed. For 25C bottles, an increase in both Ca and Mg was observed from the initial conditions to 4W, but a similar increase was observed in blank bottles containing only site water. Dissolved iron concentrations were the most variable for all experiments and were particularly high at 4W for the 4C columns amended with siderite.

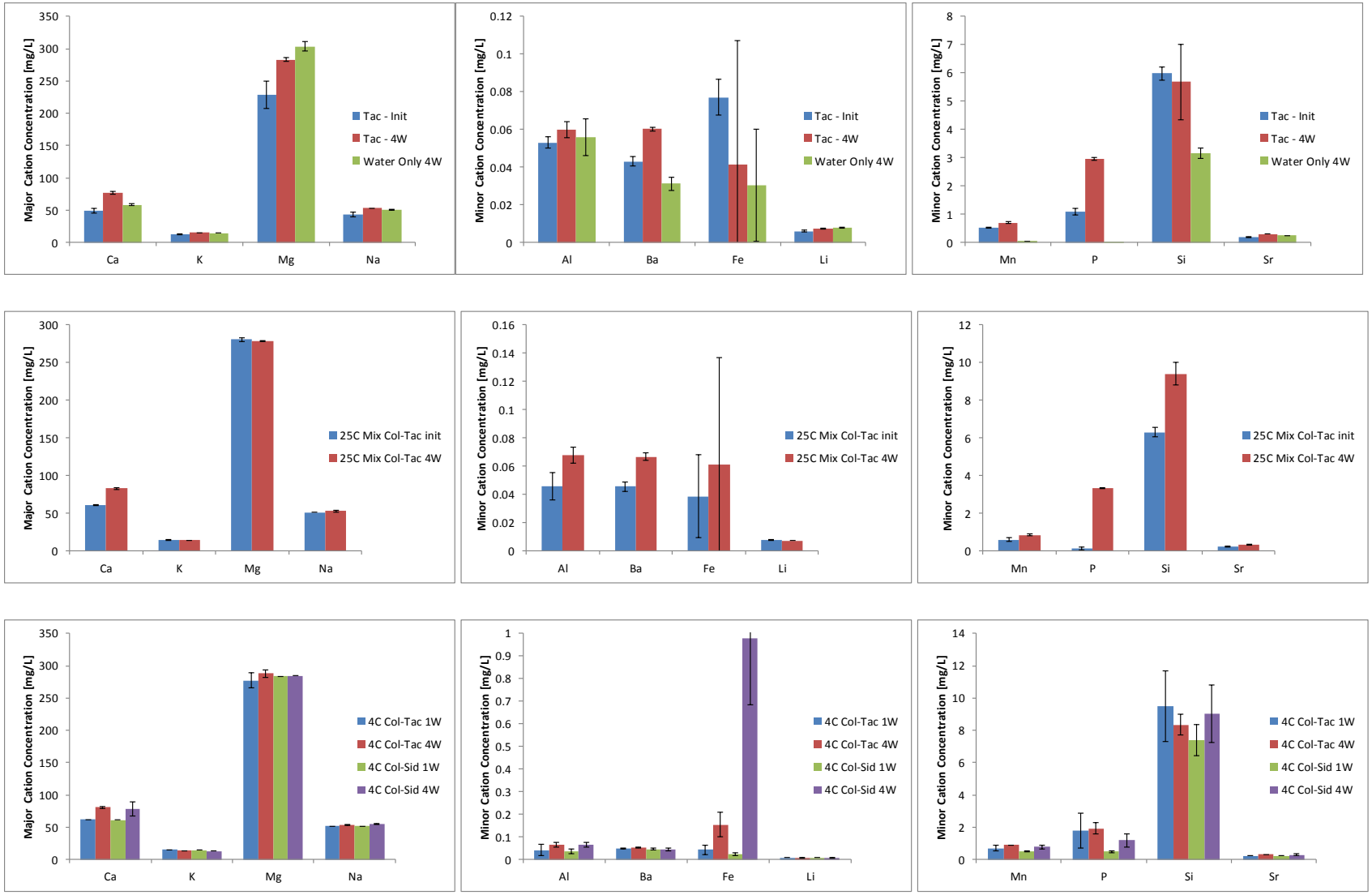

Figure 4.15 Cation concentrations at zero or one week and 4 weeks during secondary experiment. (a)-(c) – 25C bottles amended with taconite, (d)-(f) 25C columns amended with taconite, and (g)-(i) 4C columns amended with taconite and siderite.

Cost estimates for materials

A brief cost estimate was performed for iron and carbon sources. A hypothetical water body containing 1000mg/L with a volume of ~1.7 million liters (3 m deep by ~750 m square) was used as a basis (Table S.1). It was assumed that carbon was needed at just above stoichiometric requirements (110%) as ethanol appeared to be utilized readily by sulfate reducing bacteria. Two types of iron minerals were included in the analysis, siderite (~38% ferrous iron by mass) and taconite tailings (~15% iron by mass). Based on an assumption of 2x stoichiometric iron requirements for siderite and between 2 and 10 times stoichiometric iron requirements for taconite tailings, costs ranged from $0.5M to $2.5M per million cubic meters of 1000 mg/L water (Table S.2). Owing to the widespread availability of ethanol and other carbon sources, costs of carbon are be insignificant compared to the cost of iron minerals (Table S.3).

Considerations in choosing between locally available iron sources (tailings) and commercial iron sources should include the material and transportation costs but also the effectiveness of the mineral in providing iron to remove sulfide. Results presented from very short term lab studies here showed that siderite was more effective than taconite in holing HS- concentrations down. However, neither mineral was added in great (>10x) stoichiometric excess and rates of iron release may be improved if a very large quantity of mineral was added. It is possible that the performance of either material in providing iron could be imporved by physical (grinding) or chemical (activating) modifications. Cost estimate tables are included in the Appendix.

5. Summary & Conclusions

Table 5.1 presents a summary of the observations from the various experiments conducted for this study. Observed sulfate reduction rates were much slower for 4C incubations (0.022 to 0.078mM/day) regardless of iron addition method or mixing conditions. Warm (25C), mixed incubations with ethanol (0.38 to 0.61mM/day) displayed similar sulfate reduction rates as those utilizing biosolids as a carbon source (0.47mM/day); however ethanol appeared to remove sulfate more rapidly in the initial 1-2 weeks (Figure 4.1). Unmixed incubations at 4C with ethanol had a similar or even higher SRR (0.078mM/day) as those with mixed conditions (0.022-0.035mM/day), suggesting that well-mixed conditions are not a prerequisite for sustained biological sulfate reduction.

Table 5.1 Summary of experiments, observed sulfate reduction rates, effect of iron, and fraction sulfate removed.

Substrate	Mixing	Temp [degC]	Iron Addition	Sulfide Concentration [mM]	Sulfate Reduction Rate [mM/day]	Sulfate Reduction Rate [/day]	Percent sulfate removed
Biosolids	Well Mixed	25	Siderite	0.5-1.5	0.47	0.052	100 (3wks)
Ethanol	Well Mixed	25	Siderite	0.5-1.5	0.61	0.17	100 (3wks)
Ethanol	Well Mixed	25	Taconite	2.5-4.0	0.38	0.069	70 (3-4wks)
Ethanol	Well Mixed	4	Taconite	1.0-1.5	0.035	0.0029	15 (9wks)
Ethanol	Well Mixed	4	Siderite	0.5-1.0	0.022	0.0046	15 (9wks)
Ethanol	Occasional Mixing	25	Taconite	1.5-3.0	0.49	0.060	60 (4 wks)
Ethanol	Unmixed	25	Taconite	1.0-4.0	0.35	0.025	75 (4wks)
Ethanol	Unmixed	4	Taconite	0.25-1.0	0.078	0.0079	35 (8wks)
Ethanol	Unmixed	4	Siderite	0.0-0.1	0.078	0.0077	35 (8wks)

The use of taconite as an iron source (mixed Fe^{3+} and Fe^{+2}) did not appear to delay the onset of sulfate reduction (

Figure 4.5, Figure 4.9) by serving as a more energetic electron acceptor; however, experiments utilizing taconite in general displayed higher sulfide concentrations, indicating that Fe release from taconite was not able to remove the dissolved sulfide as effectively (

Figure 4.6, Figure 4.10,

Table 5.1) as was Fe release from siderite.

The inability of Fe released from taconite to deplete sulfide from solution may also have played a role in the limited extent of sulfate reduction shown in the 25C secondary experiments. Studies have shown that the level at which sulfide is toxic to sulfate reducing bacteria is dependent upon the type of carbon source being utilized. Bacteria utilizing lactate and glucose could tolerate higher sulfur concentrations than those fed acetate and propionate; however, hydrogen sulfide (H_2S) concentrations of 2-3mM caused stress in all cases (Maillacheruvu et al. 1993). Although nearly complete sulfate reduction was observed during the preliminary experiments utilizing siderite (Figure 4.1), 2-4mM sulfate (200-400mg/L) remained after 28 days in experiments using taconite (

Figure 4.5, Figure 4.9) under similar conditions (25C, mixing). The ability for taconite to provide iron for sulfide removal may also have been limited by the timescale of the experiment or the limited amount of iron added (Li et al. 2006).

Though a complete sulfur balance including gas and solid-phase measurements was not completed, evidence suggests that sulfur was removed via precipitation with iron. The loss of substantial amounts of sulfide to the gas phase is unlikely as bottles remained well-sealed throughout the experiment until they were opened in the glovebox. Columns also showed some evidence of pressurization prior to sampling. Hydrogen sulfide (H_2S) has an air-water partitioning coefficient of 0.1 [mol/L/atm] and exists as a dissolved gas at pH less than 7.0 (50% H2S, 50% HS- at pH 7.0). As a conservative estimate, if all dissolved sulfide was present as $H_2S(g)$, a sealed vessel with 20% head space would have only 10% of total sulfide present in the gas phase.

Dissolved ferrous iron remained quite high (100-200uM) in the initial set of experiments which only utilized siderite as an iron source at 25C (Figure 4.3), while sulfide concentrations remained below 1mM (Figure 4.2, excepting first week low iron addition). For the 25C incubations during the second set of experiments which utilized taconite as an iron source, sulfide rose quickly (1-2 weeks) to 1-3mM and iron concentrations remained very low (<10uM, less than reporting limits). At 4C, lower sulfide concentrations were observed in parallel experiments using siderite as an iron source under both well-mixed (

Figure 4.6) and unmixed (Figure 4.10) conditions. Slightly higher iron concentrations were also observed, although measurements were all near detection limits (

Figure 4.7, Figure 4.11). Dissolved iron concentrations (measured with cations on ICP) were substantially higher in unmixed, siderite-amended conditions after 4 weeks (1mg/L ~15uM) than in either initial conditions or in experiments using taconite as an iron source (Figure 4.15h).

Figure 4.13 shows that similar rates of sulfate removal were observed at all depths in the columns. For columns amended with either taconite or siderite, samples analyzed for sulfide at each depth did not show any pattern, suggesting that iron was equally available at all depths within the experimental columns. This could have been due to minor jostling of the columns that occurred during transport from storage to the anaerobic sampling location, or due to mixing from the production and ebullition of gas.

Major cations were not removed by the addition of CO_2 from either microbial degradation of sulfate or dissolution of iron carbonate (Figure 4.15). The ion activity product was calculated for three carbonate minerals based on carbonate estimates from alkalinity measurements (logKsp: dolomite-17.1, calcite-8.4, magnesite-7.5) and average concentrations of major cations measured at 4 weeks. Although the solubility index (SI, log of ion activity product over Ksp) was positive for dolomite magnesite, and calcite (indicating supersaturated conditions, Figure 5.1), measurements showed that magnesium and calcium were not removed from solution. Although thermodynamically favorable, the formation of carbonate minerals is often kinetically limited, especially in the presence of magnesium.

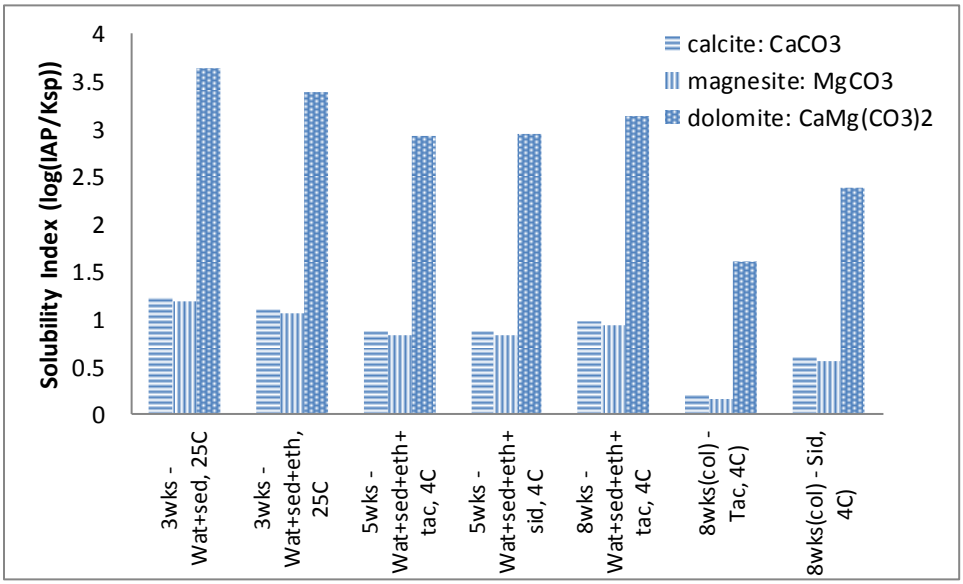

Figure 5.1 Oversaturation of carbonate minerals observed in experiments.

In summary, our results show that ethanol is a very effective carbon source for facilitating biological sulfate reduction and that reduction occurs approximately 10x more slowly at 4C than at 25C. We also found that siderite, a commercially available ferrous iron carbonate, has the potential to remove sulfide from water more effectively than taconite. Despite a large generation of carbonate during the sulfate reduction process, major cations were not removed via precipitation in carbonate minerals due to kinetic limitations.

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Acknowledgements

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Appendix

pH was measured in bottles and columns during sampling, but was not always measured immediately after sampling, so degassing of H2S or CO2 may have influenced measured pH. Results should be interpreted with caution.

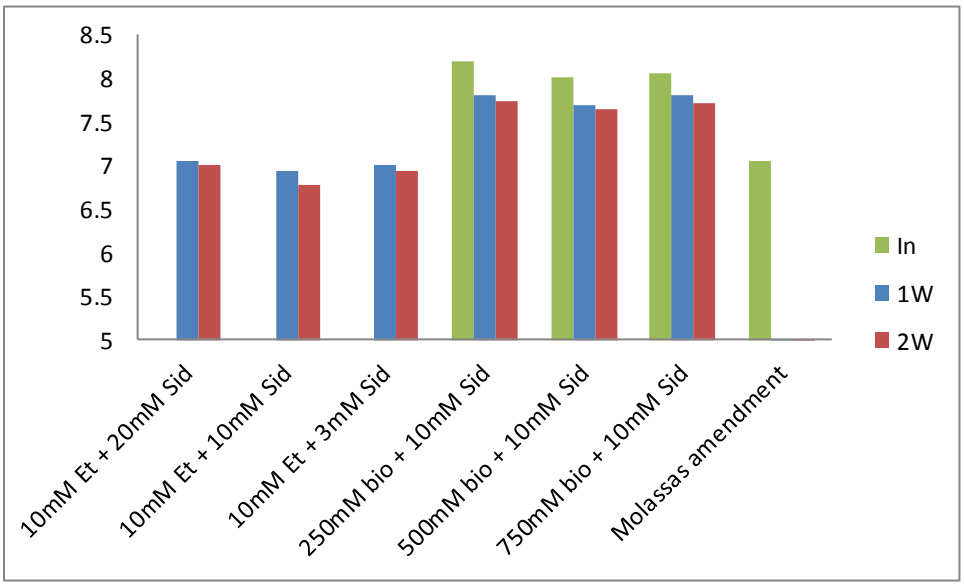

Figure S.1 pH measurements for preliminary experiments.

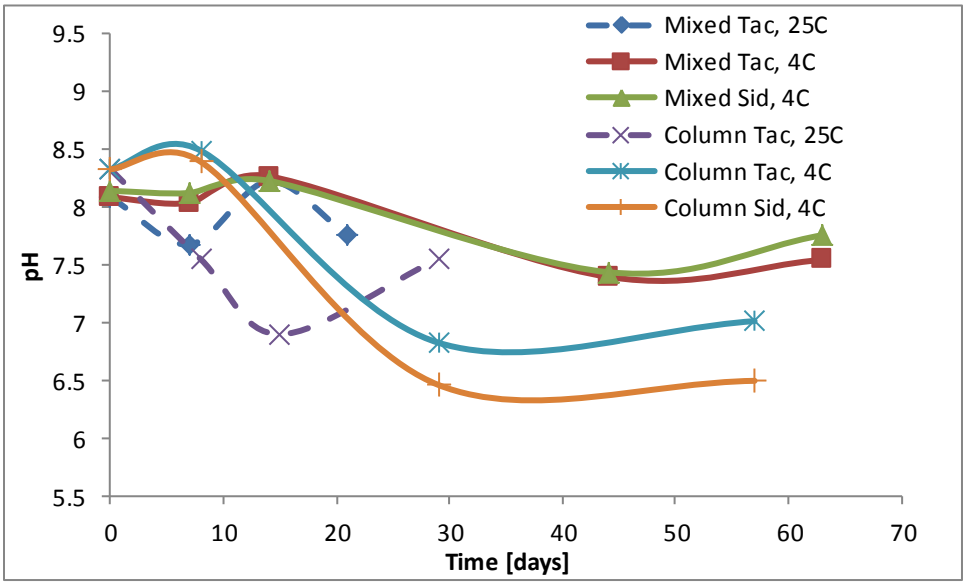

Figure S.2 pH measurements for secondary experiments.

Cost estimates for siderite, taconite, and ethanol. Results should be considered rough estimates based on unpublished and unquoted rates of shipping and material costs.

Table S.1 Estimate for mass of sulfate in lake water and consequent mass of iron required to remove sulfide if 100% is reduced.

Area	141	acres
	570606.7524	m2
Depth	10	ft
	3.048780488	m
Volume	1,739,655	m3
	1,739,654,733	L
SO4 conc in water	10.41666667	mmol/L
Max HS mass in water	18,121,403,468	mmol
	18,121,403	mol
Stoich. Fe required	18,121,403	mol

Table S.2 Estimate of mass of material required and approximate costs for material and shipping for various assumptions about stoichiometric requirements for taconite.

	Siderite	Taconite			
Stoichiometric excess	2	10	5	2	times
Estimated Fe required	36,242,807	181,214,035	90,607,017	36,242,807	mol
mol wt of Fe	55.845	55.845	55.845	55.845	g/mol
% Fe purity	0.38	0.15	0.15	0.15	fraction
Estimated mass of Fe	5,326,261,982	67,465,985,111	33,732,992,556	13,493,197,022	g
	5,326,262	67,465,985	33,732,993	13,493,197	kg
	5,859	74,213	37,106	14,843	ton
Material Costs	340	10	10	10	
Shipping Costs	400	40	40	40	
Cost per ton	740	50	50	50	$/ton
Total cost	$ 4,335,577	$ 3,710,629	$ 1,855,315	$ 742,126	
Cost per million gallons	$ 2,492,206	$ 2,132,969	$ 1,066,484	$ 426,594	

Table S.3 Estimate of volume of ethanol required and approximate costs for material.

Stoich. ethanol requird	12,080,936	mol
	Ethanol	
Stoichiometric excess	1.1	times
mass ethanol	288,891.94	g
volume ethanol	365,686	mL
	365.69	L
	96.74	gal
cost per gallon	10	$/gal
total cost	$ 967.42	
Cost per million gallons	$ 556	