# pH as a control on interactions of methanogens and iron reducers

by

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# Abstract

A growing body of evidence demonstrates that methanogenesis and Fe(III) reduction can occur simultaneously. However, environmental controls on interactions between each are poorly understood. In this study we considered pH as a control on interactions between Fe(III) reduction and methanogenesis in anoxic sediment bioreactors. The reactors consisted of 100mL of synthetic aqueous media, and 1 g of marsh sediment amended with goethite (1mmol). One set of reactors received acidic media (pH 6), and the other alkaline media (pH 7.5). Each set received media containing acetate (0.25 mM) to serve as an electron donor. Control reactors, deficient in acetate, were also included. We maintained a fluid residence time of 35 days by sampling and feeding the reactors every seven days.

For pH 6.0 and pH 7.5 reactors, the measured pH of effluent samples averaged 6.33 and 7.37, respectively. The extent of Fe(III) reduction and methanogenesis varied considerably between each set of reactors. More Fe(III) was reduced in the pH 6 reactors (646.39 µmoles on avg.) than the pH 7.5 reactors (31.32 µmoles on avg.). Conversely, more methane formed in pH 7.5 reactors (127.5 µmoles on avg.) than the pH 6 reactors (78.9 µmoles on avg.). Alkalinity concentrations during the middle and end of the experiment averaged 9.6 meq/L and 5.2 meq/L in pH 6 and pH7.5 reactors, respectively Although much less Fe(III) reduction occurred in pH 7.5 reactors, the relative abundance of Fe(III) reducers in them decreased little from levels observed in the pH 6 reactors. Sequences classified within Geobacter, a genus of bacteria known primarily as dissimilatory metal reducers, accounted for 22% and 13.45% of the sequences in the pH 6 and pH 7.5 reactors and only 0.8% of the sequences in the marsh sediment inoculum. In contrast, sequences classified within orders of methanogens were low in abundance, making up only 0.47% and 1.04% of the sequences in the pH 6 and pH 7.5 reactors, respectively.

Mass balance calculations demonstrate that the amount of electron donor consumed by each group varied considerably between the sets of reactors. Expressed as a quantity of acetate, the reactions consumed about 160µM of electron donor each in pH 6 reactors. In contrast, methanogenesis consumed over 30 times more electron donor than Fe(III) reduction in the pH 7.5 reactors. Thus, the results of our experiment indicate that the decrease in electron donor consumption by Fe(III) reduction at basic pH was nearly matched by the increase in electron donor consumption by methanogens. Results of geochemical modeling calculations indicate that more energy was available for Fe(III) reduction in the pH 6.0 reactors than the pH 7.5 reactors, matching variation in Fe(III) reduction rates, and that the density of sorbed ferrous iron was higher in pH 6 reactors than pH 7.5 reactors. Thus, the calculation results are consistent with bioenergetics, but not variation in ferrous iron sorption, as a potential mechanism driving variation in the balance between each reaction with pH.

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# Chapter 1 - Introduction

Shifts in the balance between carbon storage and carbon emission from soils as a result of climate change represent an important, but not well-understood biogeochemical feedback mechanism on global climate change (Smith and Fang, 2010). Anoxic soils (e.g., soils in wetlands and peatlands) are a particularly potent carbon reservoir because they contain high carbon densities, and serve as a major source of atmospheric methane, a greenhouse gas with 20-fold greater radiative forcing than carbon dioxide. In order to forecast how biogeochemical processes in these soils will respond to climate change we need a clear understanding of environmental controls on interactions of microorganisms within them.

In this study we considered pH as a control on the balance between microbial Fe(III) reduction, and methanogenesis in anoxic soil. During Fe(III) reduction microbes use Fe(III), most commonly found as oxides and oxyhydroxides, as a terminal electron acceptor for cellular respiration to produce Fe(II) (Weber et al., 2006). During methanogenesis microbes make methane using hydrogen and $CO_2$, methyl-containing C-1 compounds, or the methyl carbon of acetate as their substrates (Whitman et al., 2006)

Both Fe(III) reduction and methanogenesis are known to co-exist in anoxic soils. Competition between the two is common, and most often results in lower amounts of methanogens. (Roden and Wetzel, 1996, 2003; Lovley and Phillips, 1987; Reiche et al., 2008). Evidence suggests that Fe(III) reducers can successfully outcompete methanogens for electron donor in anaerobic environments, resulting in suppression in rates of methane production when there are abundant amounts of microbially reducible Fe(III) oxides (Lovley and Klug 1982, Lovley and Phillips 1987). Interaction between these two groups of microorganisms has the potential to significantly impact carbon budgets in anoxic systems. Fe(III) reduction has the potential to increase alkalinity of the system through the production of $HCO_3^-$ and $OH^-$. The increase in alkalinity can cause carbonate mineral precipitation by increasing saturation with respect to carbonate phases (Coleman et al., 1993). Roden and Lovley (1993) showed the precipitation of $FeCO_3$ during growth of Fe(III) reducing bacteria in a bicarbonate-buffered medium. This process helps to trap carbon in the soil. Methanogenesis, on the other hand, does not generate much alkalinity, and releases carbon through the formation of $CH_4$ (Kirk et al., 2013; Conrad, 1996).

Previous studies have used thermodynamic calculations to show that pH has the potential to influence interactions between Fe(III) reduction and sulfate reduction (Postma and Jakobsen, 1996; Bethke et al., 2011; Kirk et al., 2013; Kirk et al., 2015a). As shown in Figure 1, each metabolic reaction varies unequally with pH, therefore, changes in pH can alter which reaction is energetically favorable (Postma and Jakobsen, 1996; Bethke et al., 2011). At an acidic pH Fe(III) reduction has a much higher free energy yield than at alkaline pH. This allows Fe(III) reducers to have a more energetically favorable reaction than sulfate reduction at an acidic pH (Bethke et al.

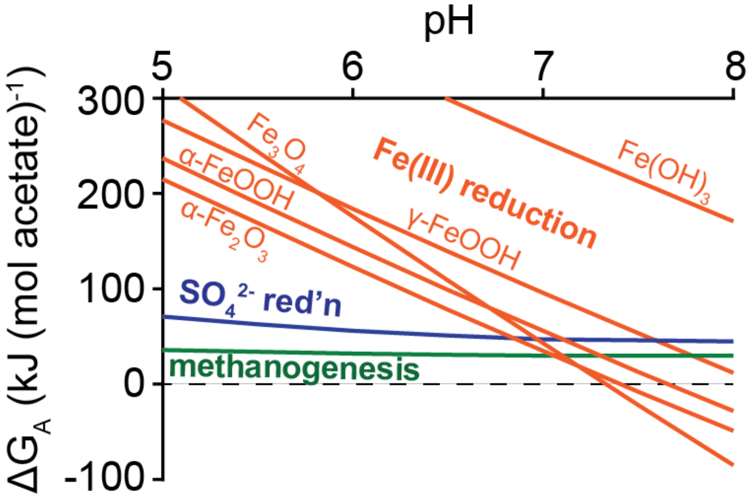

Figure 1: Variation with pH in energy available to Fe(III) reduction, sulfate reduction, and methanogenesis. $\Delta G_A$ refers to the amount of energy available to drive reactions forward.

2011; Kirk et al., 2013). We hypothesize that pH may similarly affect interactions between Fe(III) reducers and methanogens. At acidic pH, Fe(III) reduction is thermodynamically more favorable than methanogenesis (Fig. 1). At alkaline pH, however, the opposite may be true.

These changes in free energy yield occur in response to differences in the number of protons consumed by each reaction. Reduction of ferric iron in oxides and oxyhydroxides consumes several protons as shown in the following example reaction which uses goethite as the source of ferric iron and acetate as the electron donor:

$$CH_3COO^- + 8\ FeOOH(s) + 15\ H^+ \rightarrow 2\ HCO_3^- + 8\ Fe_2^+ + 12\ H_2O \qquad (1)$$

As a result of this consumption of protons, the energy yield of the reaction increases rapidly as pH decreases. In contrast, there are much fewer protons consumed during sulfate reduction and methanogenesis, so the energy yield of those reactions vary weakly with pH.

$$CH_3COO^- + SO_4^{2-} + H^+ \rightarrow 2HCO_3^- + H_2S(aq) \qquad (2)$$

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \qquad (3)$$

Reaction free energy yields can affect competition between microorganisms. Groups of microbes can conserve energy if their reactions are more energetically favorable. These groups have physiological advantages, including fast reactions and more biomass yield, over those using less favorable reactions (Lovley and Goodwin, 1988; Jin and Bethke, 2007; Roden and Jin, 2011; Jin, 2012). This increase in free energy yield that we see with decreasing pH may allow microbes capable of Fe(III) reduction to better compete with methanogens in acidic environments than in alkaline environments (Bethke et al., 2011; Kirk et al., 2013).

This study tests the hypothesis that pH can influence interactions between Fe(III) reduction and methanogenesis in a similar way to the relationship between sulfate reduction and Fe(III) reduction. We tested this hypothesis using two sets of bioreactor experiments: one receiving acidic aqueous media (pH 6), and one receiving media with basic pH (pH 7.5). Hereafter, we will refer to these as the pH 6.0 and pH 7.5 sets of bioreactors. Each set of bioreactors was divided in half. One half was fed with acetate during the weekly sampling, and the other was not. We will refer to these as our acetate-fed and control reactors.

# Chapter 2 - Materials and Methods

## Sample Collection

We collected soil for the experiment from the Big Blue river floodplain at the north end of Tuttle Creek reservoir near Olsburg Kansas (Fig 2). The samples were collected on January 30, 2016. During that time the soil was submerged in about 0.25 m of water. The soil was collected in sterile (autoclaved at 121$^{o}$C for 30 minutes) mason jars. The jars were rinsed multiple times with water from the marsh before sample collection. The jars were kept sealed in an incubator at 20$^{o}$C until the bioreactors were assembled. While collecting soil, water samples were also collected in order to analyze the water chemistry of the area. The water samples were filtered using 0.45µm filters and analyzed for major ions, as described below.

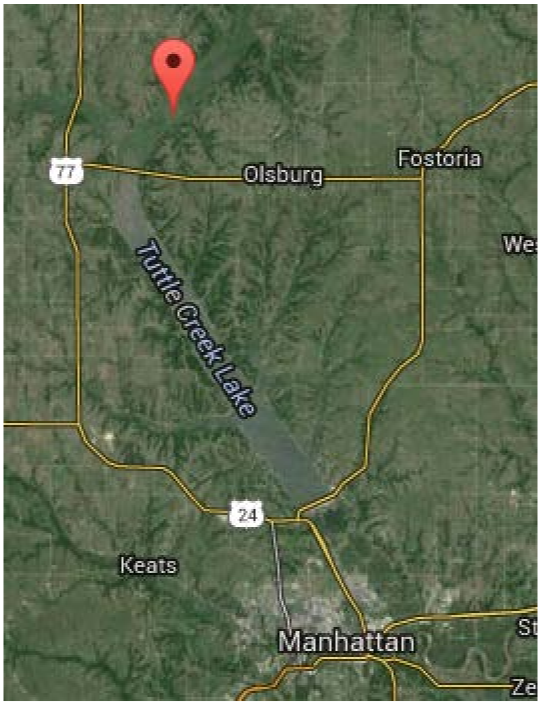

Figure 2: Map showing the location of the sampling site near Olsburg Kansas. (Latitude 039$^{o}$27’38.988”N Longitude 096$^{o}$41’25.3428”W)

## Medium

We defined a synthetic aqueous media for the experiment based on the composition of the water samples collected from the field site. The media concentrations differ slightly from the water at the sampling site in that we added ammonium and phosphate as important macronutrients (Table 1). Media for the live bioreactors also includes acetate, which can serve as an electron donor for microbes in the reactors. Control media only differed from live media in that there was no acetate added to control reactors.

To set the pH, and purge oxygen from the media, we sparged the bottles with an oxygen-free gas mixture of nitrogen and carbon dioxide. To achieve a pH of 7.5 we sparged with a gas mixture consisting of 1.1% $CO_2$ in $N_2$ for 1 hour. To achieve a pH of 6, we sparged the media with 35% $CO_2$ in $N_2$ for 1 hour. After sparging, we sealed the media bottles, autoclaved them for 30 mins at 121$^oC$, and stored them at room temperature (~22$^oC$).

**Table 1: Composition of aqueous media.**

| Medium | $NaHCO_3$ mM | $CaCl_2$ mM | $MgCl_2$*$6H_2O$ mM | KCl mM | $NH_4Cl$ mM | $HK_2PO_4$ mM | $NaCH_3COO$ mM | $CO_2$* % | final pH** |
|---|---|---|---|---|---|---|---|---|---|
| 6.0 Live | 5 | 2 | 0.5 | 0.25 | 0.05 | 0.001 | 0.25 | 35 | 6.0 |
| 6.0 Control | 5 | 2 | 0.5 | 0.25 | 0.05 | 0.001 |  | 35 | 6.0 |
| 7.5 Live | 5 | 2 | 0.5 | 0.25 | 0.05 | 0.001 | 0.25 | 1.1 | 7.6 |
| 7.5 Control | 5 | 2 | 0.5 | 0.25 | 0.05 | 0.001 |  | 1.1 | 7.6 |

*Proportion of $CO_2$ in $CO_2$:$N_2$ mix.
** These are average measured values

## Bioreactors

Each set of bioreactor experiments was performed in triplicate. The reactors consisted of 160 mL serum bottles that were filled with 100mL of media, and 1.0 mmol of goethite (Fig 3). We capped the reactors, sparged them with gas, and sterilized them using an autoclave (30 minutes at 121°C). Next, we placed the reactors in an anaerobic chamber, opened them, and added 1 gram of soil. In addition to the soil, we also added 100µM $FeCl_2$ (final concentration) to act as a reducing agent. We then recapped the reactors with sterile stoppers and seals, and a sterile needle, fitted with a gas-tight syringe valve, was inserted through the stopper. Lastly, the reactors were brought out of the anaerobic chamber to readjust the pH by sparging with the gas mixtures discussed previously. Once assembled the reactors were kept in an incubator at 20°C.

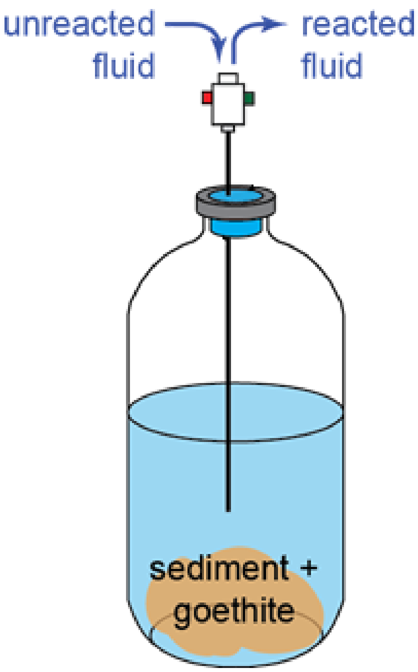

Figure 3: Schematic view of bioreactor. The long needle used for sampling/feeding fluid.

The reactors were allowed to incubate for seven days. On the seventh day, and every seventh day thereafter, the reactors were sampled and fed. Sampling and feeding consisted of withdrawing 20mL from the serum bottle and replacing the sampled volume with sterile media. Incubation and sampling persisted until electron donor consumption had stabilized for at least one month.

## Chemical Analysis

Numerous chemical analyses were performed each week on effluent samples. We also chemically analyzed each batch of fresh media to ensure consistency within our experiment. We used these data to monitor microbial activity during the experiments and provide us with constraints for conducting mass-balance and thermodynamic calculations. Fe(II) concentrations were measured in effluent samples using the ferrozine method (Stookey, 1970) with a Thermo Scientific Genesys 10S UV Vis Spectrophotometer. Total alkalinity was measured using Gran alkalinity titrations with 0.02 N sulfuric acid, and an Oakton PC-300 pH meter. Major anion concentrations ($CH_3COO^-$, $Cl^-$, $PO_4^{3-}$, and $SO_4^{2-}$) were measured in 0.45μm filtered samples using a Dionex ICS-1100 ion chromatograph. The system uses carbonate eluent (4.5mM $Na_2CO_3$, 1.4mM $NaHCO_3$) with an AS22 analytical column, and an AERS 500 suppressor. Major cation concentrations (Ca, Na, Mg, $NH_4$, K) were measured periodically using a second ICS-1100 ion chromatograph, which uses a sulfuric acid eluent (22mN $H_2SO_4$ solution) with a CS12 analytical column and a CERS 500 suppressor. For both systems, eluent was made using 18 MΩ DI $H_2O$. Fe(II) and alkalinity analyses were performed immediately after sampling. Periodically we analyzed the proportion of methane gas in the headspace of the reactors using a GOW MAC series 580 gas chromatograph equipped with a thermal conductivity detector.

## Microbial Community Analysis

In order to directly identify which groups of microorganisms were present in the reactors, and help us interpret our geochemical data, we carried out a microbial community analysis of each bioreactor. This analysis was done on samples collected at the end of the experiment for all reactors as well as on sediment collected from the marsh. To collect the microorganisms the bioreactors were thoroughly mixed, and 3mL of the slurry was removed using sterile 5mL syringes. The slurry was then filtered using a sterile 0.2µm filter. To ensure the filters were sterile, they were first placed in membrane holders, wrapped in aluminum foil, and autoclaved for 30 mins at 121$^{o}$C. After filtering, the filter was placed in a 2mL centrifuge tube, 0.2 mL of sucrose lysis buffer was added as a preservative, and the filters were frozen at -80$^{o}$C until DNA extractions were performed.

DNA was extracted from the filters using a MoBio DNA extraction kit. We followed manufacturer's instructions for each step with the exception of using an alternative lysis method to reduce DNA shearing. After extraction, the samples were kept frozen at -80$^{o}$C. A Nanodrop was used to ensure quality, and measure concentration of the DNA extracted.  DNA samples were sent to Argonne National Lab where DNA amplification was carried out using Polymerase Chain Reaction (PCR). PCR amplification was performed using universal bacterial primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT (Walters, W., 2015).  The sequences were then sequenced using the Illumina MiSeq platform. After an initial quality filtering, the lab provided raw sequence data to us in the form of a Fasta file

We analyzed sequence data using QIIME v. 1.9.1. First we split samples according to barcodes, and filtered the sequences to remove low-quality reads (script: split_libraries.py). Next, we generated BIOM formatted OTU (Operational Taxonomic Unit) tables at 97% similarity, and evaluated taxonomy with uclust (script: pick_de_novo_otus.py). This step assigned the most detailed lineage description shared by at least 90% of the sequences within each OTU. Then, singletons were removed, and we created taxonomy tables (scripts: filter_otus_from_table.py, summarize_taxa_through_plots.py).  We wanted to compare diversity between samples to examine uniformity of triplicates and influence of pH on community diversity. In order to do this we first normalized all samples to have the same number of sequences as the sample with the fewest sequences(4834 sequences) (script: single_rarefaction.py). From here we computed diversity within samples using alpha diversity (script: alpha_diversity.py). Lastly, we computed beta diversity to compare sample communities to one another. Doing this created PCoA plots as well as NMDS plots (script: beta_diversity_through_plots.py) (Kirk et al., 2015b).

# Chapter 3 - Results

## Biogeochemistry

The pH of the effluent samples throughout the experiment from the pH 6.0 and pH 7.5 control reactors averaged 6.33 and 7.37, respectively. The pH of the acetate-fed reactors did not vary substantially from the pH of their corresponding control reactors (Fig 4A).

Initial analysis of the reactor effluent showed substantially higher levels of acetate than was supplied by the media. Average initial acetate concentrations for pH 6.0 and pH 7.5 acetate-fed bioreactors was 2.12mM and 2.10mM, respectively (nearly 8.5 times weekly input value). Average intial acetate concentrations for pH 6.0 and pH 7.5 control bioreactors were 1.74 and 1.57, respectively. Effluent acetate concentrations declined steadily, dropping below input values between incubation day 35 and 42. From incubation day 42 through the end of the experiment, acetate concentrations of effluent samples from all bioreactors remained below detection limit (1mg/L) (Fig 4B).

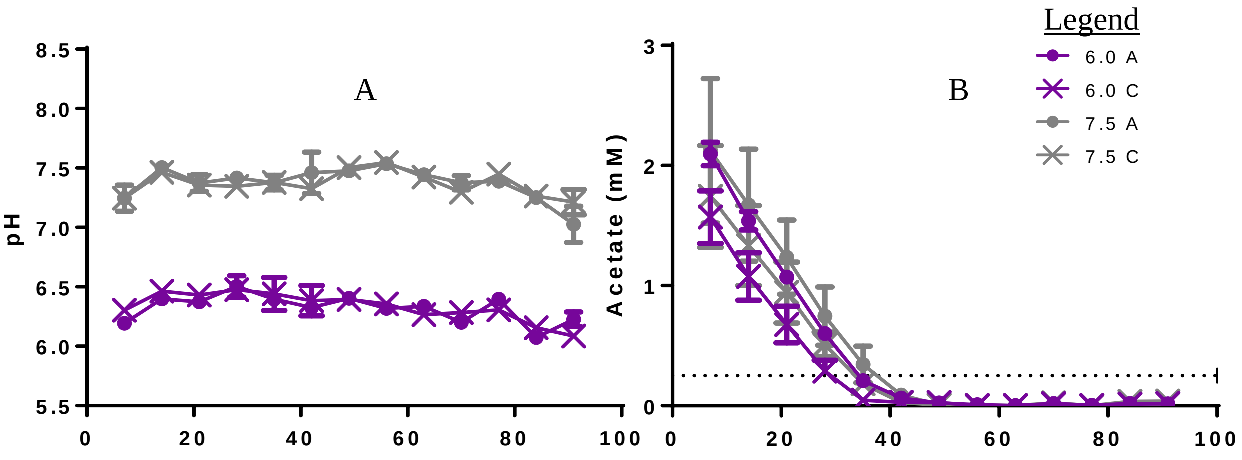

Figure 4: Graphing average pH (A), and acetate values (B) versus time in days. Dotted line illustrates average input of 0.25mM acetate into acetate-fed bioreactors.

Fe(II) concentrations in both acetate-fed and control pH 6.0 bioreactors increased rapidly from day 0, reaching their maximum on day 35 (3,425µM) and 28 (3,137µM), respectively. Following day 35, Fe(II) concentrations for pH 6.0 bioreactors continued on a downward trend with the acetate-fed bioreactors maintaining a slightly higher concentration (Fig 6A). Total amounts of Fe(III) reduced in pH 6.0 reactors averaged 6654.7 µM for acetate-fed reactors, and 6273.1 µM for control reactors.

Fe(II) concentrations in both acetate-fed and control pH 7.5 reactors are substantially lower than the pH 6.0 reactors. Maximum concentrations for acetate-fed and control reactors at pH 7.5 reached 207.2µM and 184.5µM, respectively. Total Fe(III) reduced during incubation based on mass balance calculations averaged 325.2 and 301.3 µM (Fig 5).

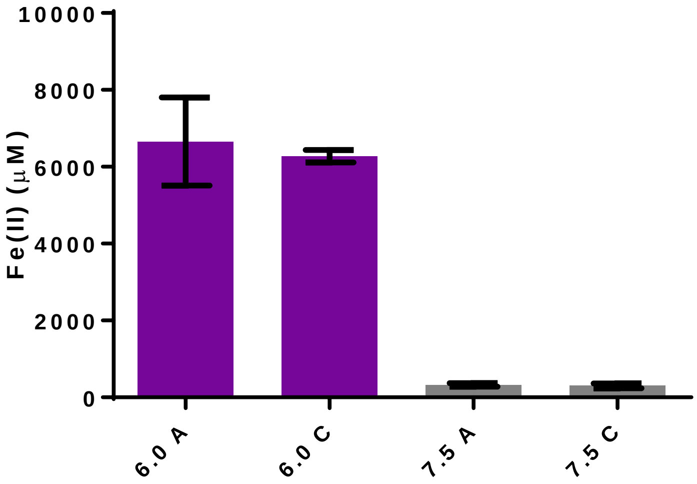

Figure 5: Total amount of Fe(II) in solution at the end of the experiment.

Initially alkalinity data were affected by organic content introduced by the soil. Due to this, reliable alkalinity data were not recorded until day 56. On day 56 average alkalinity values for acetate-fed and control reactors at pH 6.0 were 9.57 and 8.84meq/L, respectively. The average values for reactors at pH 7.5 were 5.19 and 5.04, respectively (Fig 6B). This higher alkalinity values for pH 6.0 reactors is indicative of Fe(III) reduction as seen in equation (1). On day 91 alkalinity values were substantially lower in the pH 6.0 reactors and relatively unchanged in the pH 7.5 reactors. Average alkalinity values on day 91 for acetate-fed and control reactors at pH 6.0 were 6.68 and 6.64meq/L, respectively. In pH 7.5 reactors the average values were 5.19

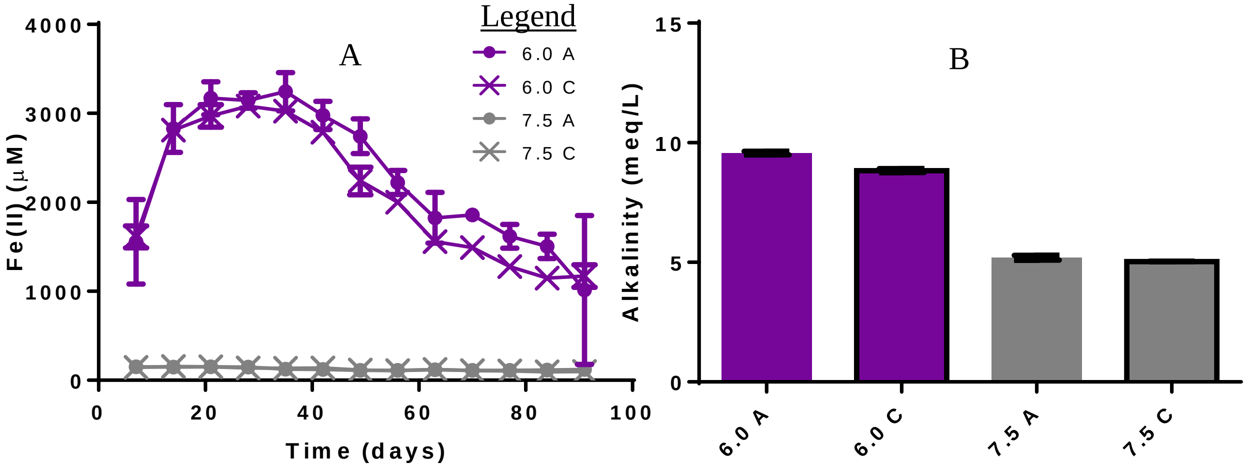

Figure 6: Graph of average Fe(II) concentration of effluent samples (A), and a column chart showing alkalinity values measured on day 56 (B).

and 5.30meq/L, respectively. This drop in alkalinity between days 56 and 91 in the pH 6.0 bioreactors indicates a decrease in the amount of Fe(III) reduction occurring.

Concentrations of both $Ca^{2+}$ and $Mg^{2+}$ were initally much higher in bioreactors at pH 6.0 versus pH 7.5. On the initial sampling day the average concentration of $Ca^{2+}$ in pH 6.0 and 7.5 bioreactors was 1.3 and 0.65mM, respectively. The $Ca^{2+}$ concentration was not tested again until day 56. On day 56 the $Ca^{2+}$ concentrations were much closer averaging 0.65 and 0.57mM, respectively. Although the concentrations converged by day 56, the concentrations remained above the weekly input value of 0.294mM throughout the entire experiment (Fig 7A).

As stated previously, $Mg^{2+}$ concentrations were initially much higher for pH 6.0 bioreactors versus reactors at pH 7.5. The initial $Mg^{2+}$ concentrations for bioreactors at pH 6.0 and 7.5 were 0.835 and 0.555mM, respectively. These values would also eventually converge. On day 56 the average concentrations were 0.53 and 0.455mM, respectively. Unlike $Ca^{2+}$ concentrations which were consistently above the weekly input value, the $Mg^{2+}$ concentrations remained close to the weekly $Mg^{2+}$ input of 0.483mM after day 56. (Fig 7B).

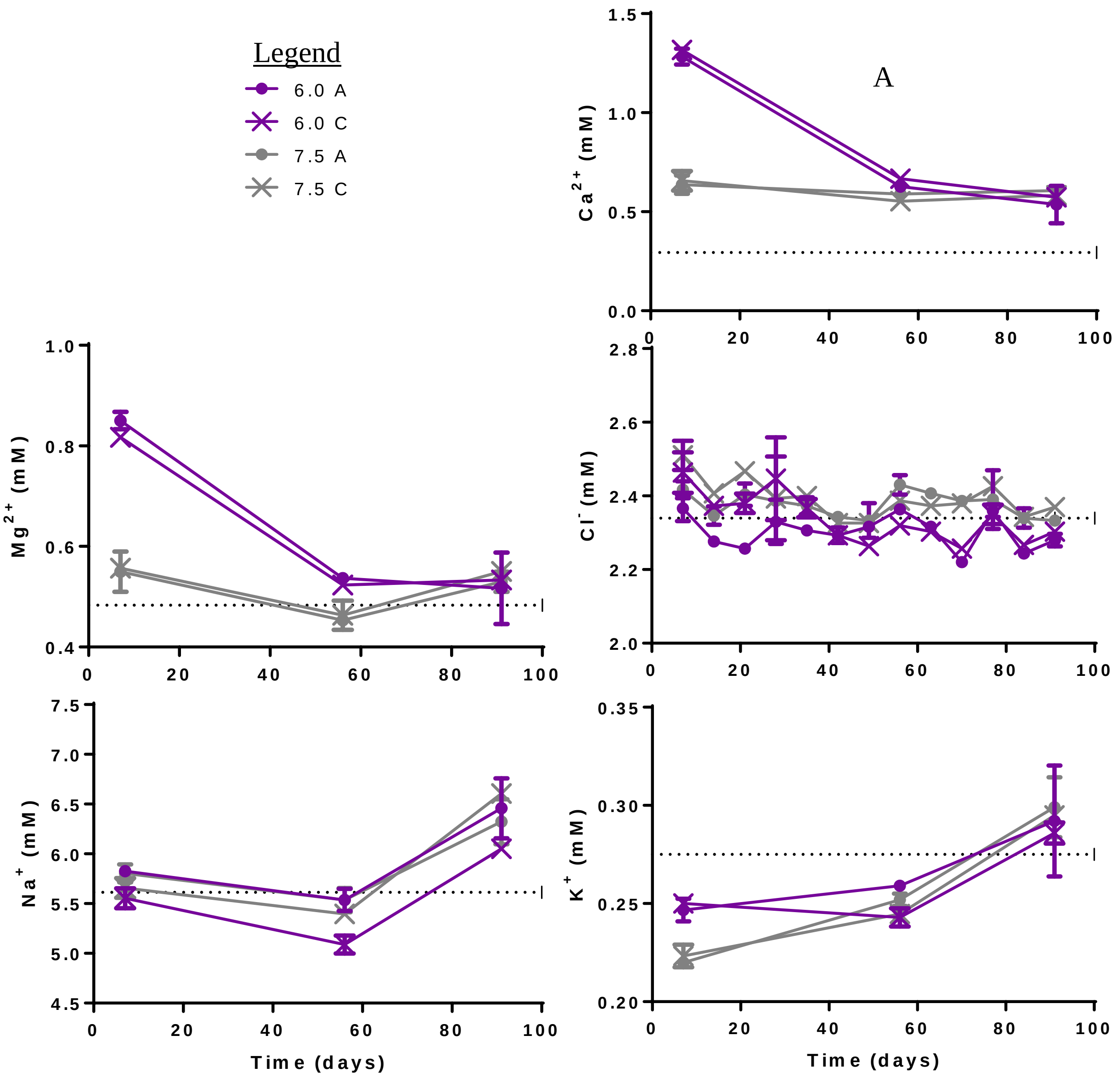

Figure 7: Average concentrations of calcium (A), magnesium (B), chloride (C), sodium (D), and Potassium (E) in effluent samples versus time in days. Average input levels were 0.294mM (A), 0.483mM (B), 2.34mM (C), 5.61mM (D), and 0.275mM (E).

Chloride concentrations throughout the experiment remained consistent. They deviated slightly from the average input of 2.34mM, but no significant trends were noticed (Fig 7C).

B

C

Sodium concentrations of effluent samples were initially near input value of 5.61mM. They dropped slightly below input during day 56 sampling, and then increased by an average of 1.0 mM on the final sampling day (Fig 7D). Potassium concentrations were below input of 0.275mM during the first two samplings. Levels increased slightly above input during the final day (Fig 7E).

Reactor headspace was sampled 3 times throughout the experiment. Methane was detected during every sampling in all 12 bioreactors. Headspace sampling took place before reactor media sampling during the 3rd, 8th, and 12th week of the experiments. Average methane amounts within all pH 6.0 reactors for all three samplings was 27.3, 79, and 78.9 µmoles, respectively. For pH 7.5 reactors, the average amount of methane was 38.5, 119.5, and 127.5 µmoles, respectively (Fig 8B). Total methane production within pH 6.0 reactors averaged 102.4µmoles for acetate-fed and 55.5µmoles for control reactors. Averages for pH 7.5 reactors were 163.9 and 91.2µmoles, respectively (Fig 8A). In order to calculate methane in terms of µmoles we used the ideal gas law (PV=nRT). Where (P) is the partial pressure of methane in kPa, (V) is the average volume of headspace within the reactors (0.0594 L), (n) is the number of moles, (R) is the ideal gas constant (8.314 L kPa/Kmol), and (T) is the temperature in Kelvin (295.5K).

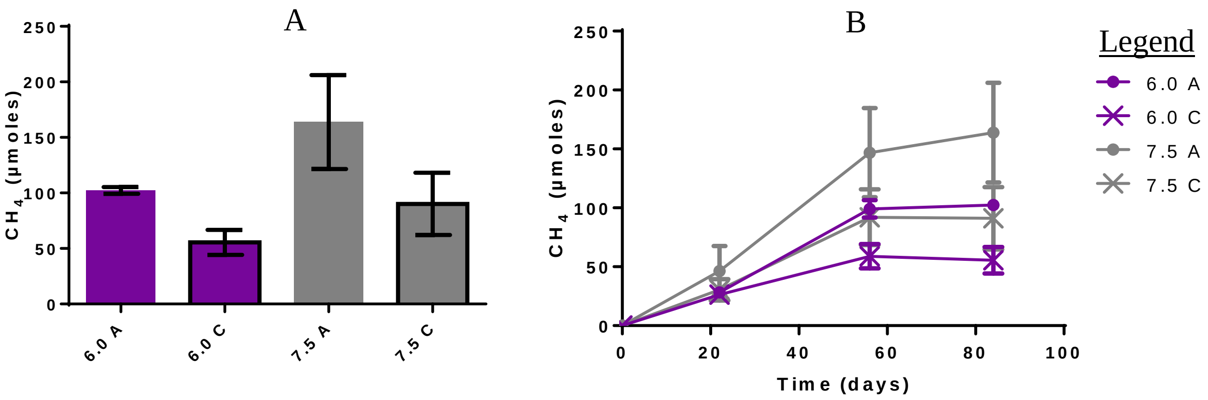

Figure 8: Column chart showing average total methane production (A), and a graph showing methane present in the headspace at each of the 3 sampling days (B).

## Microbial Community Composition

Proteobacteria accounted for over 55% of our prokaryotic community (36.4% within marsh sediment. Within this Phylum 27.6% were within the genus *Geobacter* a genus of bacteria known primarily as dissimilatory metal reducers. *Geobacter* had the highest relative abundance within all of our samples (15.29%),. Within our prokaryotic community archaea only accounted for 1.34% of sequences. Of the archaea present 51% were within orders of methanogens (0.69% relative abundance). Methanogens found in our samples were from the classes *Methanobacteria*, *Methanomicrobia*, and *Thermoplasmata*.

Relative abundance of *Geobacter* and methanogens within marsh sediment was 0.81% and 0.35%, respectively. Within pH 6.0 reactors, relative abundance of *Geobacter* and Methanogens averaged ~22% and 0.47%, respectively. In the pH 7.5 reactors, *Geobacter* averaged 13.5% and Methanogens averaged around 1% (Figs 9A,B).

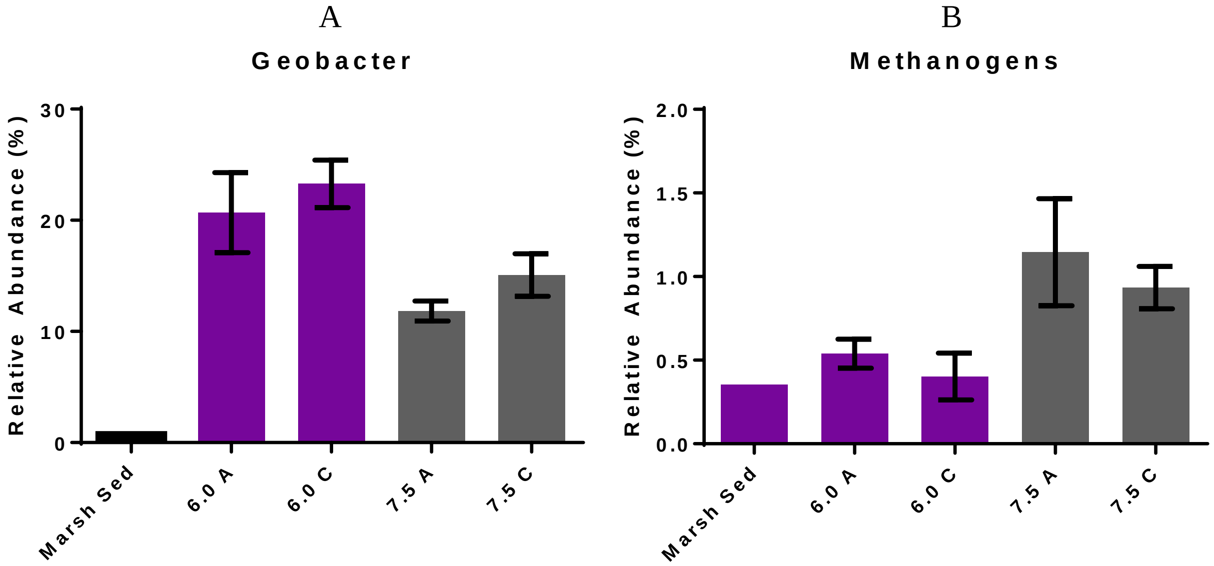

Figure 9: Column charts showing relative abundance of *Geobacter* (A) and methanogens (B) within marsh sediment and all groups of bioreactors.

# Chapter 4 - Discussion

## Microbial Reactions

The observed changes in aqueous chemistry and headspace composition are consistent with the growth of Fe(III)- reducers and methanogens. Early in the experiment there was a noticeable decrease in effluent acetate concentrations, an increase in Fe(II) and alkalinity, as well as noticeable methane production. Results indicate growth within populations of both methanogens and Fe(III) reducers. Stabilization of effluent acetate concentrations below detection (0.01mM) following day 42 indicates that the microbial community had grown enough to consume nearly all of the influent acetate each week.

Mass-balance calculations based on aqueous chemistry demonstrate that the extent to which Fe(III) reduction occurred differed considerably between each set of reactors. In bioreactors at pH 6.0, the acetate consumed for Fe(III) reduction in acetate-fed and control reactors averaged 83.18 and 78.41 µmoles, respectively. The amount of acetate consumed in pH 7.5 bioreactors for Fe(III) reduction in acetate-fed and control reactors averaged 4.06 and 3.77 µmoles, respectively (Fig 10).

On average, methanogenesis required more acetate than Fe(III) reduction. The amount of acetate consumed by methanogens in the acetate-fed and control reactors at pH 6.0 was 102.4 and 55.50 µmoles, respectively. Within the pH7.5 reactors, methanogens consumed 163.9 and 91.2 µmoles, respectively (Fig 10).

Contrary to the pH 6.0 reactors, the difference in acetate consumption for methanogenesis and Fe(III) reduction in pH 7.5 reactors was substantial. Within acetate-fed reactors at pH 7.5, methanogens consumed 163.9 µmoles of acetate and Fe(III) reducers only 5.06 µmoles. This resulted in minimal Fe(II) production, and an increased amount of methane production in pH 7.5 reactors.

We understand that acetate was not the only organic molecule being metabolized by these microbes. Degradation of organic matter within the soil most likely provided other forms of electron donor, but for simplification we are comparing them based upon 8 electron transfer. In addition we understand that acetate could have been consumed by other electron acceptors such as $NO_3^-$, $SO_4^{2-}$, or Mn(IV). Given that $SO_4^{2-}$ and $NO_3^-$ levels were below detection we feel their impact is minimal. Mn(IV) on the other hand was not quantified, so this provides possible uncertainty.

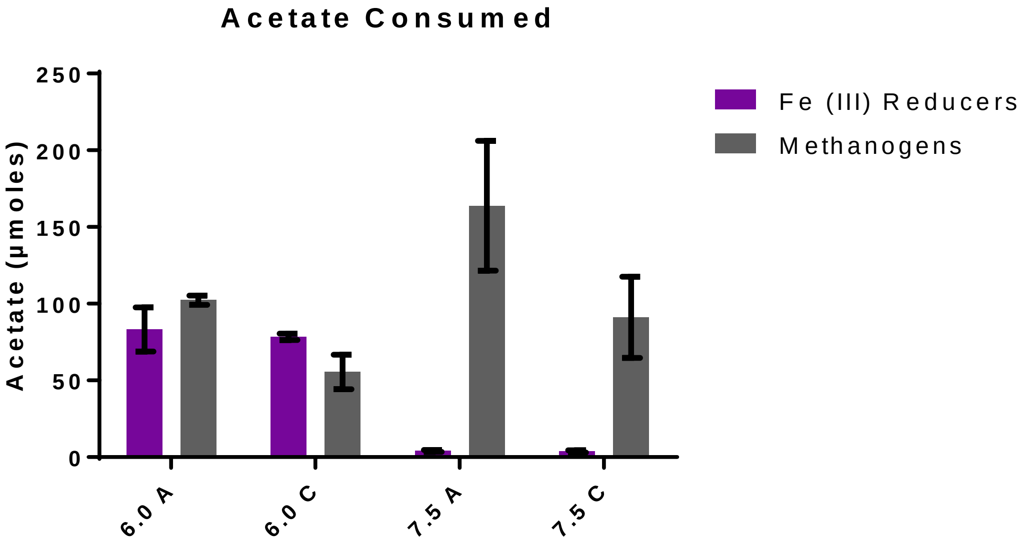

Figure 10: Amount of acetate consumed throughout the experiment by Fe(III) reducers and methanogens in all sets of bioreactors.

Although methanogens had the more energetically favorable reaction within the pH 7.5 reactors, it is evident that Fe(III) reducers were still active. The total amount of Fe(III) reduced in the pH 7.5 reactors averaged only 313.25μM (~6500 μM in pH 6.0 reactors). Evidence of Fe(III) reducer activity is shown in the growth of microorganisms within the *Geobacter* genus (Fig 11). Average relative abundance of *Geobacter* within marsh sediment was only 0.8%, but within the pH 6.0 and pH 7.5 bioreactors they averaged 22% and 13.45%, respectively. So even though very little Fe(III) was reduced within pH 7.5 reactors, the population of Fe(III) reducers increased more than 16 times. Fe(III) reducers may possess a kinetic advantage over methanogens that allows them to consume electron donor more quickly (Bethke et al., 2008). The extent to which they can exploit this advantage may depend in part upon thermodynamics (Bethke et al., 2011). It seems that even at alkaline pH, where methanogenesis is more thermodynamically favorable, Fe(III) reducers are still able to exploit this advantage, but not to reduce Fe(III). A possible explanation for this phenomena is Direct Interspecies Electron Transfer (DIET) (Lovley, 2017). While still not extensively studied, DIET and cable bacteria

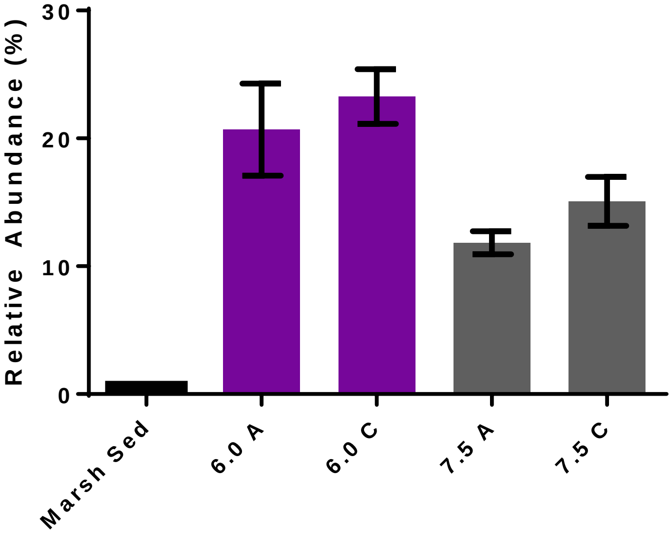

Figure 11: Relative abundance of *Geobacter* within marsh sediment and bioreactors.

have demonstrated that microbial cells can exchange electrons over distances of µm to cm lengths through electrical connections (Lovley, 2017). DIET is potentially an effective form of syntrophy in methanogenic communities (Rotaru et al., 2014a). Electrically connected microbial communities (e-communities) are currently primarily restricted to anaerobic environments in which bacteria and archaea electrically connect to either produce (Morita et al., 2011; Kato et al., 2012; Rotaru et al., 2014b) or consume (McGlynn et al., 2015; Wegener et al., 2015) methane. Under alkaline conditions *Geobacter* may be consuming acetate, but instead of utilizing it to reduce Fe(III) they may be transferring the electrons to methanogens (Fig 12).

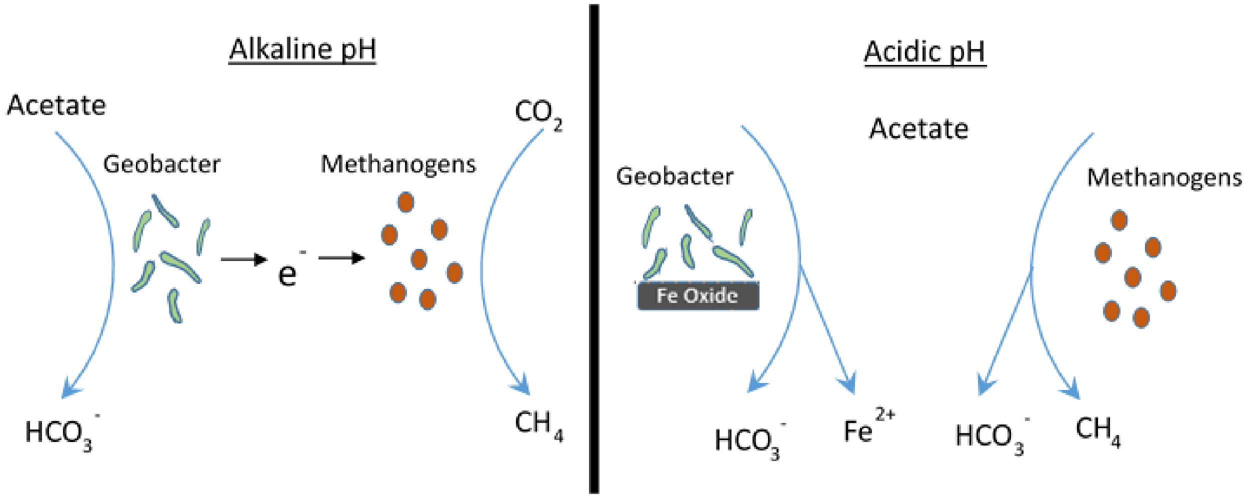

Figure 12: Diagram illustrating Direct Interspecies Electron Transfer (DIET) between *Geobacter* and methanogens. At alkaline pH Geobacter utilizes its kinetic advantage to transfer electrons to methanogens. At acidic pH Geobacter reduces Fe(III) because that reaction is more favorable.

## Potential Mechanisms

Potential mechanisms that could cause changes in the interactions between these two microbial reactions are bioenergetics, and variations in the ability and extent of sorption of Fe(II) onto goethite. In the following sections we consider each of these possibilities in more detail.

## Bioenergetics

Reduction of ferric iron oxides and oxyhydroxides consumes several protons as shown in the following reaction which exemplifies the reaction that is occurring within our bioreactors.

$$CH_3COO^- + 8\ FeOOH(s) + 15\ H^+ \rightarrow 2\ HCO_3^- + 8\ Fe_2^+ + 12\ H_2O \qquad (1)$$

As stated previously, due to this consumption of protons, the energy yield of the reaction increases rapidly as pH decreases (Fig 1). For this reason, Fe(III) reduction is more energetically favorable in acidic environments. The consumption of protons by Fe(III) reduction also leads to an increase in pH. The peak of Fe(III) reduction (~35 days) correlates with the highest pH levels recorded within the pH6.0 reactors.

Contrary to Fe(III) reduction, methanogenesis consumes few protons and thus varies little with changes in pH (Equation 3). Due to this, methanogenesis remains energetically favorable along a wide range of pH. So, although Fe(III) reduction has more energy available at low pH, methanogenesis can still occur. This is why we see methane production within our pH 6.0 reactors.

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \qquad (3)$$

We carried out thermodynamic calculations to determine amounts of energy available to drive the reactions forward. Using Geochemist’s Workbench we derived activity values for all gaseous and aqueous species, as well as log K values for each reaction. Using the log K values we calculated standard state Gibbs Free Energy ($\Delta G^{\circ}_T$) (Equation 4).

$$\Delta G^{\circ}_{T} = -2.303RT\log(K) \tag{4}$$

In this equation R represents the gas constant (8.314 J/Kmol) and T is temperature. Using the activity values we then calculated the reaction quotient (Q), and applied these values to the reaction free energy equation to find the available free energy.

$$\Delta G_{r} = \Delta G^{\circ}_{T} + RT\ln(Q) \tag{5}$$

Microbes can only run their metabolism when the energy available outside the cell exceeds the internal store (Jin and Bethke, 2009). Usable free energy ($\Delta G_U$) is the difference between the energy in the environment and within the cell. $\Delta G_U$ is calculated using the following equation (Jin and Bethke, 2002, 2003, 2005, 2007).

$$\Delta G_{U} = \Delta G_{A} - m\Delta G_{P} \tag{6}$$

Our calculations reveal that there is generally more usable energy available to Fe(III) reducers at acidic pH, and more usable energy available to methanogens at alkaline pH (Fig 13).

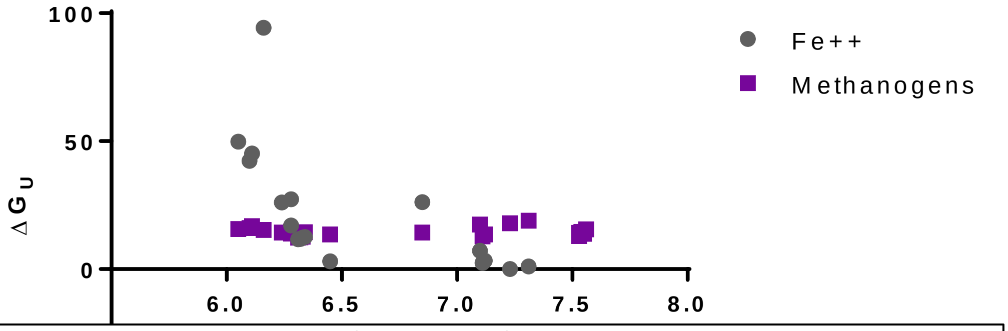

Figure 13: Graph showing shift in usable free energy available to methanogens and Fe(III) reducers with changing pH.

As the amount of usable energy decreases, the reaction rates start to slow down. As pH increases the amount of usable energy available to Fe(III) reducers begin to decrease. Fe(III) reducers are able to run their reactions at high rates at acidic pH, but at alkaline pH they are limited by thermodynamics. Methanogens are not affected in the same way by thermodynamics. Therefore, under alkaline conditions, where Fe(III) reducers are lacking in available energy, methanogens are able to thrive.

## Ferrous Iron Sorption

Another potential mechanism for controls on the microbial reactions within our experiments is Fe(II) sorption onto goethite. When Fe(II) sorbs to a ferric surface, in this case goethite, valence electrons are taken up and conducted toward high potential sites (Williams and Scherer, 2004; Larese-Casanova and Scherer, 2007). The electrons lower the effective redox potential of the surface, degrading its ability to act as an electron acceptor (Handler and other, 2009; Rosso and others, 2010). Under acidic conditions Fe(II) generally doesn't sorb as well. So as pH increases, Fe(II) sorption should increase (Dixit and Hering, 2006), and the rate of Fe(III)

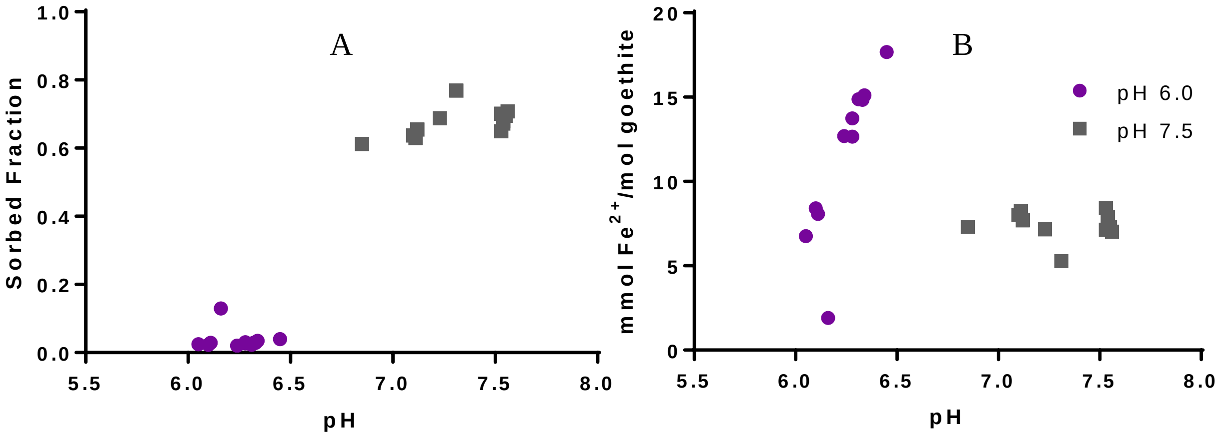

Figure 14: Graphs showing fraction of Fe(II) that has sorbed onto the goethite surface (A), and the density of Fe(II) sorbed on goethite within the bioreactors (B).

reduction should decrease. We created a sorption model using Geochemist's Workbench and from this we calculated sorbed fractions of Fe(II) as well as the density of sorbed Fe(II) on goethite (Fig 14). Sorbed fraction refers to the fraction of the total Fe(II) in solution that has sorbed onto goethite. Sorbed density is referring to the total amount of Fe(II) that sorbed. Through these calculations it is evident that there is a higher fraction of Fe(II) sorbing at basic pH, but due to the increased Fe(II) present in solution there is a much higher density of sorbed Fe(II) at acidic pH. This is inconsistent with sorption being a potential mechanism driving shifts in interactions with pH.

## Implications

Small ponds, lakes, and marshes, like the one we sampled for this study, have a large impact on our greenhouse gas budgets. A recent article discusses the contribution of small inland lakes and ponds on $CO_2$ and $CH_4$ emissions. They found that inland water actively transforms carbon, and plays an important role in natural and anthropogenic greenhouse gas budgets, including $CO_2$ and $CH_4$. According to the study, ponds that fell within the smallest size range have a disproportionally large contribution to carbon flux relative to their size. In fact, they estimate that although small ponds only make up 8.6% of the global surface are of lakes and ponds, they comprise 15.1% of all $CO_2$ emissions and 40.6% of all diffusive $CH_4$ emissions from lentic freshwaters (Holgerson and Raymond, 2016).

In order to understand how these systems will respond to changing climate, we need to understand the environmental controls on microbial reaction within them. As temperature increases, so do rates of organic matter degradation (Davidson, 2006). This increased rate of degradation could increase the levels of $CO_2$ in anoxic soil and water, and in turn lower the pH.

This change in pH has the potential to increase the ability of Fe(III) reducers to compete with methanogens. This could work to lower the amount of methane being released from these systems. Moreover, because Fe(III) reduction consumes a lot of protons, the reaction is very effective at generating carbonate alkalinity (Kirk et al., 2013), which would help these systems store additional carbon. Methanogenesis generates little alkalinity and releases carbon in the form of $CH_4$. Carbon can also be stored through precipitation of carbon bearing minerals from solution. After calculating mineral saturations using Geochemist’s Workbench we found that siderite, an iron carbonate mineral ($FeCO_3$), was super saturated in nearly 60% of samples with a higher percentage of them being in the pH 6.0 reactors.  Thus, shifts in interactions between these two groups with pH would have significant implications for carbon budgets in anoxic systems.

# Chapter 5 - Conclusions

The balance between methanogenesis and Fe(III) reduction in our experiments shifted considerably with pH. The reactions consumed nearly equivalent amounts of electron donors in reactors with acidic pH, but methanogenesis consumed 40X more at alkaline pH. Despite this shift, the abundance of Fe(III) reducers and methanogens appears to have changed relatively little. We hypothesize that Fe(III) reducer's abundance remained high despite the low extent of Fe(III) reduction because they increasingly interacted with methanogens via interspecies electron transfer at alkaline pH. Thermodynamic calculations suggest that thermodynamic controls on microbial reactions plays an important role in the shift between Fe(III) reduction and methanogenesis. Fe(II) sorption onto goethite, however, appears to have had less of an impact on the balance of each reaction based on surface complexation model calculations. Shifts in the balance between methanogenesis and Fe(III) reduction can have significant implications on carbon budgets within anoxic systems. As Fe(III) reduction increases these systems can become important carbon storage reservoirs by increasing carbonate alkalinity, decreasing methane production, and favoring siderite precipitation.

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# Appendix A - Water Chemistry

| 6.0A Water Chemistry | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | time | mass (g) | | | pH | | | Media pH | Fe(II) (μM) | | | Alk (meq/L) | | |
| date | days | 6A-1 | 6A-2 | 6A-3 | 6A-1 | 6A-2 | 6A-3 | 6.0A | 6A-1 | 6A-2 | 6A-3 | 6A-1 | 6A-2 | 6A-3 |
| 4/26/2016 | 7 | 212.32 | 212 | 212.09 | 6.21 | 6.22 | 6.15 | 6.04 | 1072.2 | 2021.4 | 1576.9 | | | |
| 6/2/2016 | 14 | 211.91 | 212.98 | 212.41 | 6.37 | 6.44 | 6.39 | 5.92 | 2563.7 | 3101.6 | 2821.7 | | | |
| 6/9/2016 | 21 | 210.92 | 212.63 | 212.08 | 6.4 | 6.37 | 6.35 | 5.9 | 3061.4 | 3383.9 | 3068.3 | | | |
| 6/16/2016 | 28 | 210.87 | 212.75 | 212.05 | 6.6 | 6.49 | 6.42 | 5.9 | 3130.0 | 3068.3 | 3239.8 | | | |
| 6/23/2016 | 35 | 212.25 | 212.84 | 212.68 | 6.4 | 6.35 | 6.44 | 6.05 | 3006.5 | 3425.0 | 3301.5 | | | |
| 6/30/2016 | 42 | 212.08 | 212.98 | 212.65 | 6.37 | 6.31 | 6.3 | 5.88 | 2800.7 | 3020.3 | 3109.5 | | | |
| 7/7/2016 | 49 | 211.46 | 212.56 | 212.37 | 6.37 | 6.42 | 6.42 | 6.05 | 2533.2 | 2917.4 | 2780.2 | | | |
| 7/14/2016 | 56 | 211.71 | 212.36 | 212.39 | 6.32 | 6.31 | 6.33 | 6.07 | 2238.2 | 2347.9 | 2080.4 | 9.48 | 9.61 | 9.62 |
| 7/21/2016 | 63 | 211.81 | 212.66 | 212.11 | 6.3 | 6.35 | 6.36 | 5.99 | 1613.9 | 1716.8 | 2149.0 | | | |
| 7/28/2016 | 70 | 211.49 | 212.66 | 211.93 | 6.17 | 6.25 | 6.18 | 6.09 | 1943.2 | 1806.0 | 1826.6 | | | |
| 8/4/2016 | 77 | 210.8 | 213.12 | 212.25 | 6.42 | 6.39 | 6.38 | 6.05 | 1469.8 | 1661.9 | 1723.6 | | | |
| 8/11/2016 | 84 | 211.81 | 212.95 | 211.88 | 6.03 | 6.07 | 6.12 | 5.97 | 1408.1 | 1449.2 | 1661.9 | | | |
| 8/18/2016 | 91 | N/A | N/A | N/A | 6.16 | 6.28 | 6.24 | N/A | 70.3 | 1312.0 | 1661.9 | 5.11 | 7.45 | 7.49 |

| | | CH3COO- | | | | | | | Adjusted Cl- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | time | 6A-1 | | 6A-2 | | 6A-3 | | | 6A-1 | | 6A-2 | | 6A-3 | |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 125.41 | 2.12 | 128.67 | 2.18 | 117.35 | 1.99 | | 82.77 | 2.33 | 85.11 | 2.40 | 84.04 | 2.37 |
| 6/2/2016 | 14 | 90.10 | 1.53 | 95.46 | 1.62 | 87.08 | 1.47 | | 80.51 | 2.27 | 80.98 | 2.28 | 80.71 | 2.28 |
| 6/9/2016 | 21 | 60.47 | 1.02 | 66.57 | 1.13 | 62.47 | 1.06 | | 79.82 | 2.25 | 79.86 | 2.25 | 80.50 | 2.27 |
| 6/16/2016 | 28 | 32.01 | 0.54 | 36.55 | 0.62 | 37.79 | 0.64 | | 80.53 | 2.27 | 82.58 | 2.33 | 84.77 | 2.39 |
| 6/23/2016 | 35 | 9.58 | 0.16 | 12.32 | 0.21 | 15.15 | 0.26 | | 81.63 | 2.30 | 81.97 | 2.31 | 82.04 | 2.31 |
| 6/30/2016 | 42 | 2.34 | 0.04 | 3.63 | 0.06 | 5.57 | 0.09 | | 81.75 | 2.31 | 80.58 | 2.27 | 81.46 | 2.30 |
| 7/7/2016 | 49 | 1.22 | 0.02 | 1.32 | 0.02 | 1.55 | 0.03 | | 81.79 | 2.31 | 82.19 | 2.32 | 82.33 | 2.32 |
| 7/14/2016 | 56 | 0.56 | 0.01 | 0.54 | 0.01 | 0.53 | 0.01 | | 83.68 | 2.36 | 83.46 | 2.35 | 84.36 | 2.38 |
| 7/21/2016 | 63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | 81.72 | 2.31 | 81.95 | 2.31 | 82.67 | 2.33 |
| 7/28/2016 | 70 | 0.91 | 0.02 | 1.07 | 0.02 | 0.89 | 0.02 | | 78.22 | 2.21 | 78.78 | 2.22 | 79.02 | 2.23 |
| 8/4/2016 | 77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.32 | 0.01 | | 83.43 | 2.35 | 84.49 | 2.38 | 83.27 | 2.35 |
| 8/11/2016 | 84 | 1.30 | 0.02 | 1.04 | 0.02 | 1.37 | 0.02 | | 78.96 | 2.23 | 79.81 | 2.25 | 79.74 | 2.25 |
| 8/18/2016 | 91 | 0.85 | 0.01 | 1.05 | 0.02 | 1.02 | 0.02 | | 80.14 | 2.26 | 81.09 | 2.29 | 81.18 | 2.29 |

|  |  | Na+ |  |  |  |  |  | K+ |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 6A-1 |  | 6A-2 |  | 6A-3 |  | 6A-1 |  | 6A-2 |  | 6A-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 133.32 | 5.80 | 134.26 | 5.84 | 134.38 | 5.84 | 9.85 | 0.25 | 9.56 | 0.24 | 9.59 | 0.25 |
| 6/2/2016 | 14 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/9/2016 | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/16/2016 | 28 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/23/2016 | 35 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/30/2016 | 42 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/7/2016 | 49 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/14/2016 | 56 | 128.42 | 5.59 | 124.38 | 5.41 | 129.08 | 5.61 | 10.15 | 0.26 | 10.10 | 0.26 | 10.14 | 0.26 |
| 7/21/2016 | 63 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/28/2016 | 70 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/4/2016 | 77 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/11/2016 | 84 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/18/2016 | 91 | 143.17 | 6.23 | 156.26 | 6.80 | 145.94 | 6.35 | 10.30 | 0.26 | 12.51 | 0.32 | 11.45 | 0.29 |

|  |  | Mg++ |  |  |  |  |  | Ca++ |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 6A-1 |  | 6A-2 |  | 6A-3 |  | 6A-1 |  | 6A-2 |  | 6A-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 20.84 | 0.86 | 20.93 | 0.86 | 20.13 | 0.83 | 51.89 | 1.29 | 53.10 | 1.32 | 49.72 | 1.24 |
| 6/2/2016 | 14 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/9/2016 | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/16/2016 | 28 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/23/2016 | 35 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/30/2016 | 42 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/7/2016 | 49 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/14/2016 | 56 | 13.08 | 0.54 | 12.87 | 0.53 | 13.24 | 0.54 | 25.41 | 0.63 | 25.01 | 0.62 | 25.13 | 0.63 |
| 7/21/2016 | 63 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/28/2016 | 70 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/4/2016 | 77 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/11/2016 | 84 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/18/2016 | 91 | 10.80 | 0.44 | 14.01 | 0.58 | 12.98 | 0.53 | 17.10 | 0.43 | 24.29 | 0.61 | 22.80 | 0.57 |

6.0C Water Chemistry

| | time | mass (g) | | | pH | | | Media pH | Fe(II) (µM) | | | Alk (meq/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| date | days | 6C-1 | 6C-2 | 6C-3 | 6C-1 | 6C-2 | 6C-3 | 6C | 6C-1 | 6C-2 | 6C-3 | 6C-1 | 6C-2 | 6C-3 |
| 4/26/2016 | 7 | 212.53 | 212.42 | 213.13 | 6.33 | 6.3 | 6.28 | 6.19 | 1634.5 | 1478.1 | 1722.3 | | | |
| 6/2/2016 | 14 | 212.32 | 212.37 | 212.74 | 6.5 | 6.43 | 6.46 | 5.99 | 2851.9 | 2876.6 | 2700.9 | | | |
| 6/9/2016 | 21 | 212.03 | 211.99 | 211.94 | 6.4 | 6.43 | 6.46 | 5.91 | 3102.6 | 2848.8 | 2965.4 | | | |
| 6/16/2016 | 28 | 212.63 | 212.74 | 211.83 | 6.41 | 6.5 | 6.52 | 5.99 | 3095.7 | 3136.9 | 3013.4 | | | |
| 6/23/2016 | 35 | 212.53 | 212.54 | 211.65 | 6.37 | 6.35 | 6.6 | 6.14 | 2944.8 | 3095.7 | 3034.0 | | | |
| 6/30/2016 | 42 | 212.77 | 212.48 | 211.77 | 6.32 | 6.53 | 6.3 | 6.87 | 2807.6 | 2711.6 | 2841.9 | | | |
| 7/7/2016 | 49 | 212.35 | 212.26 | 214.03 | 6.37 | 6.4 | 6.41 | 6.08 | 2293.1 | 2361.7 | 2066.7 | | | |
| 7/14/2016 | 56 | 212.45 | 212 | 214.04 | 6.34 | 6.28 | 6.45 | 5.88 | 1970.6 | 2025.5 | 2004.9 | 8.85 | 8.91 | 8.75 |
| 7/21/2016 | 63 | 212.13 | 212.05 | 214.34 | 6.31 | 6.26 | 6.23 | | 1545.3 | 1655.0 | 1476.7 | | | |
| 7/28/2016 | 70 | 212.76 | 212.19 | 212.36 | 6.27 | 6.27 | 6.31 | 5.89 | 1456.1 | 1511.0 | 1504.1 | | | |
| 8/4/2016 | 77 | 212.81 | 212.13 | 212.83 | 6.34 | 6.32 | 6.26 | 6.01 | 1305.2 | 1298.3 | 1229.7 | | | |
| 8/11/2016 | 84 | 213.04 | 212.41 | 213.1 | 6.21 | 6.16 | 6.1 | 5.89 | 1154.2 | 1174.8 | 1113.1 | | | |
| 8/18/2016 | 91 | N/A | N/A | N/A | 6.11 | 6.1 | 6.05 | N/A | 1092.5 | 1318.9 | 1106.2 | 6.52 | 6.82 | 6.58 |

| | | CH3COO- | | | | | | | Adjusted Cl- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | time | 6C-1 | | 6C-2 | | 6C-3 | | | 6C-1 | | 6C-2 | | 6C-3 | |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 81.73 | 1.38 | 89.64 | 1.52 | 107.01 | 1.81 | | 85.39 | 2.41 | 87.10 | 2.46 | 89.33 | 2.52 |
| 6/2/2016 | 14 | 53.40 | 0.90 | 61.54 | 1.04 | 76.33 | 1.29 | | 83.23 | 2.35 | 84.44 | 2.38 | 84.80 | 2.39 |
| 6/9/2016 | 21 | 31.86 | 0.54 | 38.42 | 0.65 | 49.44 | 0.84 | | 83.28 | 2.35 | 84.65 | 2.39 | 84.91 | 2.40 |
| 6/16/2016 | 28 | 13.22 | 0.22 | 15.60 | 0.26 | 23.12 | 0.39 | | 83.42 | 2.35 | 85.72 | 2.42 | 91.04 | 2.57 |
| 6/23/2016 | 35 | 2.32 | 0.04 | 3.55 | 0.06 | 2.47 | 0.04 | | 82.83 | 2.34 | 84.15 | 2.37 | 84.72 | 2.39 |
| 6/30/2016 | 42 | 1.05 | 0.02 | 1.57 | 0.03 | 2.35 | 0.04 | | 81.01 | 2.29 | 81.77 | 2.31 | 80.86 | 2.28 |
| 7/7/2016 | 49 | 1.48 | 0.03 | 1.58 | 0.03 | 1.06 | 0.02 | | 79.26 | 2.24 | 80.36 | 2.27 | 80.89 | 2.28 |
| 7/14/2016 | 56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | 82.14 | 2.32 | 81.94 | 2.31 | 82.56 | 2.33 |
| 7/21/2016 | 63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | 81.17 | 2.29 | 82.20 | 2.32 | 81.45 | 2.30 |
| 7/28/2016 | 70 | 0.65 | 0.01 | 0.92 | 0.02 | 0.96 | 0.02 | | 79.34 | 2.24 | 80.04 | 2.26 | 80.31 | 2.27 |
| 8/4/2016 | 77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | 84.16 | 2.37 | 83.75 | 2.36 | 82.42 | 2.32 |
| 8/11/2016 | 84 | 0.82 | 0.01 | 0.84 | 0.01 | 1.04 | 0.02 | | 80.80 | 2.28 | 80.20 | 2.26 | 80.09 | 2.26 |
| 8/18/2016 | 91 | 1.01 | 0.02 | 0.98 | 0.02 | 0.95 | 0.02 | | 81.65 | 2.30 | 82.13 | 2.32 | 81.06 | 2.29 |

|  |  | Na+ |  |  |  |  |  | K+ |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 6C-1 |  | 6C-2 |  | 6C-3 |  | 6C-1 |  | 6C-2 |  | 6C-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 125.31 | 5.45 | 130.00 | 5.65 | 127.86 | 5.56 | 9.69 | 0.25 | 9.90 | 0.25 | 9.74 | 0.25 |
| 6/2/2016 | 14 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/9/2016 | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/16/2016 | 28 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/23/2016 | 35 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/30/2016 | 42 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/7/2016 | 49 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/14/2016 | 56 | 118.78 | 5.17 | 117.44 | 5.11 | 114.74 | 4.99 | 9.67 | 0.25 | 9.51 | 0.24 | 9.31 | 0.24 |
| 7/21/2016 | 63 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/28/2016 | 70 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/4/2016 | 77 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/11/2016 | 84 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/18/2016 | 91 | 140.16 | 6.10 | 137.29 | 5.97 | 139.97 | 6.09 | 10.97 | 0.28 | 11.19 | 0.29 | 11.39 | 0.29 |

|  |  | Mg++ |  |  |  |  |  | Ca++ |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 6C-1 |  | 6C-2 |  | 6C-3 |  | 6C-1 |  | 6C-2 |  | 6C-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 19.75 | 0.81 | 20.27 | 0.83 | 19.77 | 0.81 | 54.35 | 1.36 | 52.48 | 1.31 | 51.41 | 1.28 |
| 6/2/2016 | 14 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/9/2016 | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/16/2016 | 28 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/23/2016 | 35 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/30/2016 | 42 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/7/2016 | 49 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/14/2016 | 56 | 12.85 | 0.53 | 12.84 | 0.53 | 12.44 | 0.51 | 27.22 | 0.68 | 26.78 | 0.67 | 26.10 | 0.65 |
| 7/21/2016 | 63 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/28/2016 | 70 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/4/2016 | 77 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/11/2016 | 84 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/18/2016 | 91 | 12.91 | 0.53 | 13.00 | 0.53 | 13.12 | 0.54 | 22.38 | 0.56 | 22.92 | 0.57 | 23.54 | 0.59 |

### 7.5A Water Chemistry

| date | time | mass (g) | | | pH | | | Media pH | Fe(II) (µM) | | | Alk (meq/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | days | 7.5A-1 | 7.5A-2 | 7.5A-3 | 7.5A-1 | 7.5A-2 | 7.5A-3 | 7.5A | 7.5A-1 | 7.5A-2 | 7.5A-3 | 7.5A-1 | 7.5A-2 | 7.5A-3 |
| 4/26/2016 | 7 | 212.01 | 212.41 | 212.04 | 7.37 | 7.21 | 7.16 | 8.05 | 116.9 | 128.9 | 207.2 | | | |
| 6/2/2016 | 14 | 211.06 | 212.17 | 212.06 | 7.55 | 7.5 | 7.46 | 7.61 | 124.2 | 148.9 | 176.3 | | | |
| 6/9/2016 | 21 | 211.12 | 212.35 | 211.57 | 7.44 | 7.38 | 7.3 | 7.53 | 125.2 | 139.2 | 194.6 | | | |
| 6/16/2016 | 28 | 211.32 | 212.1 | 211.59 | 7.42 | 7.46 | 7.37 | 7.6 | 157.7 | 120.3 | 164.4 | | | |
| 6/23/2016 | 35 | 211.96 | 213.01 | 212.43 | 7.31 | 7.43 | 7.39 | 7.6 | 110.9 | 117.2 | 150.4 | | | |
| 6/30/2016 | 42 | 211.86 | 212.95 | 212.23 | 7.36 | 7.66 | 7.36 | 7.5 | 110.9 | 115.6 | 142.2 | | | |
| 7/7/2016 | 49 | 211.07 | 212.75 | 211.16 | 7.45 | 7.51 | 7.47 | 7.54 | 107.4 | 104.6 | 118.9 | | | |
| 7/14/2016 | 56 | 211.19 | 212.44 | 211.29 | 7.53 | 7.55 | 7.53 | 7.59 | 101.9 | 104.9 | 128.2 | 5.28 | 5.08 | 5.22 |
| 7/21/2016 | 63 | 211.07 | 212.29 | 211.37 | 7.44 | 7.45 | 7.44 | 7.53 | 109.1 | 111.2 | 130.9 | | | |
| 7/28/2016 | 70 | 211.36 | 212.53 | 212.55 | 7.37 | 7.44 | 7.32 | 7.57 | 107.4 | 105.4 | 119.7 | | | |
| 8/4/2016 | 77 | 210.99 | 212.94 | 212.38 | 7.35 | 7.41 | 7.41 | 7.44 | 111.8 | 103.2 | 115.0 | | | |
| 8/11/2016 | 84 | 212.64 | 213.01 | 212.34 | 7.21 | 7.3 | 7.24 | 7.51 | 119.2 | 105.4 | 115.9 | | | |
| 8/18/2016 | 91 | N/A | N/A | N/A | 6.85 | 7.12 | 7.11 | N/A | 117.5 | 115.9 | 127.9 | 4.99 | 4.97 | 5.24 |

| date | time | CH3COO- | | | | | | Adjusted Cl- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7.5A-1 | | 7.5A-2 | | 7.5A-3 | | 7.5A-1 | | 7.5A-2 | | 7.5A-3 | |
| | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 99.71 | 1.69 | 110.22 | 1.87 | 165.77 | 2.81 | 84.67 | 2.39 | 86.32 | 2.43 | 86.26 | 2.43 |
| 6/2/2016 | 14 | 78.73 | 1.33 | 87.31 | 1.48 | 129.99 | 2.20 | 82.20 | 2.32 | 84.02 | 2.37 | 83.17 | 2.35 |
| 6/9/2016 | 21 | 60.19 | 1.02 | 64.68 | 1.10 | 93.87 | 1.59 | 84.06 | 2.37 | 86.08 | 2.43 | 85.41 | 2.41 |
| 6/16/2016 | 28 | 33.19 | 0.56 | 38.98 | 0.66 | 60.04 | 1.02 | 83.85 | 2.37 | 84.97 | 2.40 | 84.71 | 2.39 |
| 6/23/2016 | 35 | 12.29 | 0.21 | 18.31 | 0.31 | 30.31 | 0.51 | 83.70 | 2.36 | 84.95 | 2.40 | 83.83 | 2.36 |
| 6/30/2016 | 42 | 2.73 | 0.05 | 5.02 | 0.09 | 7.76 | 0.13 | 82.92 | 2.34 | 83.39 | 2.35 | 82.81 | 2.34 |
| 7/7/2016 | 49 | 0.65 | 0.01 | 0.48 | 0.01 | 0.83 | 0.01 | 83.13 | 2.35 | 80.95 | 2.28 | 84.14 | 2.37 |
| 7/14/2016 | 56 | 0.00 | 0.00 | 0.42 | 0.01 | 0.44 | 0.01 | 85.18 | 2.40 | 86.40 | 2.44 | 86.94 | 2.45 |
| 7/21/2016 | 63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 84.69 | 2.39 | 85.66 | 2.42 | 85.43 | 2.41 |
| 7/28/2016 | 70 | 1.02 | 0.02 | 0.88 | 0.01 | 0.77 | 0.01 | 83.90 | 2.37 | 85.08 | 2.40 | 84.58 | 2.39 |
| 8/4/2016 | 77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 81.68 | 2.30 | 87.01 | 2.45 | 85.92 | 2.42 |
| 8/11/2016 | 84 | 0.87 | 0.01 | 0.83 | 0.01 | 0.70 | 0.01 | 82.09 | 2.32 | 82.48 | 2.33 | 84.08 | 2.37 |
| 8/18/2016 | 91 | 0.70 | 0.01 | 0.56 | 0.01 | 0.64 | 0.01 | 82.29 | 2.32 | 82.83 | 2.34 | 82.81 | 2.34 |

|  |  | Na+ |  |  |  |  |  | K+ |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 7.5A-1 |  | 7.5A-2 |  | 7.5A-3 |  | 7.5A-1 |  | 7.5A-2 |  | 7.5A-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 130.98 | 5.70 | 134.88 | 5.87 | 134.15 | 5.84 | 8.47 | 0.22 | 8.64 | 0.22 | 8.72 | 0.22 |
| 6/2/2016 | 14 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/9/2016 | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/16/2016 | 28 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/23/2016 | 35 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/30/2016 | 42 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/7/2016 | 49 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/14/2016 | 56 | 128.27 | 5.58 | 124.08 | 5.40 | 129.40 | 5.63 | 9.97 | 0.25 | 9.72 | 0.25 | 9.86 | 0.25 |
| 7/21/2016 | 63 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/28/2016 | 70 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/4/2016 | 77 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/11/2016 | 84 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/18/2016 | 91 | 144.85 | 6.30 | 140.44 | 6.11 | 150.79 | 6.56 | 11.71 | 0.30 | 11.09 | 0.28 | 12.28 | 0.31 |

|  |  | Mg++ |  |  |  |  |  | Ca++ |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 7.5A-1 |  | 7.5A-2 |  | 7.5A-3 |  | 7.5A-1 |  | 7.5A-2 |  | 7.5A-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 12.40 | 0.51 | 13.42 | 0.55 | 14.37 | 0.59 | 24.65 | 0.61 | 24.32 | 0.61 | 27.66 | 0.69 |
| 6/2/2016 | 14 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/9/2016 | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/16/2016 | 28 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/23/2016 | 35 |  |  |  |  |  |  |  |  |  |  |  |  |
| 6/30/2016 | 42 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/7/2016 | 49 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/14/2016 | 56 | 11.13 | 0.46 | 10.71 | 0.44 | 11.16 | 0.46 | 23.26 | 0.58 | 23.09 | 0.58 | 24.61 | 0.61 |
| 7/21/2016 | 63 |  |  |  |  |  |  |  |  |  |  |  |  |
| 7/28/2016 | 70 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/4/2016 | 77 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/11/2016 | 84 |  |  |  |  |  |  |  |  |  |  |  |  |
| 8/18/2016 | 91 | 12.99 | 0.53 | 12.46 | 0.51 | 13.31 | 0.55 | 24.03 | 0.60 | 23.47 | 0.59 | 25.44 | 0.63 |

7.5C Water Chemistry

|  | time | mass (g) |  |  | pH |  |  | Media pH | Fe(II) (µM) |  |  | Alk (meq/L) |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| date | days | 7.5C-1 | 7.5C-2 | 7.5C-3 | 7.5C-1 | 7.5C-2 | 7.5C-3 | 7.5C | 7.5C-1 | 7.5C-2 | 7.5C-3 | 7.5C-1 | 7.5C-2 | 7.5C-3 |
| 4/26/2016 | 7 | 211.9 | 211.99 | 212.16 | 7.34 | 7.22 | 7.18 | 7.56 | 120.8 | 152.9 | 161.4 |  |  |  |
| 6/2/2016 | 14 | 210.86 | 211.68 | 211.41 | 7.54 | 7.42 | 7.43 | 7.52 | 126.9 | 151.6 | 184.5 |  |  |  |
| 6/9/2016 | 21 | 210.38 | 211.44 | 211.39 | 7.41 | 7.33 | 7.33 | 7.53 | 131.8 | 159.8 | 165.8 |  |  |  |
| 6/16/2016 | 28 | 210.55 | 212.18 | 211.72 | 7.28 | 7.39 | 7.37 | 7.41 | 124.1 | 144.7 | 149.6 |  |  |  |
| 6/23/2016 | 35 | 211.38 | 212.07 | 212.99 | 7.35 | 7.36 | 7.43 | 7.49 | 118.6 | 137.3 | 147.4 |  |  |  |
| 6/30/2016 | 42 | 211.25 | 212.07 | 212.97 | 7.38 | 7.29 | 7.31 | 7.54 | 115.6 | 157.0 | 138.6 |  |  |  |
| 7/7/2016 | 49 | 210.41 | 211.74 | 212.57 | 7.53 | 7.5 | 7.48 | 7.6 | 102.4 | 121.4 | 120.3 |  |  |  |
| 7/14/2016 | 56 | 211.37 | 212.1 | 212.46 | 7.56 | 7.54 | 7.54 | 7.57 | 98.9 | 113.1 | 117.2 | 5.04 | 5.04 | 5.03 |
| 7/21/2016 | 63 | 211.59 | 212.01 | 212.72 | 7.44 | 7.42 | 7.41 | 7.53 | 112.1 | 129.1 | 127.8 |  |  |  |
| 7/28/2016 | 70 | 211.29 | 212.05 | 212.67 | 7.34 | 7.28 | 7.27 | 7.49 | 95.0 | 114.2 | 113.4 |  |  |  |
| 8/4/2016 | 77 | 211.31 | 212.31 | 212.98 | 7.45 | 7.44 | 7.46 | 7.46 | 95.8 | 110.4 | 108.7 |  |  |  |
| 8/11/2016 | 84 | 211.84 | 212.19 | 212.09 | 7.34 | 7.19 | 7.26 | 7.42 | 73.9 | 104.6 | 104.6 |  |  |  |
| 8/18/2016 | 91 | N/A | N/A | N/A | 7.31 | 7.23 | 7.1 | N/A | 68.4 | 102.2 | 123.0 | 5.23 | 5.14 | 5.52 |

|  |  | CH3COO- |  |  |  |  |  | Adjusted Cl- |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | time | 7.5C-1 |  | 7.5C-2 |  | 7.5C-3 |  | 7.5C-1 |  | 7.5C-2 |  | 7.5C-3 |  |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 75.75 | 1.28 | 108.64 | 1.84 | 124.51 | 2.11 | 90.27 | 2.55 | 87.62 | 2.47 | 89.08 | 2.51 |
| 6/2/2016 | 14 | 57.28 | 0.97 | 83.07 | 1.41 | 95.37 | 1.62 | 85.46 | 2.41 | 85.03 | 2.40 | 85.42 | 2.41 |
| 6/9/2016 | 21 | 39.00 | 0.66 | 60.33 | 1.02 | 67.98 | 1.15 | 87.43 | 2.47 | 87.11 | 2.46 | 87.52 | 2.47 |
| 6/16/2016 | 28 | 22.78 | 0.39 | 32.84 | 0.56 | 34.54 | 0.58 | 83.66 | 2.36 | 81.48 | 2.30 | 89.41 | 2.52 |
| 6/23/2016 | 35 | 7.00 | 0.12 | 12.43 | 0.21 | 13.07 | 0.22 | 84.99 | 2.40 | 84.75 | 2.39 | 85.47 | 2.41 |
| 6/30/2016 | 42 | 1.29 | 0.02 | 1.58 | 0.03 | 1.89 | 0.03 | 82.61 | 2.33 | 82.37 | 2.32 | 82.58 | 2.33 |
| 7/7/2016 | 49 | 0.37 | 0.01 | 0.00 | 0.00 | 0.66 | 0.01 | 82.85 | 2.34 | 81.72 | 2.31 | 82.68 | 2.33 |
| 7/14/2016 | 56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 84.12 | 2.37 | 84.83 | 2.39 | 84.94 | 2.40 |
| 7/21/2016 | 63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 83.89 | 2.37 | 84.26 | 2.38 | 84.00 | 2.37 |
| 7/28/2016 | 70 | 1.32 | 0.02 | 1.56 | 0.03 | 1.32 | 0.02 | 83.70 | 2.36 | 84.23 | 2.38 | 85.02 | 2.40 |
| 8/4/2016 | 77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 86.39 | 2.44 | 86.12 | 2.43 | 85.51 | 2.41 |
| 8/11/2016 | 84 | 1.68 | 0.03 | 2.19 | 0.04 | 2.64 | 0.04 | 82.55 | 2.33 | 83.41 | 2.35 | 83.29 | 2.35 |
| 8/18/2016 | 91 | 2.26 | 0.04 | 2.31 | 0.04 | 2.37 | 0.04 | 84.02 | 2.37 | 83.66 | 2.36 | 84.31 | 2.38 |

| | time | Na+ | | | | | | K+ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7.5C-1 | | 7.5C-2 | | 7.5C-3 | | 7.5C-1 | | 7.5C-2 | | 7.5C-3 | |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 128.57 | 5.59 | 132.67 | 5.77 | 128.89 | 5.61 | 8.68 | 0.22 | 9.07 | 0.23 | 8.78 | 0.22 |
| 6/2/2016 | 14 | | | | | | | | | | | | |
| 6/9/2016 | 21 | | | | | | | | | | | | |
| 6/16/2016 | 28 | | | | | | | | | | | | |
| 6/23/2016 | 35 | | | | | | | | | | | | |
| 6/30/2016 | 42 | | | | | | | | | | | | |
| 7/7/2016 | 49 | | | | | | | | | | | | |
| 7/14/2016 | 56 | 124.73 | 5.43 | 123.83 | 5.39 | 123.54 | 5.37 | 9.64 | 0.25 | 9.55 | 0.24 | 9.52 | 0.24 |
| 7/21/2016 | 63 | | | | | | | | | | | | |
| 7/28/2016 | 70 | | | | | | | | | | | | |
| 8/4/2016 | 77 | | | | | | | | | | | | |
| 8/11/2016 | 84 | | | | | | | | | | | | |
| 8/18/2016 | 91 | 152.67 | 6.64 | 150.91 | 6.56 | 152.13 | 6.62 | 11.71 | 0.30 | 11.38 | 0.29 | 11.50 | 0.29 |

| | time | Mg++ | | | | | | Ca++ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7.5C-1 | | 7.5C-2 | | 7.5C-3 | | 7.5C-1 | | 7.5C-2 | | 7.5C-3 | |
| date | days | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM | mg/L | mM |
| 4/26/2016 | 7 | 13.17 | 0.54 | 13.97 | 0.57 | 13.70 | 0.56 | 24.05 | 0.60 | 27.72 | 0.69 | 27.45 | 0.68 |
| 6/2/2016 | 14 | | | | | | | | | | | | |
| 6/9/2016 | 21 | | | | | | | | | | | | |
| 6/16/2016 | 28 | | | | | | | | | | | | |
| 6/23/2016 | 35 | | | | | | | | | | | | |
| 6/30/2016 | 42 | | | | | | | | | | | | |
| 7/7/2016 | 49 | | | | | | | | | | | | |
| 7/14/2016 | 56 | 11.67 | 0.48 | 10.34 | 0.43 | 11.65 | 0.48 | 22.86 | 0.57 | 20.59 | 0.51 | 23.21 | 0.58 |
| 7/21/2016 | 63 | | | | | | | | | | | | |
| 7/28/2016 | 70 | | | | | | | | | | | | |
| 8/4/2016 | 77 | | | | | | | | | | | | |
| 8/11/2016 | 84 | | | | | | | | | | | | |
| 8/18/2016 | 91 | 13.32 | 0.55 | 13.35 | 0.55 | 13.41 | 0.55 | 23.29 | 0.58 | 23.43 | 0.58 | 23.75 | 0.59 |

# Appendix B - Gas Chemistry

Gas Chemistry

| 6/10/2016 | 6.0 A-1 | 6.0 A-2 | 6.0 A-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 | 7.5 C-1 | 7.5 C-2 | 7.5 C-3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| O2 (%) | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.05 | 0.02 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 |
| N2 (%) | 85.43 | 94.18 | 94.84 | 84.09 | 94.06 | 96.19 | 128.51 | 128.45 | 127.21 | 134.14 | 134.19 | 133.56 |
| CH4 (%) | 1.32 | 1.14 | 1.01 | 0.86 | 1.16 | 1.20 | 1.18 | 1.65 | 2.86 | 0.93 | 1.15 | 1.65 |
| Guage Pressure (kPa) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total Pressure (kPa) | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 | 101.33 |
| µmoles CH4 | 54.1225 | 47.0433 | 41.4459 | 35.5192 | 47.8665 | 49.4716 | 48.525 | 68.0338 | 117.547 | 38.359 | 47.1668 | 68.075 |
| partial pressure (kPa) | 2.22 | 1.93 | 1.70 | 1.46 | 1.96 | 2.03 | 1.99 | 2.79 | 4.82 | 1.57 | 1.94 | 2.79 |
| Partial Pressure (atm) | 0.01315 | 0.01143 | 0.01007 | 0.00863 | 0.01163 | 0.01202 | 0.01179 | 0.01653 | 0.02856 | 0.00932 | 0.01146 | 0.01654 |
| | | | | | | | | | | | | |
| 7/14/2016 | 6.0 A-1 | 6.0 A-2 | 6.0 A-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 | 7.5 C-1 | 7.5 C-2 | 7.5 C-3 |
| O2 (%) | 0.009 | 0.005 | 0.007 | 0.008 | 0 | 0.02 | 0.007 | 0.005 | 0.005 | 0.006 | 0.005 | 0 |
| N2 (%) | 95.259 | 93.405 | 93.006 | 94.067 | 93.065 | 93.955 | 129.615 | 130.306 | 127.96 | 133.704 | 135.133 | 121.318 |
| CH4 (%) | 3.655 | 3.856 | 3.464 | 1.858 | 2.281 | 2.627 | 4.257 | 5.088 | 7.196 | 2.135 | 3.486 | 3.93 |
| Guage Pressure (kPa) | 13.75 | 11.25 | 7 | 7 | 5 | 7.75 | 10 | 10.5 | 7 | 25.5 | 17.5 | 15 |
| Total Pressure (kPa) | 115.075 | 112.575 | 108.325 | 108.325 | 106.325 | 109.075 | 111.325 | 111.825 | 108.325 | 126.825 | 118.825 | 116.325 |
| µmoles CH4 | 102.507 | 105.795 | 91.452 | 49.0525 | 59.1081 | 69.8348 | 115.5 | 138.667 | 189.979 | 65.9917 | 100.954 | 111.417 |
| Partial Pressure (kPa) | 4.20599 | 4.34089 | 3.75238 | 2.01268 | 2.42527 | 2.8654 | 4.73911 | 5.68966 | 7.79507 | 2.70771 | 4.14224 | 4.57157 |
| Partial Pressure (atm) | 0.04151 | 0.04284 | 0.03703 | 0.01986 | 0.02394 | 0.02828 | 0.04677 | 0.05615 | 0.07693 | 0.02672 | 0.04088 | 0.04512 |
| µmolar of methane | 58.1139 | 59.9778 | 51.8463 | 27.809 | 33.5098 | 39.591 | 65.4799 | 78.6136 | 107.704 | 37.4123 | 57.233 | 63.1651 |
| | | | | | | | | | | | | |
| 8/11/2016 | 6.0 A-1 | 6.0 A-2 | 6.0 A-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 | 7.5 C-1 | 7.5 C-2 | 7.5 C-3 |
| O2 (%) | 0.007 | 0.015 | 0.023 | 0.006 | 0.006 | 0 | 0.007 | 0.005 | 0.033 | 0.01 | 0.004 | 0.005 |
| N2 (%) | 93.522 | 100.198 | 93.864 | 93.788 | 91.32 | 92.67 | 128.004 | 129.126 | 128.143 | 131.384 | 131.953 | 130.629 |
| CH4 (%) | 3.596 | 3.408 | 3.977 | 1.678 | 2.088 | 2.519 | 4.847 | 5.207 | 7.884 | 2.024 | 3.358 | 4.103 |
| Guage Pressure (kPa) | 17 | 19 | 8 | 8 | 8 | 8.5 | 9.75 | 18 | 9.5 | 27.5 | 15.75 | 15 |
| Total Pressure (kPa) | 118.325 | 120.325 | 109.325 | 109.325 | 109.325 | 109.825 | 111.075 | 119.325 | 110.825 | 128.825 | 117.075 | 116.325 |
| uMoles CH4 | 103.701 | 99.9406 | 105.965 | 44.7093 | 55.6335 | 67.4242 | 131.213 | 151.428 | 212.947 | 63.5473 | 95.8145 | 116.322 |
| Partial Pressure (kPa) | 4.25497 | 4.10068 | 4.34786 | 1.83447 | 2.28271 | 2.76649 | 5.38381 | 6.21325 | 8.73744 | 2.60742 | 3.93138 | 4.77281 |
| Partial Pressure (atm) | 0.04199 | 0.04047 | 0.04291 | 0.0181 | 0.02253 | 0.0273 | 0.05313 | 0.06132 | 0.08623 | 0.02573 | 0.0388 | 0.0471 |
| µmolar | 58.7906 | 56.6587 | 60.074 | 25.3468 | 31.54 | 38.2244 | 74.3876 | 85.8481 | 120.725 | 36.0265 | 54.3196 | 65.9456 |

# Appendix C - Mass Balance Calculations

6.0A Mass Balance Calculations

| | | Acetate Consumed Weekly | | | Rate of Acetate Oxidation | | | Total Acetate Consumed | | | Acetate consumption by Fe reducers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | 6A-1 | 6A-2 | 6A-3 | 6A-1 | 6A-2 | 6A-3 | 6A-1 | 6A-2 | 6A-3 | 6.0 A-1 | 6.0 A-2 | 6.0 A-3 |
| date | Days | uM | uM | uM | uM/Day | uM/Day | uM/Day | uM | uM | uM | uM/Day | uM/Day | uM/Day |
| 4/26/2016 | 7 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 6/2/2016 | 14 | 1747.65 | 1791.75 | 1638.57 | 249.66 | 255.96 | 234.08 | 1747.65 | 1791.75 | 1638.57 | 30.46 | 26.51 | 27.86 |
| 6/9/2016 | 21 | 1269.70 | 1342.25 | 1228.79 | 181.39 | 191.75 | 175.54 | 3017.35 | 3134.01 | 2867.36 | 18.04 | 16.12 | 14.48 |
| 6/16/2016 | 28 | 868.83 | 951.33 | 895.77 | 124.12 | 135.90 | 127.97 | 3886.18 | 4085.34 | 3763.13 | 12.16 | 6.45 | 14.02 |
| 6/23/2016 | 35 | 483.56 | 545.05 | 561.73 | 69.08 | 77.86 | 80.25 | 4369.74 | 4630.38 | 4324.86 | 8.97 | 17.33 | 12.67 |
| 6/30/2016 | 42 | 179.70 | 216.86 | 255.13 | 25.67 | 30.98 | 36.45 | 4549.44 | 4847.24 | 4579.99 | 7.06 | 5.00 | 8.36 |
| 7/7/2016 | 49 | 81.75 | 99.14 | 125.50 | 11.68 | 14.16 | 17.93 | 4631.19 | 4946.39 | 4705.49 | 5.22 | 8.95 | 5.22 |
| 7/14/2016 | 56 | 66.58 | 67.86 | 70.96 | 9.51 | 9.69 | 10.14 | 4697.76 | 5014.25 | 4776.45 | 3.78 | 0.25 | -2.57 |
| 7/21/2016 | 63 | 57.64 | 57.37 | 57.15 | 8.23 | 8.20 | 8.16 | 4755.41 | 5071.63 | 4833.60 | -3.15 | -2.89 | 8.66 |
| 7/28/2016 | 70 | 49.98 | 49.98 | 49.98 | 7.14 | 7.14 | 7.14 | 4805.39 | 5121.61 | 4883.58 | 11.64 | 7.72 | 1.92 |
| 8/4/2016 | 77 | 62.39 | 64.48 | 62.03 | 8.91 | 9.21 | 8.86 | 4867.78 | 5186.09 | 4945.62 | -1.51 | 3.88 | 4.69 |
| 8/11/2016 | 84 | 49.98 | 49.98 | 54.32 | 7.14 | 7.14 | 7.76 | 4917.76 | 5236.07 | 4999.93 | 4.15 | 2.14 | 5.05 |
| 8/18/2016 | 91 | 67.62 | 64.04 | 68.56 | 9.66 | 9.15 | 9.79 | 4985.37 | 5300.11 | 5068.49 | -18.86 | 2.73 | 5.94 |

6.0A Mass Balance Calculations

| | | Fe Reduced Weekly | | | Rate of Iron reduction | | | Total Iron reduced | | | Total Methane Produced | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| date | Time Days | 6.0 A-1 uM | 6.0 A-2 uM | 6.0 A-3 uM | 6.0 A-1 uM/Day | 6.0 A-2 uM/Day | 6.0 A-3 uM/Day | 6.0 A-1 uM | 6.0 A-2 uM | 6.0 A-3 uM | 6.0 A-1 µmoles | 6.0 A-2 µmoles | 6.0 A-3 µmoles |
| 4/26/2016 | 7 | 972.218 | 1921.42 | 1476.87 | 138.888 | 274.489 | 210.981 | 972.218 | 1921.42 | 1476.87 | | | |
| 6/2/2016 | 14 | 1705.96 | 1484.45 | 1560.19 | 243.708 | 212.065 | 222.885 | 2678.18 | 3405.88 | 3037.06 | | | |
| 6/9/2016 | 21 | 1010.45 | 902.601 | 810.945 | 144.35 | 128.943 | 115.849 | 3688.62 | 4308.48 | 3848 | 53.6920585 | 46.6692189 | 41.116276 |
| 6/16/2016 | 28 | 680.891 | 361.194 | 785.17 | 97.2702 | 51.5992 | 112.167 | 4369.51 | 4669.67 | 4633.17 | | | |
| 6/23/2016 | 35 | 502.519 | 970.402 | 709.705 | 71.7885 | 138.629 | 101.386 | 4872.03 | 5640.07 | 5342.88 | | | |
| 6/30/2016 | 42 | 395.496 | 280.241 | 468.217 | 56.4995 | 40.0344 | 66.8882 | 5267.53 | 5920.31 | 5811.1 | | | |
| 7/7/2016 | 49 | 292.59 | 501.147 | 292.59 | 41.7985 | 71.5925 | 41.7985 | 5560.12 | 6421.46 | 6103.69 | | | |
| 7/14/2016 | 56 | 211.636 | 14.0555 | -143.73 | 30.2338 | 2.00793 | -20.534 | 5771.76 | 6435.52 | 5959.95 | 101.692121 | 104.95374 | 90.7246958 |
| 7/21/2016 | 63 | -176.66 | -161.57 | 484.682 | -25.238 | -23.082 | 69.2403 | 5595.09 | 6273.94 | 6444.63 | | | |
| 7/28/2016 | 70 | 652.077 | 432.543 | 107.358 | 93.1539 | 61.7918 | 15.3368 | 6247.17 | 6706.49 | 6551.99 | | | |
| 8/4/2016 | 77 | -84.735 | 217.125 | 262.404 | -12.105 | 31.0178 | 37.4862 | 6162.43 | 6923.61 | 6814.39 | | | |
| 8/11/2016 | 84 | 232.218 | 119.706 | 282.985 | 33.174 | 17.1009 | 40.4264 | 6394.65 | 7043.32 | 7097.38 | | | |
| 8/18/2016 | 91 | -1056.2 | 152.637 | 332.38 | -150.88 | 21.8052 | 47.4829 | 5338.48 | 7195.96 | 7429.76 | 102.876253 | 99.145817 | 105.122097 |
| | | | | | | | | Acetate needed | | 83.1841 | Acetate Needed | | 102.381389 |

6.0C Mass Balance Calculations

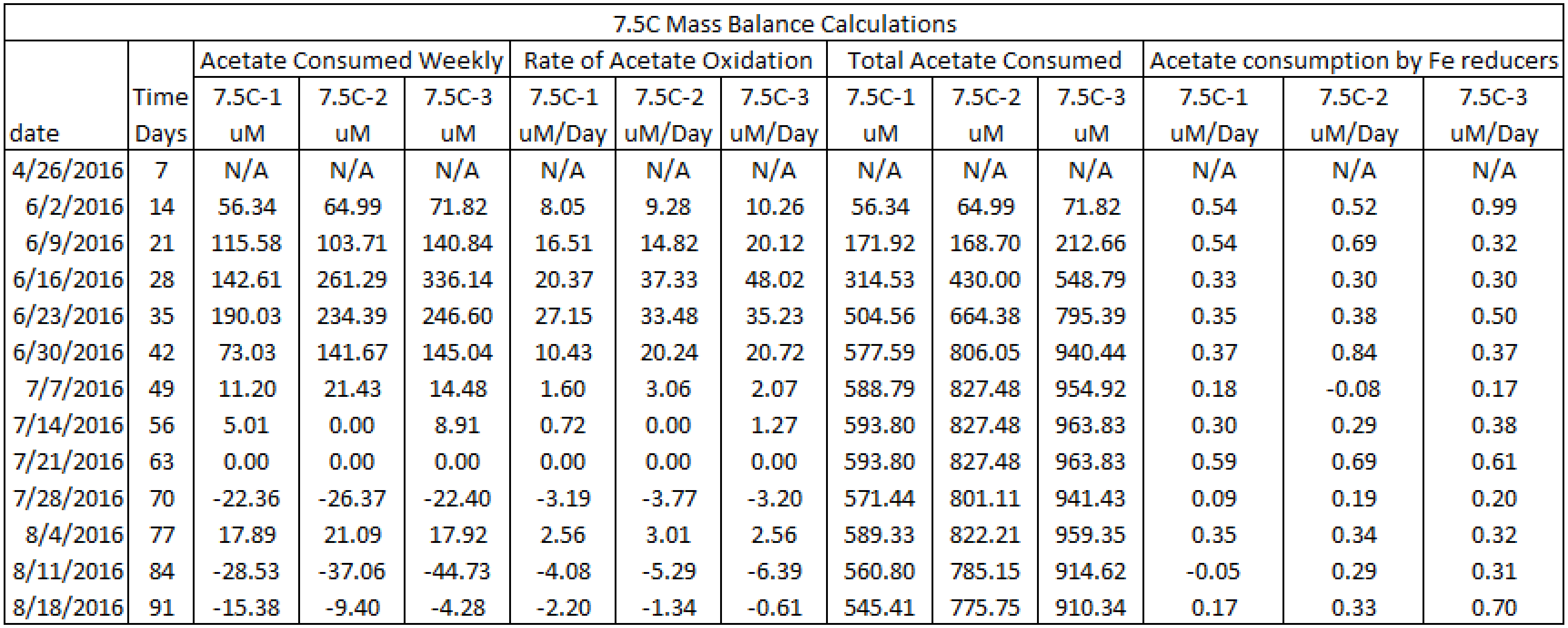
| | | Acetate Consumed Weekly | | | Rate of Acetate Oxidation | | | Total Acetate Consumed | | | Acetate consumption by Fe reducers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | 6C-1 | 6C-2 | 6C-3 | 6C-1 | 6C-2 | 6C-3 | 6C-1 | 6C-2 | 6C-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 |
| date | Days | uM | uM | uM | uM/Day | uM/Day | uM/Day | uM | uM | uM | uM/Day | uM/Day | uM/Day |
| 4/26/2016 | 7 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 6/2/2016 | 14 | 202.95 | 172.21 | 157.05 | 28.99 | 24.60 | 22.44 | 202.95 | 172.21 | 157.05 | 27.58 | 30.25 | 23.63 |
| 6/9/2016 | 21 | 183.92 | 183.25 | 196.85 | 26.27 | 26.18 | 28.12 | 386.87 | 355.46 | 353.90 | 14.66 | 9.78 | 14.37 |
| 6/16/2016 | 28 | 207.74 | 256.35 | 278.33 | 29.68 | 36.62 | 39.76 | 594.61 | 611.81 | 632.23 | 10.96 | 15.32 | 11.45 |
| 6/23/2016 | 35 | 139.85 | 151.20 | 271.50 | 19.98 | 21.60 | 38.79 | 734.45 | 763.01 | 903.73 | 8.36 | 10.47 | 11.13 |
| 6/30/2016 | 42 | 13.70 | 21.45 | -6.36 | 1.96 | 3.06 | -0.91 | 748.16 | 784.47 | 897.36 | 8.07 | 4.20 | 7.41 |
| 7/7/2016 | 49 | -10.87 | -5.40 | 13.87 | -1.55 | -0.77 | 1.98 | 737.29 | 779.06 | 911.23 | 0.84 | 3.44 | -3.69 |
| 7/14/2016 | 56 | 20.07 | 21.37 | 14.36 | 2.87 | 3.05 | 2.05 | 757.35 | 800.44 | 925.59 | 2.43 | 2.43 | 6.28 |
| 7/21/2016 | 63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 757.35 | 800.44 | 925.59 | -0.56 | 0.62 | -2.27 |
| 7/28/2016 | 70 | -10.98 | -15.54 | -16.28 | -1.57 | -2.22 | -2.33 | 746.37 | 784.90 | 909.31 | 3.93 | 3.34 | 5.76 |
| 8/4/2016 | 77 | 8.79 | 12.43 | 13.02 | 1.26 | 1.78 | 1.86 | 755.16 | 797.33 | 922.34 | 2.51 | 1.60 | 0.47 |
| 8/11/2016 | 84 | -13.93 | -14.30 | -17.67 | -1.99 | -2.04 | -2.52 | 741.23 | 783.02 | 904.67 | 1.97 | 2.43 | 2.31 |
| 8/18/2016 | 91 | -5.91 | -5.22 | -1.97 | -0.84 | -0.75 | -0.28 | 735.32 | 777.81 | 902.70 | 3.02 | 6.77 | 3.85 |

| 6.0C Mass Balance Calculations | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fe Reduced Weekly | | | Rate of Iron reduction | | | Total Iron reduced | | | Total Methane Produced | | |
| | Time | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 | 6.0 C-1 | 6.0 C-2 | 6.0 C-3 |
| date | Days | uM | uM | uM | uM/Day | uM/Day | uM/Day | uM/Day | uM/Day | uM/Day | µmoles | µmoles | µmoles |
| 4/26/2016 | 7 | 1534.49 | 1378.08 | 1622.31 | 219.213 | 196.868 | 231.758 | 1534.49 | 1378.08 | 1622.31 | | | |
| 6/2/2016 | 14 | 1544.28 | 1694.11 | 1323.1 | 220.611 | 242.016 | 189.014 | 3078.77 | 3072.18 | 2945.4 | | | |
| 6/9/2016 | 21 | 821.098 | 547.504 | 804.633 | 117.3 | 78.2148 | 114.948 | 3899.87 | 3619.69 | 3750.04 | 35.2366893 | 47.4858281 | 49.0782162 |
| 6/16/2016 | 28 | 613.659 | 857.891 | 641.1 | 87.6655 | 122.556 | 91.5858 | 4513.53 | 4477.58 | 4391.14 | | | |
| 6/23/2016 | 35 | 468.217 | 586.217 | 623.263 | 66.8882 | 83.7453 | 89.0376 | 4981.74 | 5063.8 | 5014.4 | | | |
| 6/30/2016 | 42 | 451.752 | 234.962 | 414.706 | 64.536 | 33.566 | 59.2437 | 5433.5 | 5298.76 | 5429.11 | | | |
| 7/7/2016 | 49 | 46.9856 | 192.427 | -206.85 | 6.71223 | 27.4896 | -29.55 | 5480.48 | 5491.18 | 5222.25 | | | |
| 7/14/2016 | 56 | 136.171 | 136.171 | 351.59 | 19.4531 | 19.4531 | 50.2271 | 5616.65 | 5627.36 | 5573.84 | 48.6623801 | 58.6380631 | 69.2794185 |
| 7/21/2016 | 63 | -31.223 | 34.6368 | -127.27 | -4.4605 | 4.94812 | -18.181 | 5585.43 | 5661.99 | 5446.57 | | | |
| 7/28/2016 | 70 | 219.869 | 186.939 | 322.776 | 31.4098 | 26.7055 | 46.1108 | 5805.3 | 5848.93 | 5769.35 | | | |
| 8/4/2016 | 77 | 140.288 | 89.5204 | 26.4043 | 20.0411 | 12.7886 | 3.77204 | 5945.59 | 5938.45 | 5795.75 | | | |
| 8/11/2016 | 84 | 110.102 | 136.171 | 129.311 | 15.7288 | 19.4531 | 18.473 | 6055.69 | 6074.62 | 5925.07 | | | |
| 8/18/2016 | 91 | 169.102 | 379.031 | 215.753 | 24.1574 | 54.1473 | 30.8218 | 6224.79 | 6453.65 | 6140.82 | 44.3537539 | 55.1910835 | 66.8880167 |
| | | | | | | | | Acetate needed | | 78.4136 | Acetate Needed | | 55.477618 |

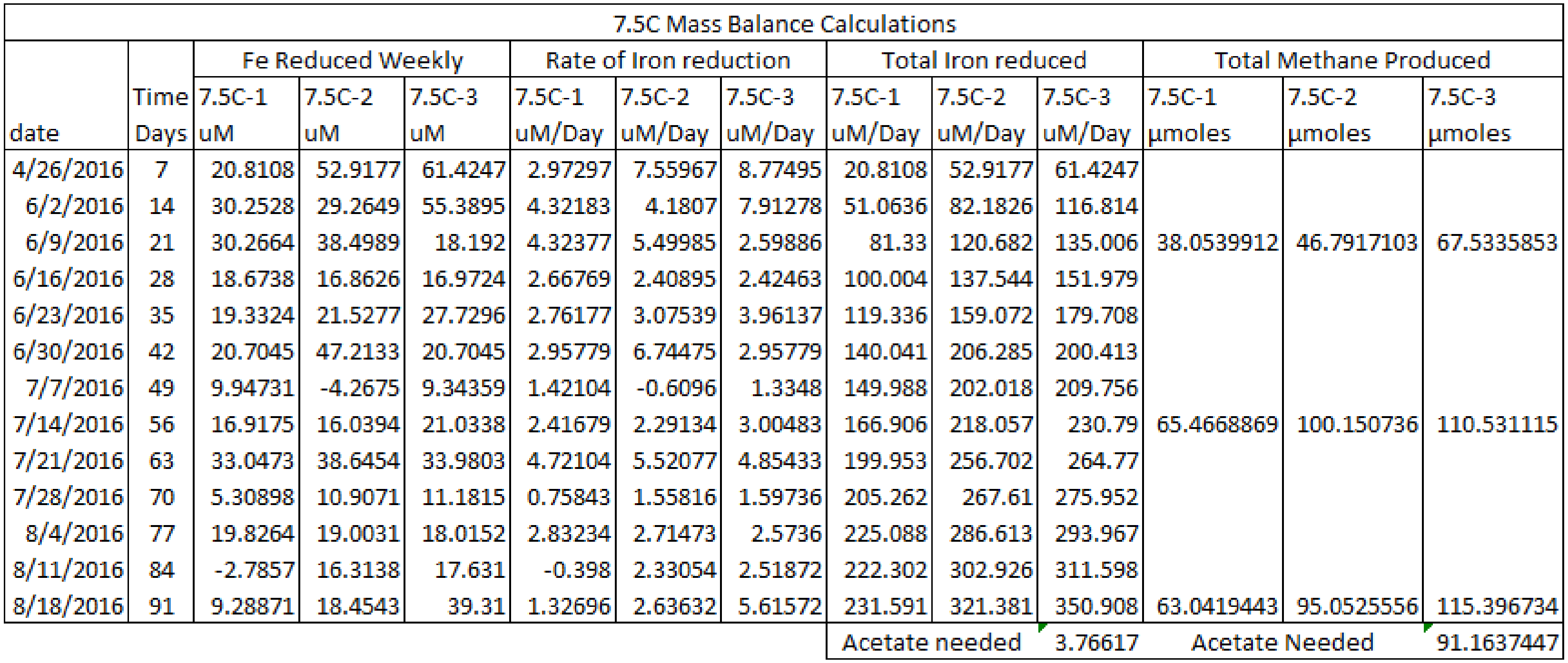
7.5A Mass Balance Calculations

| | | Acetate Consumed Weekly | | | Rate of Acetate Oxidation | | | Total Acetate Consumed | | | Acetate consumption by Fe reducers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 | 7.5 A-1 | 7.5 A-2 | 7.5 A-3 |
| date | Days | uM | uM | uM | uM/Day | uM/Day | uM/Day | uM | uM | uM | uM/Day | uM/Day | uM/Day |
| 4/26/2016 | 7 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 6/2/2016 | 14 | 1399.69 | 1541.94 | 2293.91 | 199.96 | 220.28 | 327.70 | 1399.69 | 1541.94 | 2293.91 | 0.55 | 0.82 | 0.19 |
| 6/9/2016 | 21 | 1115.67 | 1231.86 | 1809.66 | 159.38 | 175.98 | 258.52 | 2515.36 | 2773.80 | 4103.57 | 0.46 | 0.36 | 0.96 |
| 6/16/2016 | 28 | 864.97 | 925.73 | 1320.85 | 123.57 | 132.25 | 188.69 | 3380.33 | 3699.53 | 5424.42 | 1.03 | 0.16 | 0.16 |
| 6/23/2016 | 35 | 499.45 | 577.87 | 862.92 | 71.35 | 82.55 | 123.27 | 3879.77 | 4277.40 | 6287.33 | -0.27 | 0.38 | 0.34 |
| 6/30/2016 | 42 | 216.53 | 297.96 | 460.58 | 30.93 | 42.57 | 65.80 | 4096.31 | 4575.35 | 6747.92 | 0.40 | 0.39 | 0.39 |
| 7/7/2016 | 49 | 87.03 | 118.00 | 155.14 | 12.43 | 16.86 | 22.16 | 4183.34 | 4693.36 | 6903.05 | 0.33 | 0.22 | 0.09 |
| 7/14/2016 | 56 | 58.84 | 56.49 | 61.25 | 8.41 | 8.07 | 8.75 | 4242.19 | 4749.84 | 6964.30 | 0.29 | 0.38 | 0.59 |
| 7/21/2016 | 63 | 50.00 | 55.63 | 55.96 | 7.14 | 7.95 | 7.99 | 4292.19 | 4805.48 | 7020.26 | 0.49 | 0.49 | 0.51 |
| 7/28/2016 | 70 | 49.98 | 49.99 | 49.99 | 7.14 | 7.14 | 7.14 | 4342.17 | 4855.46 | 7070.25 | 0.36 | 0.29 | 0.27 |
| 8/4/2016 | 77 | 63.76 | 61.88 | 60.39 | 9.11 | 8.84 | 8.63 | 4405.93 | 4917.34 | 7130.64 | 0.46 | 0.34 | 0.34 |
| 8/11/2016 | 84 | 49.99 | 49.99 | 49.99 | 7.14 | 7.14 | 7.14 | 4455.91 | 4967.32 | 7180.63 | 0.53 | 0.41 | 0.43 |
| 8/18/2016 | 91 | 61.76 | 61.28 | 59.44 | 8.82 | 8.75 | 8.49 | 4517.67 | 5028.61 | 7240.07 | 0.40 | 0.56 | 0.63 |

| 7.5A Mass Balance Calculations | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fe Reduced Weekly | | | Rate of Iron reduction | | | Total Iron reduced | | | Total Methane Produced | | |
| date | Time Days | 7.5 A-1 uM | 7.5 A-2 uM | 7.5 A-3 uM | 7.5 A-1 uM/Day | 7.5 A-2 uM/Day | 7.5 A-3 uM/Day | 7.5 A-1 uM/Day | 7.5 A-2 uM/Day | 7.5 A-3 uM/Day | 7.5 A-1 µmoles | 7.5 A-2 µmoles | 7.5 A-3 µmoles |
| 4/26/2016 | 7 | 16.9355 | 28.8727 | 107.219 | 2.41936 | 4.12467 | 15.317 | 16.9355 | 28.8727 | 107.219 | | | |
| 6/2/2016 | 14 | 30.6089 | 45.7567 | 10.5215 | 4.37269 | 6.53667 | 1.50307 | 47.5444 | 74.6294 | 117.74 | | | |
| 6/9/2016 | 21 | 25.8757 | 20.1129 | 53.5919 | 3.69653 | 2.87327 | 7.65599 | 73.42 | 94.7423 | 171.332 | 48.1391155 | 67.4927549 | 116.611802 |
| 6/16/2016 | 28 | 57.5196 | 8.90452 | 8.73987 | 8.21709 | 1.27207 | 1.24855 | 130.94 | 103.647 | 180.072 | | | |
| 6/23/2016 | 35 | -15.213 | 21.0338 | 18.8933 | -2.1733 | 3.00483 | 2.69905 | 115.727 | 124.681 | 198.966 | | | |
| 6/30/2016 | 42 | 22.1864 | 21.8022 | 21.8571 | 3.16948 | 3.1146 | 3.12244 | 137.913 | 146.483 | 220.823 | | | |
| 7/7/2016 | 49 | 18.6189 | 12.1427 | 5.11756 | 2.65985 | 1.73467 | 0.73108 | 156.532 | 158.625 | 225.94 | | | |
| 7/14/2016 | 56 | 15.9845 | 21.1984 | 33.1082 | 2.2835 | 3.02835 | 4.72974 | 172.517 | 179.824 | 259.048 | 114.581708 | 137.564049 | 188.468508 |
| 7/21/2016 | 63 | 27.6138 | 27.2571 | 28.3547 | 3.94483 | 3.89387 | 4.05068 | 200.13 | 207.081 | 287.403 | | | |
| 7/28/2016 | 70 | 20.0727 | 16.5052 | 14.9685 | 2.86752 | 2.35789 | 2.13836 | 220.203 | 223.586 | 302.372 | | | |
| 8/4/2016 | 77 | 25.8636 | 18.8933 | 19.2775 | 3.69479 | 2.69905 | 2.75393 | 246.067 | 242.48 | 321.649 | | | |
| 8/11/2016 | 84 | 29.7603 | 22.845 | 23.8329 | 4.25147 | 3.26357 | 3.40469 | 275.827 | 265.325 | 345.482 | | | |
| 8/18/2016 | 91 | 22.1864 | 31.5166 | 35.2486 | 3.16948 | 4.50237 | 5.03552 | 298.013 | 296.841 | 380.731 | 130.169214 | 150.223529 | 211.253199 |
| | | | | | | | | Acetate needed | | 4.06494 | Acetate Needed | | 163.881981 |



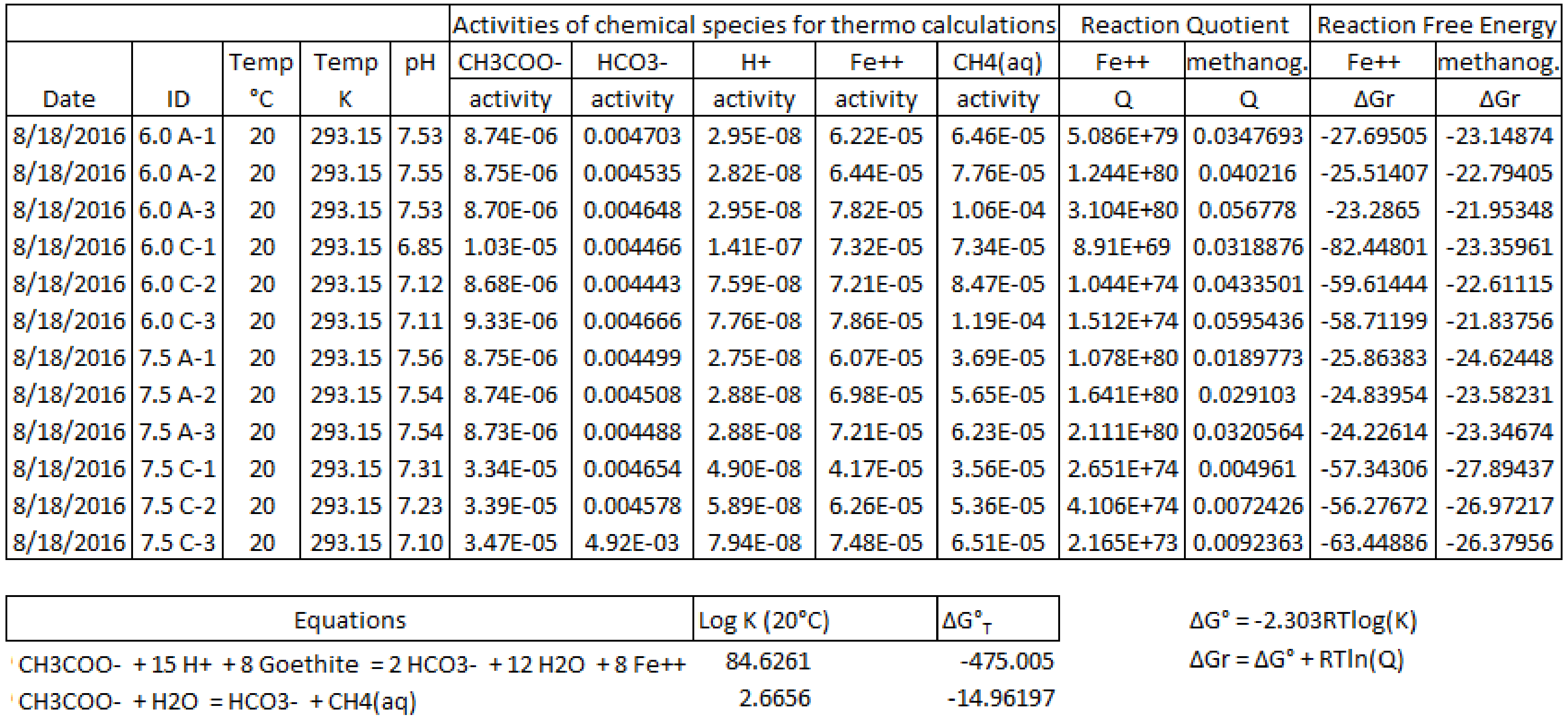
| 7.5C Mass Balance Calculations | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Acetate Consumed Weekly | | | Rate of Acetate Oxidation | | | Total Acetate Consumed | | | Acetate consumption by Fe reducers | | |
| | Time | 7.5C-1 | 7.5C-2 | 7.5C-3 | 7.5C-1 | 7.5C-2 | 7.5C-3 | 7.5C-1 | 7.5C-2 | 7.5C-3 | 7.5C-1 | 7.5C-2 | 7.5C-3 |
| date | Days | uM | uM | uM | uM/Day | uM/Day | uM/Day | uM | uM | uM | uM/Day | uM/Day | uM/Day |
| 4/26/2016 | 7 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| 6/2/2016 | 14 | 56.34 | 64.99 | 71.82 | 8.05 | 9.28 | 10.26 | 56.34 | 64.99 | 71.82 | 0.54 | 0.52 | 0.99 |
| 6/9/2016 | 21 | 115.58 | 103.71 | 140.84 | 16.51 | 14.82 | 20.12 | 171.92 | 168.70 | 212.66 | 0.54 | 0.69 | 0.32 |
| 6/16/2016 | 28 | 142.61 | 261.29 | 336.14 | 20.37 | 37.33 | 48.02 | 314.53 | 430.00 | 548.79 | 0.33 | 0.30 | 0.30 |
| 6/23/2016 | 35 | 190.03 | 234.39 | 246.60 | 27.15 | 33.48 | 35.23 | 504.56 | 664.38 | 795.39 | 0.35 | 0.38 | 0.50 |
| 6/30/2016 | 42 | 73.03 | 141.67 | 145.04 | 10.43 | 20.24 | 20.72 | 577.59 | 806.05 | 940.44 | 0.37 | 0.84 | 0.37 |
| 7/7/2016 | 49 | 11.20 | 21.43 | 14.48 | 1.60 | 3.06 | 2.07 | 588.79 | 827.48 | 954.92 | 0.18 | -0.08 | 0.17 |
| 7/14/2016 | 56 | 5.01 | 0.00 | 8.91 | 0.72 | 0.00 | 1.27 | 593.80 | 827.48 | 963.83 | 0.30 | 0.29 | 0.38 |
| 7/21/2016 | 63 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 593.80 | 827.48 | 963.83 | 0.59 | 0.69 | 0.61 |
| 7/28/2016 | 70 | -22.36 | -26.37 | -22.40 | -3.19 | -3.77 | -3.20 | 571.44 | 801.11 | 941.43 | 0.09 | 0.19 | 0.20 |
| 8/4/2016 | 77 | 17.89 | 21.09 | 17.92 | 2.56 | 3.01 | 2.56 | 589.33 | 822.21 | 959.35 | 0.35 | 0.34 | 0.32 |
| 8/11/2016 | 84 | -28.53 | -37.06 | -44.73 | -4.08 | -5.29 | -6.39 | 560.80 | 785.15 | 914.62 | -0.05 | 0.29 | 0.31 |
| 8/18/2016 | 91 | -15.38 | -9.40 | -4.28 | -2.20 | -1.34 | -0.61 | 545.41 | 775.75 | 910.34 | 0.17 | 0.33 | 0.70 |



| 7.5C Mass Balance Calculations | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fe Reduced Weekly | | | Rate of Iron reduction | | | Total Iron reduced | | | Total Methane Produced | | |
| date | Time Days | 7.5C-1 uM | 7.5C-2 uM | 7.5C-3 uM | 7.5C-1 uM/Day | 7.5C-2 uM/Day | 7.5C-3 uM/Day | 7.5C-1 uM/Day | 7.5C-2 uM/Day | 7.5C-3 uM/Day | 7.5C-1 µmoles | 7.5C-2 µmoles | 7.5C-3 µmoles |
| 4/26/2016 | 7 | 20.8108 | 52.9177 | 61.4247 | 2.97297 | 7.55967 | 8.77495 | 20.8108 | 52.9177 | 61.4247 | | | |
| 6/2/2016 | 14 | 30.2528 | 29.2649 | 55.3895 | 4.32183 | 4.1807 | 7.91278 | 51.0636 | 82.1826 | 116.814 | | | |
| 6/9/2016 | 21 | 30.2664 | 38.4989 | 18.192 | 4.32377 | 5.49985 | 2.59886 | 81.33 | 120.682 | 135.006 | 38.0539912 | 46.7917103 | 67.5335853 |
| 6/16/2016 | 28 | 18.6738 | 16.8626 | 16.9724 | 2.66769 | 2.40895 | 2.42463 | 100.004 | 137.544 | 151.979 | | | |
| 6/23/2016 | 35 | 19.3324 | 21.5277 | 27.7296 | 2.76177 | 3.07539 | 3.96137 | 119.336 | 159.072 | 179.708 | | | |
| 6/30/2016 | 42 | 20.7045 | 47.2133 | 20.7045 | 2.95779 | 6.74475 | 2.95779 | 140.041 | 206.285 | 200.413 | | | |
| 7/7/2016 | 49 | 9.94731 | -4.2675 | 9.34359 | 1.42104 | -0.6096 | 1.3348 | 149.988 | 202.018 | 209.756 | | | |
| 7/14/2016 | 56 | 16.9175 | 16.0394 | 21.0338 | 2.41679 | 2.29134 | 3.00483 | 166.906 | 218.057 | 230.79 | 65.4668869 | 100.150736 | 110.531115 |
| 7/21/2016 | 63 | 33.0473 | 38.6454 | 33.9803 | 4.72104 | 5.52077 | 4.85433 | 199.953 | 256.702 | 264.77 | | | |
| 7/28/2016 | 70 | 5.30898 | 10.9071 | 11.1815 | 0.75843 | 1.55816 | 1.59736 | 205.262 | 267.61 | 275.952 | | | |
| 8/4/2016 | 77 | 19.8264 | 19.0031 | 18.0152 | 2.83234 | 2.71473 | 2.5736 | 225.088 | 286.613 | 293.967 | | | |
| 8/11/2016 | 84 | -2.7857 | 16.3138 | 17.631 | -0.398 | 2.33054 | 2.51872 | 222.302 | 302.926 | 311.598 | | | |
| 8/18/2016 | 91 | 9.28871 | 18.4543 | 39.31 | 1.32696 | 2.63632 | 5.61572 | 231.591 | 321.381 | 350.908 | 63.0419443 | 95.0525556 | 115.396734 |
| | | | | | | | | Acetate needed | | 3.76617 | Acetate Needed | | 91.1637447 |

# Appendix D - Thermodynamic Calculations

|  |  |  |  |  | Activities of chemical species for thermo calculations |  |  |  |  | Reaction Quotient |  | Reaction Free Energy |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | Temp | Temp | pH | CH3COO- | HCO3- | H+ | Fe++ | CH4(aq) | Fe++ | methanog. | Fe++ | methanog. |
| Date | ID | °C | K |  | activity | activity | activity | activity | activity | Q | Q | ΔGr | ΔGr |
| 7/14/2016 | 6.0 A-1 | 20 | 293.15 | 6.32 | 6.66E-06 | 8.26E-03 | 4.79E-07 | 1.23E-03 | 5.74E-05 | 3.301E+72 | 0.0712456 | -68.03199 | -21.40026 |
| 7/14/2016 | 6.0 A-2 | 20 | 293.15 | 6.31 | 6.59E-06 | 8.36E-03 | 4.90E-07 | 1.28E-03 | 5.93E-05 | 3.458E+72 | 0.0752772 | -67.91922 | -21.26611 |
| 7/14/2016 | 6.0 A-3 | 20 | 293.15 | 6.33 | 6.77E-06 | 8.39E-03 | 4.68E-07 | 1.14E-03 | 5.13E-05 | 2.645E+72 | 0.0635209 | -68.57182 | -21.67997 |
| 7/14/2016 | 6.0 C-1 | 20 | 293.15 | 6.16 | 1.22E-05 | 4.63E-03 | 6.92E-07 | 4.43E-05 | 5.80E-05 | 6.54E+57 | 0.0221011 | -150.5456 | -24.25308 |
| 7/14/2016 | 6.0 C-2 | 20 | 293.15 | 6.28 | 1.29E-05 | 6.56E-03 | 5.25E-07 | 7.54E-04 | 5.60E-05 | 5.513E+69 | 0.0284938 | -83.6183 | -23.63387 |
| 7/14/2016 | 6.0 C-3 | 20 | 293.15 | 6.24 | 1.20E-05 | 6.59E-03 | 5.75E-07 | 9.51E-04 | 5.94E-05 | 9.647E+69 | 0.0324798 | -82.25458 | -23.31476 |
| 7/14/2016 | 7.5 A-1 | 20 | 293.15 | 6.34 | 6.84E-06 | 7.73E-03 | 4.57E-07 | 1.10E-03 | 2.75E-05 | 2.344E+72 | 0.0310944 | -68.86714 | -23.421 |
| 7/14/2016 | 7.5 A-2 | 20 | 293.15 | 6.28 | 6.78E-06 | 7.79E-03 | 5.25E-07 | 1.13E-03 | 3.31E-05 | 3.722E+71 | 0.0380677 | -73.35162 | -22.92785 |
| 7/14/2016 | 7.5 A-3 | 20 | 293.15 | 6.45 | 6.84E-06 | 7.63E-03 | 3.55E-07 | 1.12E-03 | 3.91E-05 | 1.194E+74 | 0.0436319 | -59.28673 | -22.59536 |
| 7/14/2016 | 7.5 C-1 | 20 | 293.15 | 6.11 | 1.26E-05 | 5.80E-03 | 7.76E-07 | 6.47E-04 | 2.50E-05 | 3.645E+66 | 0.0114899 | -101.4627 | -25.84743 |
| 7/14/2016 | 7.5 C-2 | 20 | 293.15 | 6.10 | 1.20E-05 | 6.05E-03 | 7.94E-07 | 7.72E-04 | 3.12E-05 | 1.218E+67 | 0.0156787 | -98.52197 | -25.08985 |
| 7/14/2016 | 7.5 C-3 | 20 | 293.15 | 6.05 | 1.18E-05 | 0.005853 | 8.91E-07 | 6.53E-04 | 3.78E-05 | 5.397E+65 | 0.018693 | -106.1179 | -24.66127 |

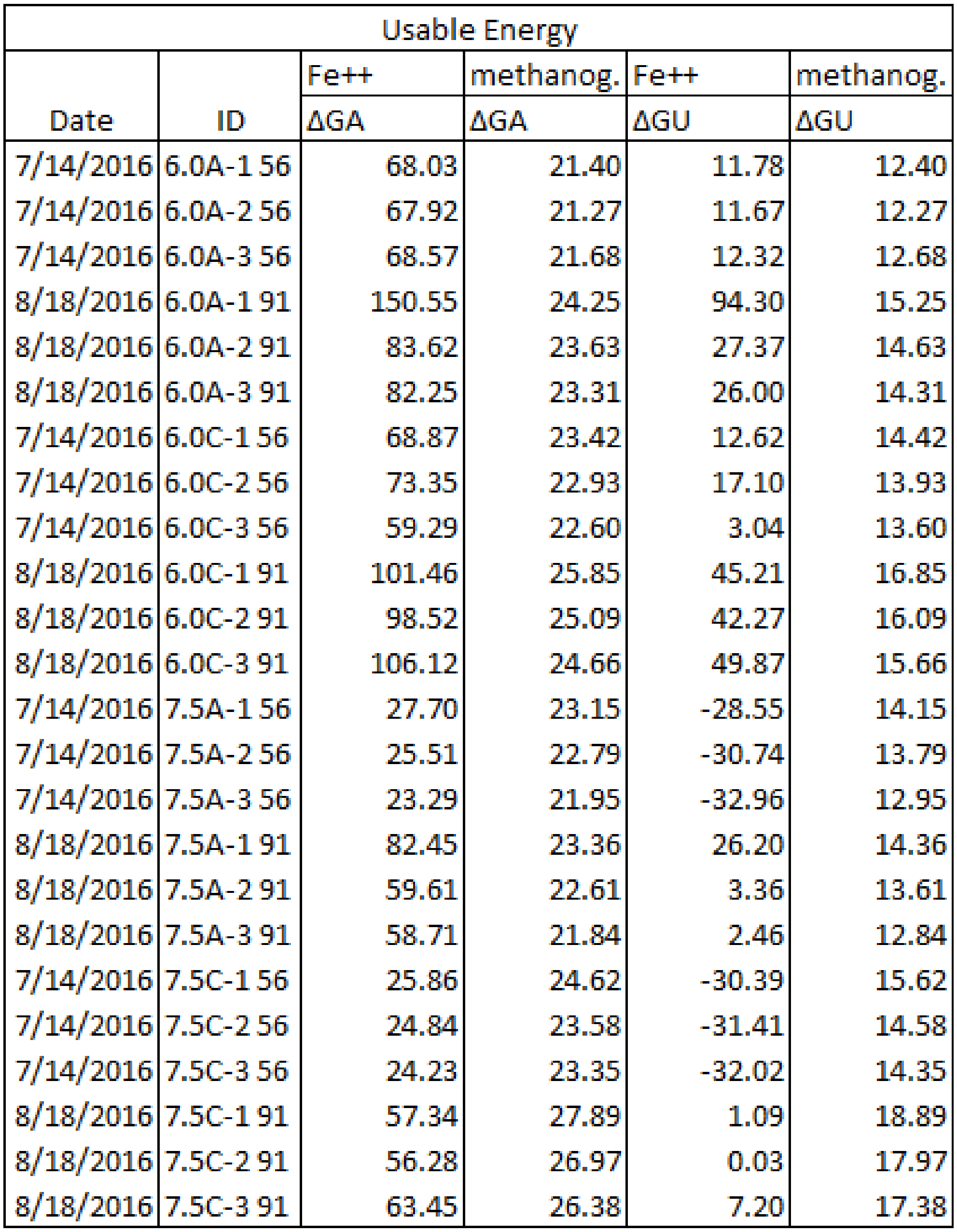


| | | | | | Activities of chemical species for thermo calculations | | | | | Reaction Quotient | | Reaction Free Energy | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Temp | Temp | pH | CH3COO- | HCO3- | H+ | Fe++ | CH4(aq) | Fe++ | methanog. | Fe++ | methanog. |
| Date | ID | °C | K | | activity | activity | activity | activity | activity | Q | Q | ΔGr | ΔGr |
| 8/18/2016 | 6.0 A-1 | 20 | 293.15 | 7.53 | 8.74E-06 | 0.004703 | 2.95E-08 | 6.22E-05 | 6.46E-05 | 5.086E+79 | 0.0347693 | -27.69505 | -23.14874 |
| 8/18/2016 | 6.0 A-2 | 20 | 293.15 | 7.55 | 8.75E-06 | 0.004535 | 2.82E-08 | 6.44E-05 | 7.76E-05 | 1.244E+80 | 0.040216 | -25.51407 | -22.79405 |
| 8/18/2016 | 6.0 A-3 | 20 | 293.15 | 7.53 | 8.70E-06 | 0.004648 | 2.95E-08 | 7.82E-05 | 1.06E-04 | 3.104E+80 | 0.056778 | -23.2865 | -21.95348 |
| 8/18/2016 | 6.0 C-1 | 20 | 293.15 | 6.85 | 1.03E-05 | 0.004466 | 1.41E-07 | 7.32E-05 | 7.34E-05 | 8.91E+69 | 0.0318876 | -82.44801 | -23.35961 |
| 8/18/2016 | 6.0 C-2 | 20 | 293.15 | 7.12 | 8.68E-06 | 0.004443 | 7.59E-08 | 7.21E-05 | 8.47E-05 | 1.044E+74 | 0.0433501 | -59.61444 | -22.61115 |
| 8/18/2016 | 6.0 C-3 | 20 | 293.15 | 7.11 | 9.33E-06 | 0.004666 | 7.76E-08 | 7.86E-05 | 1.19E-04 | 1.512E+74 | 0.0595436 | -58.71199 | -21.83756 |
| 8/18/2016 | 7.5 A-1 | 20 | 293.15 | 7.56 | 8.75E-06 | 0.004499 | 2.75E-08 | 6.07E-05 | 3.69E-05 | 1.078E+80 | 0.0189773 | -25.86383 | -24.62448 |
| 8/18/2016 | 7.5 A-2 | 20 | 293.15 | 7.54 | 8.74E-06 | 0.004508 | 2.88E-08 | 6.98E-05 | 5.65E-05 | 1.641E+80 | 0.029103 | -24.83954 | -23.58231 |
| 8/18/2016 | 7.5 A-3 | 20 | 293.15 | 7.54 | 8.73E-06 | 0.004488 | 2.88E-08 | 7.21E-05 | 6.23E-05 | 2.111E+80 | 0.0320564 | -24.22614 | -23.34674 |
| 8/18/2016 | 7.5 C-1 | 20 | 293.15 | 7.31 | 3.34E-05 | 0.004654 | 4.90E-08 | 4.17E-05 | 3.56E-05 | 2.651E+74 | 0.004961 | -57.34306 | -27.89437 |
| 8/18/2016 | 7.5 C-2 | 20 | 293.15 | 7.23 | 3.39E-05 | 0.004578 | 5.89E-08 | 6.26E-05 | 5.36E-05 | 4.106E+74 | 0.0072426 | -56.27672 | -26.97217 |
| 8/18/2016 | 7.5 C-3 | 20 | 293.15 | 7.10 | 3.47E-05 | 4.92E-03 | 7.94E-08 | 7.48E-05 | 6.51E-05 | 2.165E+73 | 0.0092363 | -63.44886 | -26.37956 |

| Equations | Log K (20°C) | $\Delta G°_T$ |
|---|---|---|
| CH3COO- + 15 H+ + 8 Goethite = 2 HCO3- + 12 H2O + 8 Fe++ | 84.6261 | -475.005 |
| CH3COO- + H2O = HCO3- + CH4(aq) | 2.6656 | -14.96197 |

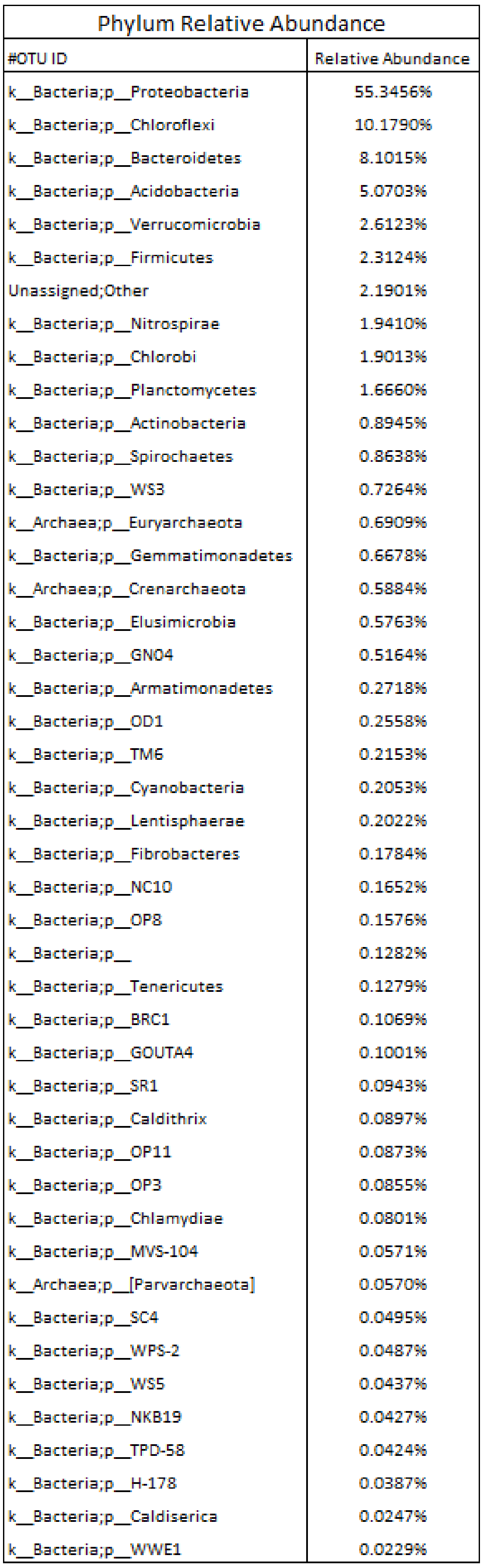
$\Delta G° = -2.303RT\log(K)$

$\Delta Gr = \Delta G° + RT\ln(Q)$



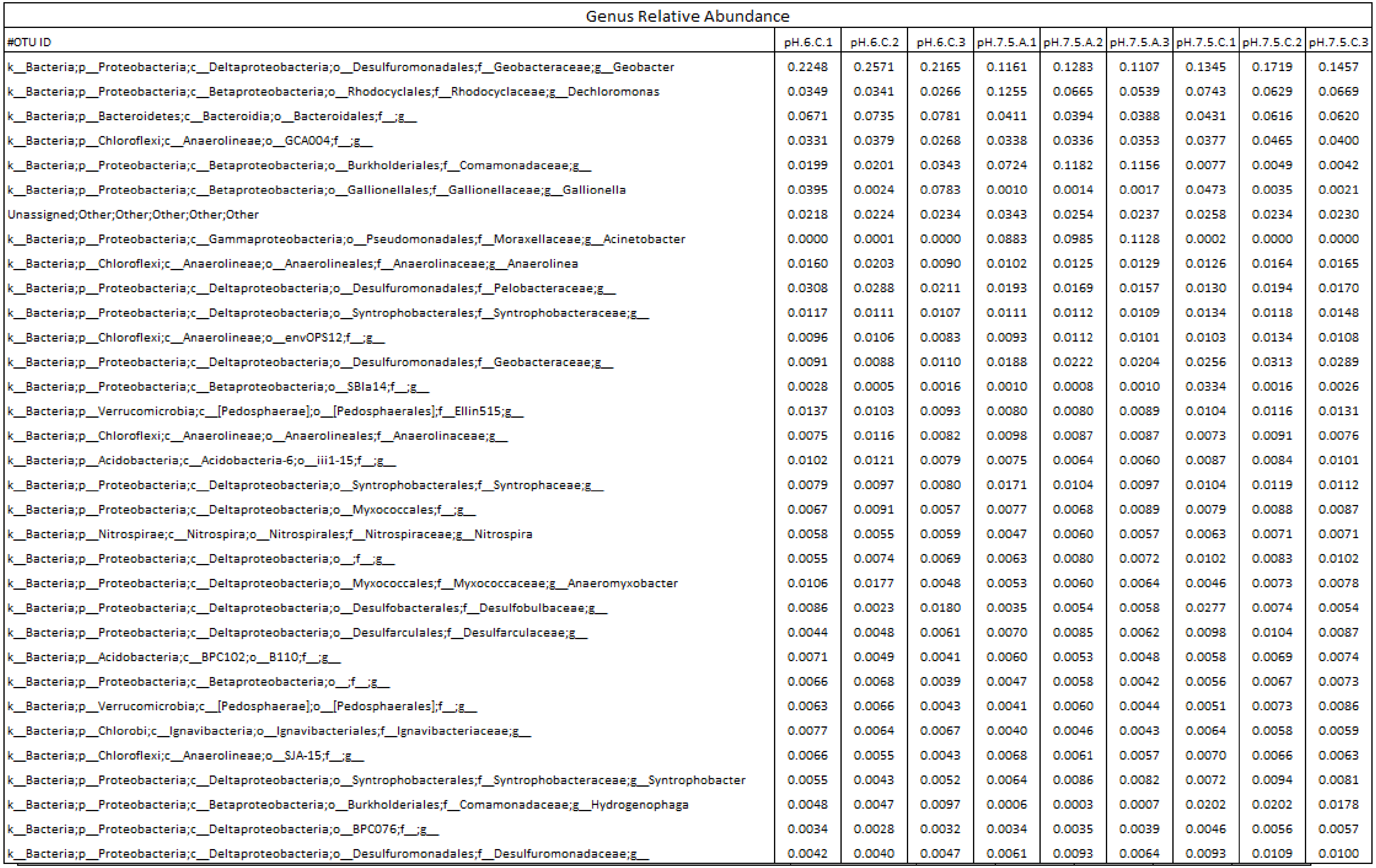
| Usable Energy | | | | | |
|---|---|---|---|---|---|
| | | Fe++ | methanog. | Fe++ | methanog. |
| Date | ID | ΔGA | ΔGA | ΔGU | ΔGU |
| 7/14/2016 | 6.0A-1 56 | 68.03 | 21.40 | 11.78 | 12.40 |
| 7/14/2016 | 6.0A-2 56 | 67.92 | 21.27 | 11.67 | 12.27 |
| 7/14/2016 | 6.0A-3 56 | 68.57 | 21.68 | 12.32 | 12.68 |
| 8/18/2016 | 6.0A-1 91 | 150.55 | 24.25 | 94.30 | 15.25 |
| 8/18/2016 | 6.0A-2 91 | 83.62 | 23.63 | 27.37 | 14.63 |
| 8/18/2016 | 6.0A-3 91 | 82.25 | 23.31 | 26.00 | 14.31 |
| 7/14/2016 | 6.0C-1 56 | 68.87 | 23.42 | 12.62 | 14.42 |
| 7/14/2016 | 6.0C-2 56 | 73.35 | 22.93 | 17.10 | 13.93 |
| 7/14/2016 | 6.0C-3 56 | 59.29 | 22.60 | 3.04 | 13.60 |
| 8/18/2016 | 6.0C-1 91 | 101.46 | 25.85 | 45.21 | 16.85 |
| 8/18/2016 | 6.0C-2 91 | 98.52 | 25.09 | 42.27 | 16.09 |
| 8/18/2016 | 6.0C-3 91 | 106.12 | 24.66 | 49.87 | 15.66 |
| 7/14/2016 | 7.5A-1 56 | 27.70 | 23.15 | -28.55 | 14.15 |
| 7/14/2016 | 7.5A-2 56 | 25.51 | 22.79 | -30.74 | 13.79 |
| 7/14/2016 | 7.5A-3 56 | 23.29 | 21.95 | -32.96 | 12.95 |
| 8/18/2016 | 7.5A-1 91 | 82.45 | 23.36 | 26.20 | 14.36 |
| 8/18/2016 | 7.5A-2 91 | 59.61 | 22.61 | 3.36 | 13.61 |
| 8/18/2016 | 7.5A-3 91 | 58.71 | 21.84 | 2.46 | 12.84 |
| 7/14/2016 | 7.5C-1 56 | 25.86 | 24.62 | -30.39 | 15.62 |
| 7/14/2016 | 7.5C-2 56 | 24.84 | 23.58 | -31.41 | 14.58 |
| 7/14/2016 | 7.5C-3 56 | 24.23 | 23.35 | -32.02 | 14.35 |
| 8/18/2016 | 7.5C-1 91 | 57.34 | 27.89 | 1.09 | 18.89 |
| 8/18/2016 | 7.5C-2 91 | 56.28 | 26.97 | 0.03 | 17.97 |
| 8/18/2016 | 7.5C-3 91 | 63.45 | 26.38 | 7.20 | 17.38 |

# Appendix E - Microbial Data



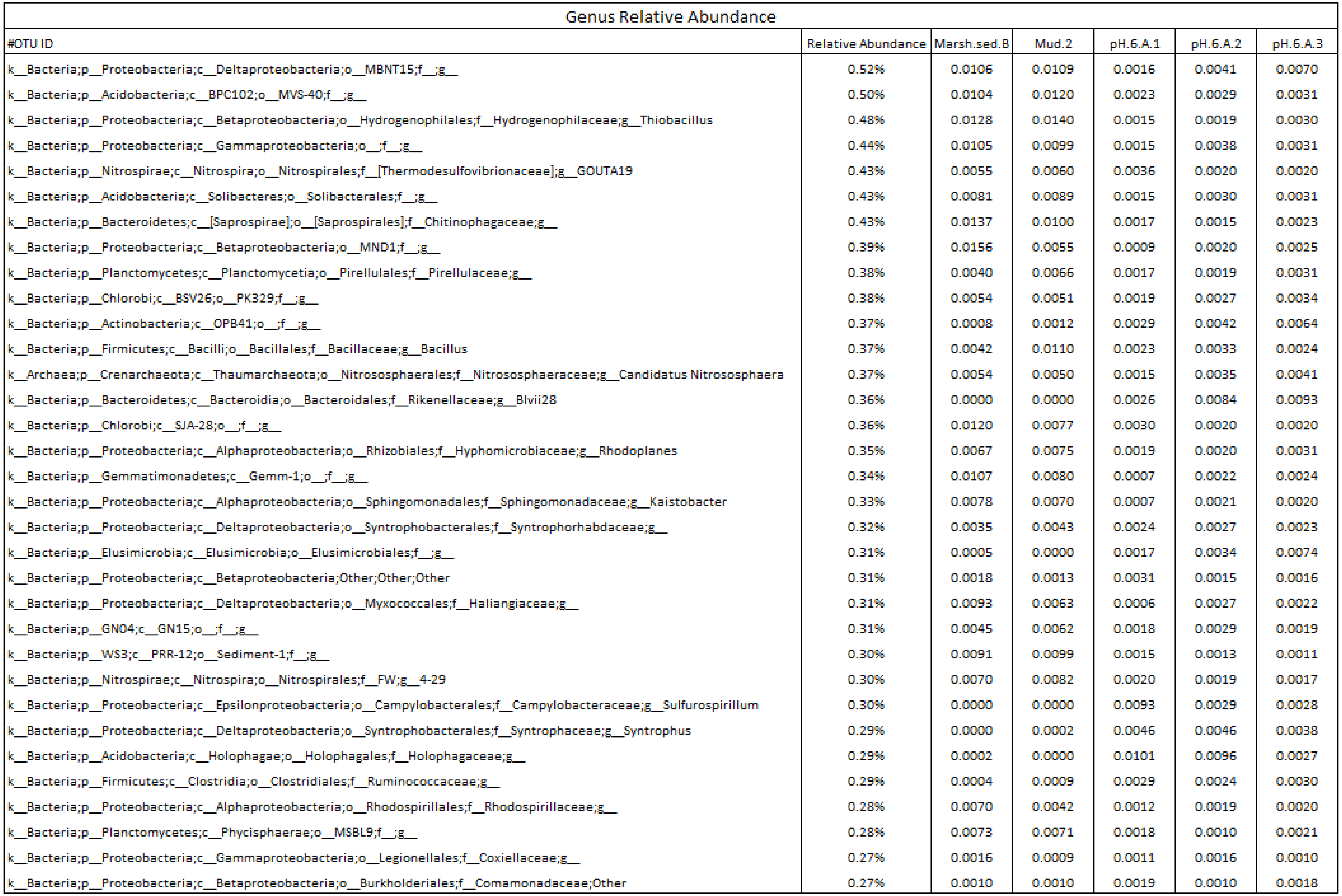
Phylum Relative Abundance

| #OTU ID | Relative Abundance |
|---|---|
| k__Bacteria;p__Proteobacteria | 55.3456% |
| k__Bacteria;p__Chloroflexi | 10.1790% |
| k__Bacteria;p__Bacteroidetes | 8.1015% |
| k__Bacteria;p__Acidobacteria | 5.0703% |
| k__Bacteria;p__Verrucomicrobia | 2.6123% |
| k__Bacteria;p__Firmicutes | 2.3124% |
| Unassigned;Other | 2.1901% |
| k__Bacteria;p__Nitrospirae | 1.9410% |
| k__Bacteria;p__Chlorobi | 1.9013% |
| k__Bacteria;p__Planctomycetes | 1.6660% |
| k__Bacteria;p__Actinobacteria | 0.8945% |
| k__Bacteria;p__Spirochaetes | 0.8638% |
| k__Bacteria;p__WS3 | 0.7264% |
| k__Archaea;p__Euryarchaeota | 0.6909% |
| k__Bacteria;p__Gemmatimonadetes | 0.6678% |
| k__Archaea;p__Crenarchaeota | 0.5884% |
| k__Bacteria;p__Elusimicrobia | 0.5763% |
| k__Bacteria;p__GN04 | 0.5164% |
| k__Bacteria;p__Armatimonadetes | 0.2718% |
| k__Bacteria;p__OD1 | 0.2558% |
| k__Bacteria;p__TM6 | 0.2153% |
| k__Bacteria;p__Cyanobacteria | 0.2053% |
| k__Bacteria;p__Lentisphaerae | 0.2022% |
| k__Bacteria;p__Fibrobacteres | 0.1784% |
| k__Bacteria;p__NC10 | 0.1652% |
| k__Bacteria;p__OP8 | 0.1576% |
| k__Bacteria;p__ | 0.1282% |
| k__Bacteria;p__Tenericutes | 0.1279% |
| k__Bacteria;p__BRC1 | 0.1069% |
| k__Bacteria;p__GOUTA4 | 0.1001% |
| k__Bacteria;p__SR1 | 0.0943% |
| k__Bacteria;p__Caldithrix | 0.0897% |
| k__Bacteria;p__OP11 | 0.0873% |
| k__Bacteria;p__OP3 | 0.0855% |
| k__Bacteria;p__Chlamydiae | 0.0801% |
| k__Bacteria;p__MVS-104 | 0.0571% |
| k__Archaea;p__[Parvarchaeota] | 0.0570% |
| k__Bacteria;p__SC4 | 0.0495% |
| k__Bacteria;p__WPS-2 | 0.0487% |
| k__Bacteria;p__WS5 | 0.0437% |
| k__Bacteria;p__NKB19 | 0.0427% |
| k__Bacteria;p__TPD-58 | 0.0424% |
| k__Bacteria;p__H-178 | 0.0387% |
| k__Bacteria;p__Caldiserica | 0.0247% |
| k__Bacteria;p__WWE1 | 0.0229% |



Genus Relative Abundance

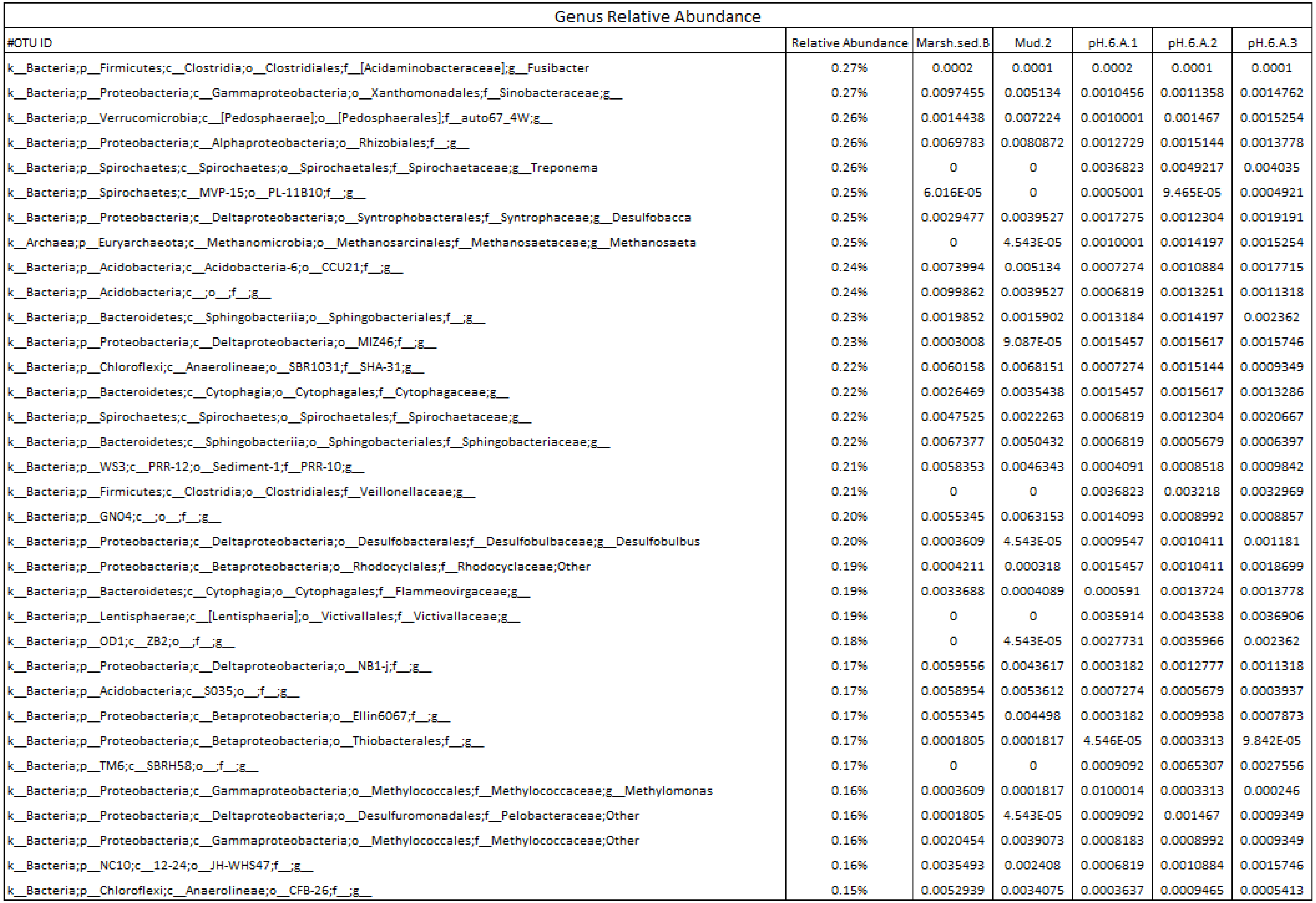
| #OTU ID | pH.6.C.1 | pH.6.C.2 | pH.6.C.3 | pH.7.5.A.1 | pH.7.5.A.2 | pH.7.5.A.3 | pH.7.5.C.1 | pH.7.5.C.2 | pH.7.5.C.3 |
|---|---|---|---|---|---|---|---|---|---|
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter | 0.2248 | 0.2571 | 0.2165 | 0.1161 | 0.1283 | 0.1107 | 0.1345 | 0.1719 | 0.1457 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Dechloromonas | 0.0349 | 0.0341 | 0.0266 | 0.1255 | 0.0665 | 0.0539 | 0.0743 | 0.0629 | 0.0669 |
| k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__;g__ | 0.0671 | 0.0735 | 0.0781 | 0.0411 | 0.0394 | 0.0388 | 0.0431 | 0.0616 | 0.0620 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__GCA004;f__;g__ | 0.0331 | 0.0379 | 0.0268 | 0.0338 | 0.0336 | 0.0353 | 0.0377 | 0.0465 | 0.0400 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__ | 0.0199 | 0.0201 | 0.0343 | 0.0724 | 0.1182 | 0.1156 | 0.0077 | 0.0049 | 0.0042 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Gallionellales;f__Gallionellaceae;g__Gallionella | 0.0395 | 0.0024 | 0.0783 | 0.0010 | 0.0014 | 0.0017 | 0.0473 | 0.0035 | 0.0021 |
| Unassigned;Other;Other;Other;Other;Other | 0.0218 | 0.0224 | 0.0234 | 0.0343 | 0.0254 | 0.0237 | 0.0258 | 0.0234 | 0.0230 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter | 0.0000 | 0.0001 | 0.0000 | 0.0883 | 0.0985 | 0.1128 | 0.0002 | 0.0000 | 0.0000 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolinaceae;g__Anaerolinea | 0.0160 | 0.0203 | 0.0090 | 0.0102 | 0.0125 | 0.0129 | 0.0126 | 0.0164 | 0.0165 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae;g__ | 0.0308 | 0.0288 | 0.0211 | 0.0193 | 0.0169 | 0.0157 | 0.0130 | 0.0194 | 0.0170 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__ | 0.0117 | 0.0111 | 0.0107 | 0.0111 | 0.0112 | 0.0109 | 0.0134 | 0.0118 | 0.0148 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__envOPS12;f__;g__ | 0.0096 | 0.0106 | 0.0083 | 0.0093 | 0.0112 | 0.0101 | 0.0103 | 0.0134 | 0.0108 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__ | 0.0091 | 0.0088 | 0.0110 | 0.0188 | 0.0222 | 0.0204 | 0.0256 | 0.0313 | 0.0289 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__SBla14;f__;g__ | 0.0028 | 0.0005 | 0.0016 | 0.0010 | 0.0008 | 0.0010 | 0.0334 | 0.0016 | 0.0026 |
| k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__Ellin515;g__ | 0.0137 | 0.0103 | 0.0093 | 0.0080 | 0.0080 | 0.0089 | 0.0104 | 0.0116 | 0.0131 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolinaceae;g__ | 0.0075 | 0.0116 | 0.0082 | 0.0098 | 0.0087 | 0.0087 | 0.0073 | 0.0091 | 0.0076 |
| k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15;f__;g__ | 0.0102 | 0.0121 | 0.0079 | 0.0075 | 0.0064 | 0.0060 | 0.0087 | 0.0084 | 0.0101 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae;g__ | 0.0079 | 0.0097 | 0.0080 | 0.0171 | 0.0104 | 0.0097 | 0.0104 | 0.0119 | 0.0112 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__;g__ | 0.0067 | 0.0091 | 0.0057 | 0.0077 | 0.0068 | 0.0089 | 0.0079 | 0.0088 | 0.0087 |
| k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira | 0.0058 | 0.0055 | 0.0059 | 0.0047 | 0.0060 | 0.0057 | 0.0063 | 0.0071 | 0.0071 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__;f__;g__ | 0.0055 | 0.0074 | 0.0069 | 0.0063 | 0.0080 | 0.0072 | 0.0102 | 0.0083 | 0.0102 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Anaeromyxobacter | 0.0106 | 0.0177 | 0.0048 | 0.0053 | 0.0060 | 0.0064 | 0.0046 | 0.0073 | 0.0078 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae;g__ | 0.0086 | 0.0023 | 0.0180 | 0.0035 | 0.0054 | 0.0058 | 0.0277 | 0.0074 | 0.0054 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales;f__Desulfarculaceae;g__ | 0.0044 | 0.0048 | 0.0061 | 0.0070 | 0.0085 | 0.0062 | 0.0098 | 0.0104 | 0.0087 |
| k__Bacteria;p__Acidobacteria;c__BPC102;o__B110;f__;g__ | 0.0071 | 0.0049 | 0.0041 | 0.0060 | 0.0053 | 0.0048 | 0.0058 | 0.0069 | 0.0074 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__;f__;g__ | 0.0066 | 0.0068 | 0.0039 | 0.0047 | 0.0058 | 0.0042 | 0.0056 | 0.0067 | 0.0073 |
| k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__;g__ | 0.0063 | 0.0066 | 0.0043 | 0.0041 | 0.0060 | 0.0044 | 0.0051 | 0.0073 | 0.0086 |
| k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__Ignavibacteriaceae;g__ | 0.0077 | 0.0064 | 0.0067 | 0.0040 | 0.0046 | 0.0043 | 0.0064 | 0.0058 | 0.0059 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SJA-15;f__;g__ | 0.0066 | 0.0055 | 0.0043 | 0.0068 | 0.0061 | 0.0057 | 0.0070 | 0.0066 | 0.0063 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae;g__Syntrophobacter | 0.0055 | 0.0043 | 0.0052 | 0.0064 | 0.0086 | 0.0082 | 0.0072 | 0.0094 | 0.0081 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Hydrogenophaga | 0.0048 | 0.0047 | 0.0097 | 0.0006 | 0.0003 | 0.0007 | 0.0202 | 0.0202 | 0.0178 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__BPC076;f__;g__ | 0.0034 | 0.0028 | 0.0032 | 0.0034 | 0.0035 | 0.0039 | 0.0046 | 0.0056 | 0.0057 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Desulfuromonadaceae;g__ | 0.0042 | 0.0040 | 0.0047 | 0.0061 | 0.0093 | 0.0064 | 0.0093 | 0.0109 | 0.0100 |



Genus Relative Abundance

| #OTU ID | Relative Abundance | Marsh.sed.B | Mud.2 | pH.6.A.1 | pH.6.A.2 | pH.6.A.3 |
|---|---|---|---|---|---|---|
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MBNT15;f__;g__ | 0.52% | 0.0106 | 0.0109 | 0.0016 | 0.0041 | 0.0070 |
| k__Bacteria;p__Acidobacteria;c__BPC102;o__MVS-40;f__;g__ | 0.50% | 0.0104 | 0.0120 | 0.0023 | 0.0029 | 0.0031 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Hydrogenophilales;f__Hydrogenophilaceae;g__Thiobacillus | 0.48% | 0.0128 | 0.0140 | 0.0015 | 0.0019 | 0.0030 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__;f__;g__ | 0.44% | 0.0105 | 0.0099 | 0.0015 | 0.0038 | 0.0031 |
| k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__[Thermodesulfovibrionaceae];g__GOUTA19 | 0.43% | 0.0055 | 0.0060 | 0.0036 | 0.0020 | 0.0020 |
| k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__;g__ | 0.43% | 0.0081 | 0.0089 | 0.0015 | 0.0030 | 0.0031 |
| k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__ | 0.43% | 0.0137 | 0.0100 | 0.0017 | 0.0015 | 0.0023 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__;g__ | 0.39% | 0.0156 | 0.0055 | 0.0009 | 0.0020 | 0.0025 |
| k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__ | 0.38% | 0.0040 | 0.0066 | 0.0017 | 0.0019 | 0.0031 |
| k__Bacteria;p__Chlorobi;c__BSV26;o__PK329;f__;g__ | 0.38% | 0.0054 | 0.0051 | 0.0019 | 0.0027 | 0.0034 |
| k__Bacteria;p__Actinobacteria;c__OPB41;o__;f__;g__ | 0.37% | 0.0008 | 0.0012 | 0.0029 | 0.0042 | 0.0064 |
| k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus | 0.37% | 0.0042 | 0.0110 | 0.0023 | 0.0033 | 0.0024 |
| k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera | 0.37% | 0.0054 | 0.0050 | 0.0015 | 0.0035 | 0.0041 |
| k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Blvii28 | 0.36% | 0.0000 | 0.0000 | 0.0026 | 0.0084 | 0.0093 |
| k__Bacteria;p__Chlorobi;c__SJA-28;o__;f__;g__ | 0.36% | 0.0120 | 0.0077 | 0.0030 | 0.0020 | 0.0020 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes | 0.35% | 0.0067 | 0.0075 | 0.0019 | 0.0020 | 0.0031 |
| k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__;f__;g__ | 0.34% | 0.0107 | 0.0080 | 0.0007 | 0.0022 | 0.0024 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter | 0.33% | 0.0078 | 0.0070 | 0.0007 | 0.0021 | 0.0020 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophorhabdaceae;g__ | 0.32% | 0.0035 | 0.0043 | 0.0024 | 0.0027 | 0.0023 |
| k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__Elusimicrobiales;f__;g__ | 0.31% | 0.0005 | 0.0000 | 0.0017 | 0.0034 | 0.0074 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other;Other;Other | 0.31% | 0.0018 | 0.0013 | 0.0031 | 0.0015 | 0.0016 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae;g__ | 0.31% | 0.0093 | 0.0063 | 0.0006 | 0.0027 | 0.0022 |
| k__Bacteria;p__GN04;c__GN15;o__;f__;g__ | 0.31% | 0.0045 | 0.0062 | 0.0018 | 0.0029 | 0.0019 |
| k__Bacteria;p__WS3;c__PRR-12;o__Sediment-1;f__;g__ | 0.30% | 0.0091 | 0.0099 | 0.0015 | 0.0013 | 0.0011 |
| k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__FW;g__4-29 | 0.30% | 0.0070 | 0.0082 | 0.0020 | 0.0019 | 0.0017 |
| k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Sulfurospirillum | 0.30% | 0.0000 | 0.0000 | 0.0093 | 0.0029 | 0.0028 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae;g__Syntrophus | 0.29% | 0.0000 | 0.0002 | 0.0046 | 0.0046 | 0.0038 |
| k__Bacteria;p__Acidobacteria;c__Holophagae;o__Holophagales;f__Holophagaceae;g__ | 0.29% | 0.0002 | 0.0000 | 0.0101 | 0.0096 | 0.0027 |
| k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__ | 0.29% | 0.0004 | 0.0009 | 0.0029 | 0.0024 | 0.0030 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__ | 0.28% | 0.0070 | 0.0042 | 0.0012 | 0.0019 | 0.0020 |
| k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__MSBL9;f__;g__ | 0.28% | 0.0073 | 0.0071 | 0.0018 | 0.0010 | 0.0021 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__ | 0.27% | 0.0016 | 0.0009 | 0.0011 | 0.0016 | 0.0010 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;Other | 0.27% | 0.0010 | 0.0010 | 0.0019 | 0.0010 | 0.0018 |

| Genus Relative Abundance | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| #OTU ID | pH.6.C.1 | pH.6.C.2 | pH.6.C.3 | pH.7.5.A.1 | pH.7.5.A.2 | pH.7.5.A.3 | pH.7.5.C.1 | pH.7.5.C.2 | pH.7.5.C.3 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MBNT15;f__;g__ | 0.0051 | 0.0069 | 0.0051 | 0.0028 | 0.0039 | 0.0041 | 0.0030 | 0.0028 | 0.0048 |
| k__Bacteria;p__Acidobacteria;c__BPC102;o__MVS-40;f__;g__ | 0.0037 | 0.0044 | 0.0039 | 0.0050 | 0.0047 | 0.0046 | 0.0042 | 0.0042 | 0.0052 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Hydrogenophilales;f__Hydrogenophilaceae;g__Thiobacillus | 0.0034 | 0.0041 | 0.0025 | 0.0030 | 0.0026 | 0.0028 | 0.0049 | 0.0050 | 0.0058 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__;f__;g__ | 0.0032 | 0.0029 | 0.0033 | 0.0029 | 0.0037 | 0.0015 | 0.0040 | 0.0061 | 0.0049 |
| k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__[Thermodesulfovibrionaceae];g__GOUTA19 | 0.0074 | 0.0028 | 0.0076 | 0.0028 | 0.0042 | 0.0031 | 0.0049 | 0.0045 | 0.0045 |
| k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__;g__ | 0.0049 | 0.0038 | 0.0020 | 0.0030 | 0.0034 | 0.0037 | 0.0043 | 0.0056 | 0.0047 |
| k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__ | 0.0024 | 0.0026 | 0.0037 | 0.0030 | 0.0038 | 0.0031 | 0.0050 | 0.0037 | 0.0034 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__;g__ | 0.0035 | 0.0033 | 0.0018 | 0.0026 | 0.0022 | 0.0028 | 0.0036 | 0.0041 | 0.0037 |
| k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__ | 0.0045 | 0.0047 | 0.0028 | 0.0030 | 0.0042 | 0.0030 | 0.0048 | 0.0045 | 0.0049 |
| k__Bacteria;p__Chlorobi;c__BSV26;o__PK329;f__;g__ | 0.0051 | 0.0043 | 0.0041 | 0.0045 | 0.0028 | 0.0027 | 0.0029 | 0.0042 | 0.0038 |
| k__Bacteria;p__Actinobacteria;c__OPB41;o__;f__;g__ | 0.0040 | 0.0058 | 0.0042 | 0.0039 | 0.0053 | 0.0032 | 0.0027 | 0.0042 | 0.0036 |
| k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus | 0.0029 | 0.0027 | 0.0035 | 0.0030 | 0.0024 | 0.0020 | 0.0040 | 0.0035 | 0.0051 |
| k__Archaea;p__Crenarchaeota;c__Thaumarchaeota;o__Nitrososphaerales;f__Nitrososphaeraceae;g__Candidatus Nitrososphaera | 0.0057 | 0.0041 | 0.0059 | 0.0023 | 0.0030 | 0.0021 | 0.0024 | 0.0036 | 0.0035 |
| k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__Blvii28 | 0.0082 | 0.0092 | 0.0020 | 0.0011 | 0.0013 | 0.0030 | 0.0009 | 0.0031 | 0.0018 |
| k__Bacteria;p__Chlorobi;c__SJA-28;o__;f__;g__ | 0.0033 | 0.0026 | 0.0028 | 0.0035 | 0.0020 | 0.0029 | 0.0017 | 0.0024 | 0.0027 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes | 0.0033 | 0.0033 | 0.0028 | 0.0024 | 0.0018 | 0.0032 | 0.0036 | 0.0032 | 0.0036 |
| k__Bacteria;p__Gemmatimonadetes;c__Gemm-1;o__;f__;g__ | 0.0028 | 0.0033 | 0.0015 | 0.0021 | 0.0024 | 0.0027 | 0.0032 | 0.0032 | 0.0030 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Kaistobacter | 0.0032 | 0.0031 | 0.0026 | 0.0023 | 0.0021 | 0.0028 | 0.0027 | 0.0042 | 0.0030 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophorhabdaceae;g__ | 0.0021 | 0.0032 | 0.0025 | 0.0028 | 0.0030 | 0.0044 | 0.0039 | 0.0040 | 0.0045 |
| k__Bacteria;p__Elusimicrobia;c__Elusimicrobia;o__Elusimicrobiales;f__;g__ | 0.0033 | 0.0037 | 0.0044 | 0.0030 | 0.0022 | 0.0017 | 0.0043 | 0.0033 | 0.0049 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other;Other;Other | 0.0146 | 0.0045 | 0.0106 | 0.0004 | 0.0002 | 0.0006 | 0.0012 | 0.0008 | 0.0014 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae;g__ | 0.0029 | 0.0033 | 0.0015 | 0.0017 | 0.0020 | 0.0023 | 0.0021 | 0.0031 | 0.0036 |
| k__Bacteria;p__GN04;c__GN15;o__;f__;g__ | 0.0024 | 0.0030 | 0.0019 | 0.0027 | 0.0031 | 0.0023 | 0.0035 | 0.0038 | 0.0031 |
| k__Bacteria;p__WS3;c__PRR-12;o__Sediment-1;f__;g__ | 0.0023 | 0.0024 | 0.0026 | 0.0022 | 0.0010 | 0.0027 | 0.0021 | 0.0021 | 0.0022 |
| k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__FW;g__4-29 | 0.0023 | 0.0016 | 0.0035 | 0.0021 | 0.0015 | 0.0015 | 0.0023 | 0.0031 | 0.0033 |
| k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Sulfurospirillum | 0.0027 | 0.0024 | 0.0050 | 0.0020 | 0.0039 | 0.0027 | 0.0014 | 0.0028 | 0.0036 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae;g__Syntrophus | 0.0027 | 0.0057 | 0.0047 | 0.0022 | 0.0012 | 0.0019 | 0.0030 | 0.0039 | 0.0027 |
| k__Bacteria;p__Acidobacteria;c__Holophagae;o__Holophagales;f__Holophagaceae;g__ | 0.0043 | 0.0022 | 0.0023 | 0.0018 | 0.0014 | 0.0022 | 0.0007 | 0.0012 | 0.0021 |
| k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__ | 0.0040 | 0.0048 | 0.0057 | 0.0021 | 0.0017 | 0.0023 | 0.0033 | 0.0035 | 0.0036 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__ | 0.0029 | 0.0024 | 0.0029 | 0.0023 | 0.0020 | 0.0017 | 0.0026 | 0.0022 | 0.0032 |
| k__Bacteria;p__Planctomycetes;c__Phycisphaerae;o__MSBL9;f__;g__ | 0.0016 | 0.0025 | 0.0019 | 0.0015 | 0.0022 | 0.0015 | 0.0028 | 0.0030 | 0.0024 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae;g__ | 0.0010 | 0.0017 | 0.0023 | 0.0015 | 0.0041 | 0.0087 | 0.0033 | 0.0043 | 0.0054 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;Other | 0.0048 | 0.0061 | 0.0101 | 0.0019 | 0.0018 | 0.0017 | 0.0014 | 0.0014 | 0.0024 |



| Genus Relative Abundance | | | | | | |
|---|---|---|---|---|---|---|
| #OTU ID | Relative Abundance | Marsh.sed.B | Mud.2 | pH.6.A.1 | pH.6.A.2 | pH.6.A.3 |
| k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae];g__Fusibacter | 0.27% | 0.0002 | 0.0001 | 0.0002 | 0.0001 | 0.0001 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__ | 0.27% | 0.0097455 | 0.005134 | 0.0010456 | 0.0011358 | 0.0014762 |
| k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__auto67_4W;g__ | 0.26% | 0.0014438 | 0.007224 | 0.0010001 | 0.001467 | 0.0015254 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__;g__ | 0.26% | 0.0069783 | 0.0080872 | 0.0012729 | 0.0015144 | 0.0013778 |
| k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__Treponema | 0.26% | 0 | 0 | 0.0036823 | 0.0049217 | 0.004035 |
| k__Bacteria;p__Spirochaetes;c__MVP-15;o__PL-11B10;f__;g__ | 0.25% | 6.016E-05 | 0 | 0.0005001 | 9.465E-05 | 0.0004921 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae;g__Desulfobacca | 0.25% | 0.0029477 | 0.0039527 | 0.0017275 | 0.0012304 | 0.0019191 |
| k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosaetaceae;g__Methanosaeta | 0.25% | 0 | 4.543E-05 | 0.0010001 | 0.0014197 | 0.0015254 |
| k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__CCU21;f__;g__ | 0.24% | 0.0073994 | 0.005134 | 0.0007274 | 0.0010884 | 0.0017715 |
| k__Bacteria;p__Acidobacteria;c__;o__;f__;g__ | 0.24% | 0.0099862 | 0.0039527 | 0.0006819 | 0.0013251 | 0.0011318 |
| k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__;g__ | 0.23% | 0.0019852 | 0.0015902 | 0.0013184 | 0.0014197 | 0.002362 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46;f__;g__ | 0.23% | 0.0003008 | 9.087E-05 | 0.0015457 | 0.0015617 | 0.0015746 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__SHA-31;g__ | 0.22% | 0.0060158 | 0.0068151 | 0.0007274 | 0.0015144 | 0.0009349 |
| k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__ | 0.22% | 0.0026469 | 0.0035438 | 0.0015457 | 0.0015617 | 0.0013286 |
| k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__ | 0.22% | 0.0047525 | 0.0022263 | 0.0006819 | 0.0012304 | 0.0020667 |
| k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__ | 0.22% | 0.0067377 | 0.0050432 | 0.0006819 | 0.0005679 | 0.0006397 |
| k__Bacteria;p__WS3;c__PRR-12;o__Sediment-1;f__PRR-10;g__ | 0.21% | 0.0058353 | 0.0046343 | 0.0004091 | 0.0008518 | 0.0009842 |
| k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__ | 0.21% | 0 | 0 | 0.0036823 | 0.003218 | 0.0032969 |
| k__Bacteria;p__GN04;c__;o__;f__;g__ | 0.20% | 0.0055345 | 0.0063153 | 0.0014093 | 0.0008992 | 0.0008857 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae;g__Desulfobulbus | 0.20% | 0.0003609 | 4.543E-05 | 0.0009547 | 0.0010411 | 0.001181 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;Other | 0.19% | 0.0004211 | 0.000318 | 0.0015457 | 0.0010411 | 0.0018699 |
| k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae;g__ | 0.19% | 0.0033688 | 0.0004089 | 0.000591 | 0.0013724 | 0.0013778 |
| k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae;g__ | 0.19% | 0 | 0 | 0.0035914 | 0.0043538 | 0.0036906 |
| k__Bacteria;p__OD1;c__ZB2;o__;f__;g__ | 0.18% | 0 | 4.543E-05 | 0.0027731 | 0.0035966 | 0.002362 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__;g__ | 0.17% | 0.0059556 | 0.0043617 | 0.0003182 | 0.0012777 | 0.0011318 |
| k__Bacteria;p__Acidobacteria;c__S035;o__;f__;g__ | 0.17% | 0.0058954 | 0.0053612 | 0.0007274 | 0.0005679 | 0.0003937 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067;f__;g__ | 0.17% | 0.0055345 | 0.004498 | 0.0003182 | 0.0009938 | 0.0007873 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Thiobacterales;f__;g__ | 0.17% | 0.0001805 | 0.0001817 | 4.546E-05 | 0.0003313 | 9.842E-05 |
| k__Bacteria;p__TM6;c__SBRH58;o__;f__;g__ | 0.17% | 0 | 0 | 0.0009092 | 0.0065307 | 0.0027556 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas | 0.16% | 0.0003609 | 0.0001817 | 0.0100014 | 0.0003313 | 0.000246 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae;Other | 0.16% | 0.0001805 | 4.543E-05 | 0.0009092 | 0.001467 | 0.0009349 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;Other | 0.16% | 0.0020454 | 0.0039073 | 0.0008183 | 0.0008992 | 0.0009349 |
| k__Bacteria;p__NC10;c__12-24;o__JH-WHS47;f__;g__ | 0.16% | 0.0035493 | 0.002408 | 0.0006819 | 0.0010884 | 0.0015746 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__CFB-26;f__;g__ | 0.15% | 0.0052939 | 0.0034075 | 0.0003637 | 0.0009465 | 0.0005413 |

Genus Relative Abundance

| #OTU ID | pH.6.C.1 | pH.6.C.2 | pH.6.C.3 | pH.7.5.A.1 | pH.7.5.A.2 | pH.7.5.A.3 | pH.7.5.C.1 | pH.7.5.C.2 | pH.7.5.C.3 |
|---|---|---|---|---|---|---|---|---|---|
| k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae];g__Fusibacter | 0.0008 | 0.0011 | 0.0012 | 0.0038 | 0.0053 | 0.0078 | 0.0042 | 0.0059 | 0.0075 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__ | 0.0023 | 0.0024 | 0.0019 | 0.0011 | 0.0023 | 0.0015 | 0.0018 | 0.0027 | 0.0028 |
| k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__auto67_4W;g__ | 0.0033 | 0.0027 | 0.0036 | 0.0017 | 0.0023 | 0.0022 | 0.0025 | 0.0024 | 0.0036 |
| k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__;g__ | 0.0020 | 0.0017 | 0.0015 | 0.0022 | 0.0019 | 0.0013 | 0.0021 | 0.0021 | 0.0019 |
| k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__Treponema | 0.0024 | 0.0019 | 0.0044 | 0.0025 | 0.0015 | 0.0031 | 0.0018 | 0.0029 | 0.0028 |
| k__Bacteria;p__Spirochaetes;c__MVP-15;o__PL-11B10;f__;g__ | 0.0004 | 0.0002 | 0.0006 | 0.0092 | 0.0055 | 0.0125 | 0.0037 | 0.0007 | 0.0008 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae;g__Desulfobacca | 0.0020 | 0.0021 | 0.0042 | 0.0011 | 0.0020 | 0.0018 | 0.0033 | 0.0033 | 0.0031 |
| k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__Methanosarcinales;f__Methanosaetaceae;g__Methanosaeta | 0.0010 | 0.0002 | 0.0006 | 0.0050 | 0.0047 | 0.0094 | 0.0031 | 0.0037 | 0.0028 |
| k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__CCU21;f__;g__ | 0.0020 | 0.0032 | 0.0010 | 0.0014 | 0.0010 | 0.0018 | 0.0017 | 0.0029 | 0.0020 |
| k__Bacteria;p__Acidobacteria;c__;o__;f__;g__ | 0.0021 | 0.0018 | 0.0020 | 0.0015 | 0.0014 | 0.0019 | 0.0015 | 0.0012 | 0.0027 |
| k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__;g__ | 0.0014 | 0.0022 | 0.0015 | 0.0018 | 0.0027 | 0.0025 | 0.0034 | 0.0048 | 0.0035 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MIZ46;f__;g__ | 0.0040 | 0.0033 | 0.0040 | 0.0027 | 0.0025 | 0.0035 | 0.0028 | 0.0028 | 0.0013 |
| k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031;f__SHA-31;g__ | 0.0022 | 0.0024 | 0.0015 | 0.0018 | 0.0015 | 0.0011 | 0.0015 | 0.0012 | 0.0018 |
| k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__ | 0.0020 | 0.0009 | 0.0010 | 0.0027 | 0.0021 | 0.0023 | 0.0027 | 0.0036 | 0.0030 |
| k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__ | 0.0014 | 0.0019 | 0.0004 | 0.0026 | 0.0025 | 0.0025 | 0.0019 | 0.0035 | 0.0032 |
| k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__ | 0.0019 | 0.0017 | 0.0017 | 0.0016 | 0.0018 | 0.0026 | 0.0014 | 0.0021 | 0.0017 |
| k__Bacteria;p__WS3;c__PRR-12;o__Sediment-1;f__PRR-10;g__ | 0.0011 | 0.0020 | 0.0012 | 0.0016 | 0.0016 | 0.0023 | 0.0016 | 0.0027 | 0.0029 |
| k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__ | 0.0023 | 0.0031 | 0.0041 | 0.0033 | 0.0012 | 0.0016 | 0.0006 | 0.0016 | 0.0011 |
| k__Bacteria;p__GN04;c__;o__;f__;g__ | 0.0020 | 0.0018 | 0.0014 | 0.0012 | 0.0012 | 0.0011 | 0.0009 | 0.0017 | 0.0019 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae;g__Desulfobulbus | 0.0011 | 0.0015 | 0.0010 | 0.0041 | 0.0027 | 0.0037 | 0.0042 | 0.0034 | 0.0028 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;Other | 0.0009 | 0.0011 | 0.0005 | 0.0018 | 0.0014 | 0.0019 | 0.0047 | 0.0032 | 0.0063 |
| k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae;g__ | 0.0021 | 0.0016 | 0.0010 | 0.0021 | 0.0024 | 0.0019 | 0.0025 | 0.0033 | 0.0028 |
| k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Victivallales;f__Victivallaceae;g__ | 0.0022 | 0.0026 | 0.0047 | 0.0012 | 0.0009 | 0.0014 | 0.0005 | 0.0007 | 0.0006 |
| k__Bacteria;p__OD1;c__ZB2;o__;f__;g__ | 0.0031 | 0.0025 | 0.0041 | 0.0008 | 0.0001 | 0.0009 | 0.0031 | 0.0006 | 0.0008 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__;g__ | 0.0013 | 0.0013 | 0.0013 | 0.0015 | 0.0006 | 0.0017 | 0.0013 | 0.0005 | 0.0016 |
| k__Bacteria;p__Acidobacteria;c__S035;o__;f__;g__ | 0.0014 | 0.0010 | 0.0017 | 0.0012 | 0.0009 | 0.0009 | 0.0009 | 0.0013 | 0.0018 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Ellin6067;f__;g__ | 0.0018 | 0.0026 | 0.0006 | 0.0008 | 0.0015 | 0.0009 | 0.0007 | 0.0014 | 0.0013 |
| k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Thiobacterales;f__;g__ | 0.0011 | 0.0000 | 0.0002 | 0.0001 | 0.0007 | 0.0009 | 0.0055 | 0.0014 | 0.0127 |
| k__Bacteria;p__TM6;c__SBRH58;o__;f__;g__ | 0.0005 | 0.0008 | 0.0003 | 0.0004 | 0.0030 | 0.0011 | 0.0014 | 0.0015 | 0.0039 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas | 0.0007 | 0.0002 | 0.0004 | 0.0003 | 0.0002 | 0.0002 | 0.0093 | 0.0002 | 0.0004 |
| k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae;Other | 0.0023 | 0.0023 | 0.0030 | 0.0016 | 0.0015 | 0.0015 | 0.0020 | 0.0029 | 0.0023 |
| k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;Other | 0.0010 | 0.0007 | 0.0011 | 0.0015 | 0.0015 | 0.0016 | 0.0015 | 0.0016 | 0.0027 |
| k__Bacteria;p__NC10;c__12-24;o__JH-WHS47;f__;g__ | 0.0008 | 0.0014 | 0.0009 | 0.0015 | 0.0012 | 0.0019 | 0.0014 | 0.0012 | 0.0020 |

# Appendix F - Sorption Calculations

| Reactor | Days | pH | Goethite moles | >(s)FeOH moles | >(w)FeOH moles | tot Fe++ moles | Fe++ sorbed fraction | sorbed Fe++ mmoles | sorbed Fe++/goeth mmol/mol |
|---|---|---|---|---|---|---|---|---|---|
| 6A 1 | 56 | 6.32 | 0.004206 | 3.79E-06 | 1.51E-04 | 0.0022 | 0.02849 | 0.062678 | 14.9020447 |
| 6A 1 | 91 | 6.16 | 0.004704 | 4.23E-06 | 1.69E-04 | 6.92E-05 | 0.1295 | 0.0089614 | 1.905059524 |
| 6A 2 | 56 | 6.31 | 0.003605 | 3.24E-06 | 1.30E-04 | 0.00231 | 0.02322 | 0.0536382 | 14.87883495 |
| 6A 2 | 91 | 6.28 | 0.002803 | 2.52E-06 | 1.01E-04 | 0.00129 | 0.0275 | 0.035475 | 12.65608277 |
| 6A 3 | 56 | 6.33 | 0.004006 | 3.61E-06 | 1.44E-04 | 0.00205 | 0.02899 | 0.0594295 | 14.83512232 |
| 6A 3 | 91 | 6.24 | 0.002603 | 2.34E-06 | 9.37E-05 | 0.00164 | 0.02015 | 0.033046 | 12.69535152 |
| 6C 1 | 56 | 6.34 | 0.004406 | 3.97E-06 | 1.59E-04 | 0.00194 | 0.03433 | 0.0666002 | 15.11579664 |
| 6C 1 | 91 | 6.11 | 0.003805 | 3.42E-06 | 1.37E-04 | 0.00108 | 0.02847 | 0.0307476 | 8.080840999 |
| 6C 2 | 56 | 6.28 | 0.004406 | 3.97E-06 | 1.59E-04 | 0.002 | 0.03026 | 0.06052 | 13.7358148 |
| 6C 2 | 91 | 6.1 | 0.003505 | 3.15E-06 | 1.26E-04 | 0.0013 | 0.02269 | 0.029497 | 8.415691869 |
| 6C 3 | 56 | 6.45 | 0.004405 | 3.96E-06 | 1.59E-04 | 0.00197 | 0.03951 | 0.0778347 | 17.66962543 |
| 6C 3 | 91 | 6.05 | 0.003905 | 3.51E-06 | 1.41E-04 | 0.00109 | 0.02421 | 0.0263889 | 6.757720871 |
| 7.5A 1 | 56 | 7.53 | 0.009806 | 8.83E-06 | 0.000353 | 0.0001 | 0.701 | 0.0701 | 7.148684479 |
| 7.5A 1 | 91 | 6.85 | 0.009706 | 8.74E-06 | 0.000349 | 0.000116 | 0.6122 | 0.0710152 | 7.316628889 |
| 7.5A 2 | 56 | 7.55 | 0.009806 | 8.83E-06 | 0.000353 | 0.000103 | 0.6955 | 0.0716365 | 7.305374261 |
| 7.5A 2 | 91 | 7.12 | 0.009706 | 8.74E-06 | 0.000349 | 1.14E-04 | 0.6547 | 0.0746358 | 7.689655883 |
| 7.5A 3 | 56 | 7.53 | 0.009706 | 8.74E-06 | 0.000349 | 1.26E-04 | 0.6498 | 0.0818748 | 8.435483206 |
| 7.5A 3 | 91 | 7.11 | 0.009606 | 8.65E-06 | 0.000346 | 1.26E-04 | 0.6299 | 0.0793674 | 8.262273579 |
| 7.5C 1 | 56 | 7.56 | 0.009806 | 8.83E-06 | 0.000353 | 9.73E-05 | 0.7078 | 0.06886894 | 7.023142974 |
| 7.5C 1 | 91 | 7.31 | 0.009806 | 8.83E-06 | 0.000353 | 6.73E-05 | 0.7688 | 0.05174024 | 5.276385886 |
| 7.5C 2 | 56 | 7.54 | 0.009806 | 8.83E-06 | 0.000353 | 1.11E-04 | 0.6797 | 0.0754467 | 7.693932286 |
| 7.5C 2 | 91 | 7.23 | 0.009706 | 8.74E-06 | 0.000349 | 1.01E-04 | 0.6881 | 0.0694981 | 7.160323511 |
| 7.5C 3 | 56 | 7.54 | 0.009806 | 8.83E-06 | 0.000353 | 1.15E-04 | 0.672 | 0.07728 | 7.880889251 |
| 7.5C 3 | 91 | 7.1 | 0.009606 | 8.65E-06 | 0.000346 | 1.21E-04 | 0.6367 | 0.0770407 | 8.020060379 |