Seasonal Sulfur Biogeochemistry of Oil Sands Composite Tailings Undergoing Fen Reclamation

Seasonal Sulfur Biogeochemistry of Oil Sands Composite Tailings Undergoing Fen Reclamation

By

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Abstract

The Athabasca oil sands produce 20% of Canada's oil, which in turn creates trillions of cubic meters of waste. The Alberta government mandates that oil sands land be reclaimed to its natural state after mining has occurred. Syncrude Canada is currently creating a novel freshwater fen on top of a composite tailings (CT) deposit as a pilot large-scale reclamation project. CT are both microbially and sulfur rich, in addition, the fen could be a potential source of labile organics and sulfate reducing bacteria which could further stimulate sulfur cycling by microorganisms with the potential to stimulate $H_2S_{(g)}$ generation, a health and safety concern. Therefore, this thesis examines three main research questions regarding this H_2S production within the Sandhill reclamation fen: 1) Is H_2S generation widespread within the porewaters of the CT and sand cap of the developing Sandhill Fen reclamation project? 2) Do microbial metabolisms capable of metabolizing Fe and S linked to H_2S generation occur within CT and sand cap of the developing Sandhill Fen? and 3) Will seasonality and ongoing fen construction impact H_2S generation?

Field and experimental results herein discuss potential microbial and abiotic metabolisms and pathways that effect sulfur and iron cycling that could affect hydrogen sulfide generation within the composite tailings and developing fen during three seasonal sampling campaigns from June 2010 to July 2011. Results indicate that detectable $H_2S_{(aq)}$ occurred in the fen porewaters during each sampling campaign, with a trend of increasing $H_2S_{(aq)}$ concentrations as construction of the fen progressed. Further, enrichment results indicate that microbial sulfur and iron redox reactions are likely affecting the $H_2S_{(aq)}$

generation. Experimental microcosm results indicate that the CT may contain unstable sulfur species that can contribute to $H_2S_{(aq)}$ generation and sequestration in the CT as pyrite. Additionally, the evolution of the Sandhill Fen changed the microbial communities that were present *in situ* as well as shifted dominance of species type in environmental microbial enrichments. The putative function of these bacteria show a shift from autotrophy to increased heterotrophic metabolisms as the fen is being constructed, suggesting the addition of labile organic substrates from the peat and woody debris are both changing the dominant metabolisms and well as increasing microbial diversity to the underlying CT and sand cap of Sandhill Fen. Results of this thesis established widespread microbial Fe and S metabolisms within CT for the first time and indicated that fen reclamation will alter microbial activity with implications for S cycling within CT. Although this thesis covers a short sampling time frame, it is clear that $H_2S_{(aq)}$ generation is an important factor to consider during large scale CT reclamation. While microorganisms are present and could be impacting Fe and S cycling, the CT materials should be investigated further in regards to their potential for $H_2S_{(aq)}$ generation. More consideration should be given to inhibiting $H_2S_{(aq)}$ generation or supporting FeS formation within the reclamation fen.

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1.0 – Introduction

1.1 – Sulfur Biogeochemistry

Sulfur (S) redox has been proposed to be the earliest of the metabolisms and microbes coupling energy conservation to the oxidation and/or reduction of sulfur species are widespread across the tree of life (Canfield and Raiswell, 1999). Sulfur exists in multiple oxidation states, ranging from (-II to +VI), and thus S cycling of both oxidized and reductive species can occur (Sturman et al., 2008). Sulfur oxidation and reduction as well as sulfur fluxes between solid and solution phases in freshwater, marine, and mine systems is controlled both by abiotic and biotic processes and pathways (Jorgensen, 1990; Holmer and Storkholm, 2001; Drushel et al., 2003a; Drushel et al., 2003b). The behaviour of S in any system is dictated in part by its aqueous speciation, pH of the environment, and the reductive formation and oxidative dissolution of sulfur minerals (Schippers et al., 1996; Druschel et al., 2003a, Druschel et al., 2003b, Borda et al., 2003).

The S cycle is linked to other globally important elemental cycles including carbon, iron and nitrogen (Muyzer and Stams, 2008). Its interaction with these elements plays essential roles in the speciation of sulfur and its role in microbial metabolisms, especially in mining environments. Metal mine waste is commonly high in reduced sulfur and iron compounds (e.g. pyrite (FeS_2), the most abundant mineral on earth's surface and pyrrhotite ($Fe_{1-x}S$), the most abundant iron sulfide in the earth and solar

system (Rickard and Luther, 2007)). These compounds are rendered reactive under earth surface conditions and are typically microbially accessible. Given the importance of sulfur minerals in mining and acid mine drainage (AMD) processes, the study of sulfur redox cycling, especially sulfur oxidation, and the microbial communities controlling these reactions have largely been restricted to acidic (pH < 4) environments. Less work has been done on more circumneutral pH mines, including the Alberta oil sands bitumen mines. Understanding the sulfur cycling in these less studied systems is important to the petroleum industry, with specific consideration to reports of hydrogen sulfide gas ($H_2S_{(g)}$) release, a frequent product in ground water systems, during reclamation processes and future waste management practices.

1.2 – Sulfur Redox Pathways in Acid Mine Drainage

Acid mine drainage (AMD) is described as highly acidic (pH < 3), metal-rich waste waters and is of great environmental concern to the metal mining industry (Akcil and Koldas, 2006; Baker and Banfield, 2003). It is well established that sulfur- and iron-oxidizing bacteria increase the rates of iron (Fe) and sulfur (S) mineral oxidation and subsequent proton release creating AMD. Figure 1.1 represents a schematic of AMD focusing on Fe, S, and microbial interactions. The overall reaction for AMD can be described in the following equations (Akcil and Koldas, 2006; Eby, 2004; Baker and Banfield, 2003):

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad (1)$$

$$14Fe^{2+} + 3.5O_2 + 14H^+ \rightarrow 14Fe^{3+} + 7H_2O \qquad (2)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (3)$$

Pyrite is oxidized by dissolved oxygen, generating sulfate, ferrous iron, and hydrogen ions (Equation 1). Further, it has been established that under acidic conditions ferric iron can oxidize pyrite-sulfur 3 to 100 times faster than does oxygen and is important at pH values < 4 since ferric iron has low solubility at higher pH values (Edwards et al., 1999). This is due to the fact that the energy of the lowest unoccupied molecular orbital is lower for ferric iron than it is for oxygen, decreasing the activation energy barrier of the reaction (Luther, 2002). Therefore, this process begins with the oxidation of ferrous iron by oxygen, followed by further oxidation of pyrite-sulfur by ferric iron (Equations 2 and 3) (Baker and Banfield, 2003).

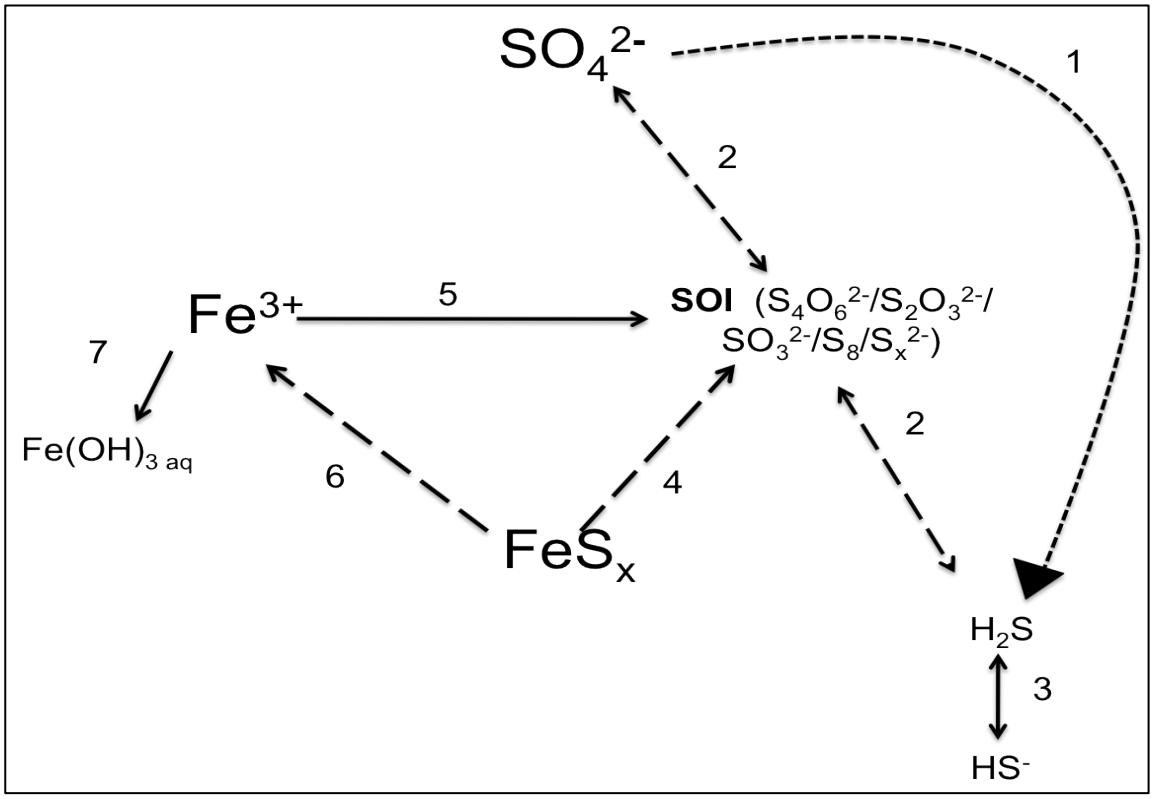


Figure 1.1: Schematic of an AMD environment. Solid lines indicate abiotic process, small hashed lines indicated a process only feasible microbially, and larger hashed lines represent a process that has been documented be both abiotic and microbially accomplished. **1** – Microbial reduction of sulfate to H_2S. **2** – Oxidation or disproportionation of sulfur oxidation intermediates (SOI) by oxygen or sulfur oxidizing or disproportionating bacteria. **3** – Speciation of $H_2S_{(aq)}$ depends on pH. At pH values < 7 $H_2S_{(aq)}$ will be in solution, above pH 7 the dominate species will be $HS^-_{(aq)}$. **4** – Microbial and abiotic oxidative dissolution of sulfur from reduced sulfur and iron compounds. Oxidation of sulfur releases protons into solution. **5** – Abiotic oxidation of SOI by Fe^{3+}. **6** – Microbial and abiotic oxidation of Fe^{2+} from reduced sulfur and iron compounds. Abiotically, in AMD situations, oxygen will oxidize the Fe first then the Fe^{3+} will continue to oxidize the reduced Fe at a quicker rate than the oxygen. **7** – at pH values above 3.5 iron oxyhydroxides will form, precipitating out of solution. These precipitates can sorb heavy metals including S, Ni, Cu, Pb, etc.

This re-oxidation of Fe^{2+} is slow under acidic conditions, but can be catalyzed by microorganisms or oxygen increasing the rate of pyrite dissolution and acid generation (Baker and Banfield, 2003; Druschel et al., 2004). *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) has been widely used as a model organism for the

study of biologic oxidation of pyrite as it can oxidize both iron and sulfur (Kelly and Wood, 2000; Pronk et al., 1990) but it is clear there is a large diversity in the microbial species that are present in AMD environments (Baker and Banfield, 2003; Bernier and Warren, 2005).

Sulfide oxidation to sulfate can abiotically occur quickly in one single step; however it can also occur via a succession of sulfur oxidation intermediates (SOI), in which protons are produced for each reaction. Since SOI speciation is often insensitive to pH, the abiotic processes that oxidize SOI are often controlled by the concentrations of Fe^{3+} in the system (Druschel et al., 2003b). These pathways are displayed in Figure 1.2. Microbes can utilize these intermediate sulfur compounds and can significantly affect rates of acidification and reduced mineral dissolution (Baker and Banfield, 2003) for example, Equations 4 and 5 (Bernier, 2007).

$$S_2O_3^{2-} + 0.5O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ \quad (4)$$

$$S_4O_6^{2-} + 3.5O_2 + 3H_2O \rightarrow 4SO_4^{2-} + 6H^+ \quad (5)$$

Ferric iron can catalyze the oxidation of pyrite-sulfur, as mentioned above, but it can also oxidize SOIs, such as polythionates to sulfate and $S_2O_3^{2-}$ (thiosulfate) to $S_4O_6^{2-}$ (tetrathionate) faster than oxygen (Druschel et al., 2003b). Polysulfides are the most common sulfur oxidation intermediates resulting from sulfide mineral dissolution (Borda et al., 2003; McGuire et al., 2001); they subsequently can be further oxidized into SOI with higher oxidation states, ultimately ending as sulfate.

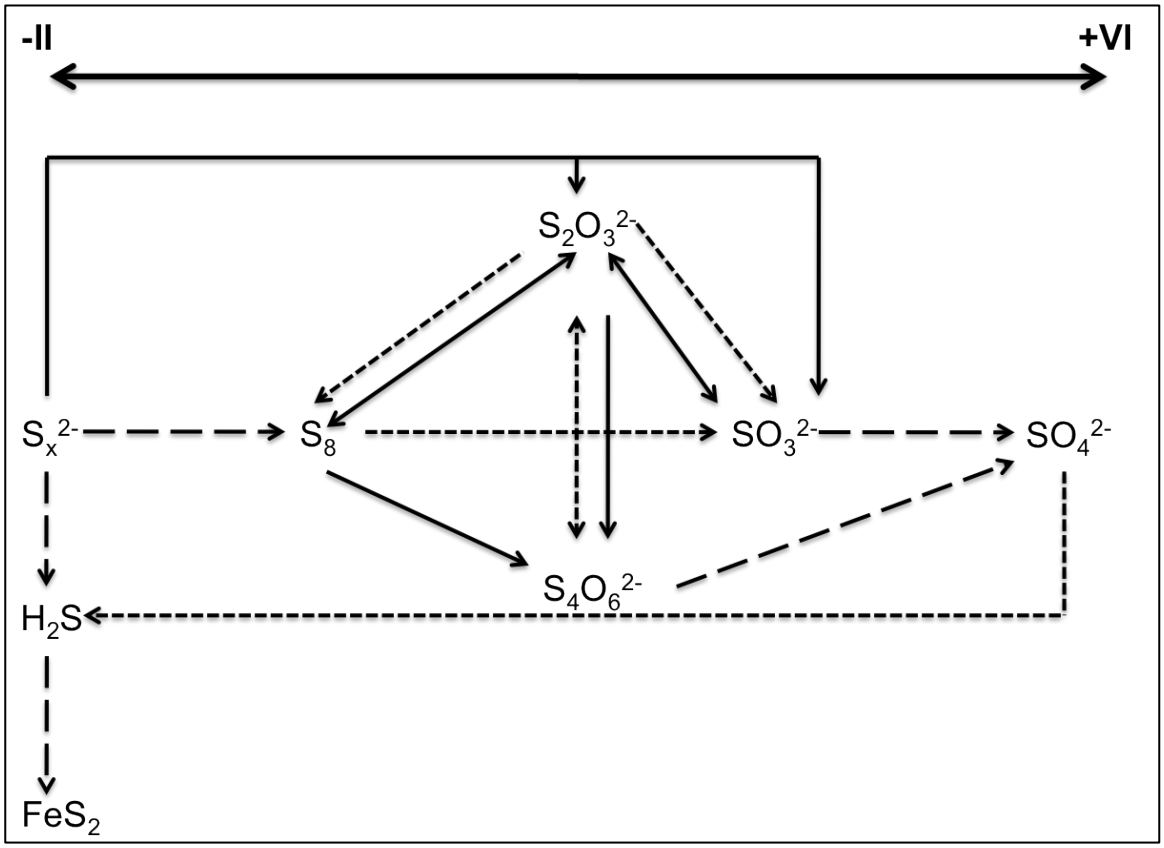


Figure 1.2: Sulfur pathways in aquatic systems, showing sulfur oxidation intermediates. Thick solid line represents the direction of the oxidation state of sulfur from –II to +VI. Thin solid line represents reactions only abiotically produced. Small hashed lines represent reactions only possible through microbial catalysis. Larger hashed lines represent reactions that can occur both abiotically and microbially. These reactions utilize oxygen and ferric iron as election acceptors and organic carbon as electron donors. Modified from Zopfi et al. (2004) and Bernier (2007).

Sulfur intermediates produced during the oxidation of sulfides from pyrite can also undergo sulfur disproportionation which was discovered by Bak and Cypionka (1987). Disproportionation is considered a type of inorganic fermentation process in which organisms obtain energy for growth, but is not considered a respiratory redox process (Jorgensen, 1990). Disproportionation refers to the splitting of sulfur compounds into one that is more oxidized and one more reduced than the reactant sulfur compound for example, Equation 6 from Zopfi et al. (2004).

$$4S_4O_6^{2-} + 4H_2O \rightarrow 6S_2O_3^{2-} + S_3O_6 + SO_4^{2-} + 8H^+ \quad (6)$$

Sulfur disproportionation is of important environmental relevance due to the recycling of SOI and the potential to produce protons, decreasing the pH of the system and creating mining water quality issues (Norlund et al., 2009). However, Norlund et al. (2009) found that microbial consortia and sulfur disproportionating bacteria can produce less acid than predicted based on abiotic models. The forward and back reactions continuously feed the sulfur cycle in aqueous systems, making it hard to pin point specific interactions and pathways.

1.2.1 – Microbiology of Sulfur Oxidation

The important catalytic role bacteria play in sulfur oxidation, especially in metal mining environments, has long been recognized (e.g. Colmer and Hinkle, 1947) but the actual oxidative pathways these bacteria utilize only have more recently become the focus of research and these pathways are regulated differently depending on the bacterial species (Kelly et al., 1997). Currently, there are two known fundamental pathways for the oxidation of reduced S species (i.e. sulfide, sulfur and thiosulfate) by bacteria, (1) the sulfur oxidation pathway and (2) the tetrathionate intermediate pathway (S_4I) (Kelly et al., 1997). The main difference between these pathways is that the sulfur oxidation pathway involves polythionates, while the S_4I pathway does not (Kelly et al., 1997).

The sulfur oxidation pathway, governed by the conservative *sox* operon, is found in photo- and chemolithotrophic *Alphaproteobacteria* and has evolved through several organisms beginning with *Starkeya novella* (Oh and Suzuki, 1977). Oh and Suzuki

(1977) thought the sulfur oxidation pathway was located in the cell membrane. It was later established by Kelly et al. (1997) in the organism *Paracaccus verstus* that the pathway was located in the cell's periplasm (Friedrich et al., 2001). The pathway described in Kelly et al. (1997) is termed the thiosulfate oxidase multienzyme complex (TOMES). The TOMES complex is very similar in structure and protein complex to the Sox complex described by Friedrich et al. (2001) (Friedrich et al., 2001; Kelly et al., 1997; Muller et al., 2004). The Sox complex has been studied in *Paracoccus pantotophus,* a gram negative, neutrophilic, facultative lithoautotroph and is comprised of a gene cluster of 15 genes in two transcriptional units (Friedrich et al., 2001). These genes include *sox*XYZABCD (code for the oxidation of sulfur within the cell), *sox*E (predicts a diheme c-type cytochrome) and four open reading frames (*orf*1, *orf*2, *shx*V, *shx*W) that code for the actual proteins (Friedrich et al., 2001). It should be noted that the Sox and TOMES complexes have only been shown to be present in microbes living in neutral or near neutral pHs (Kelly et al., 1997). Microbial species utilizing these pathways could be present in many environments within these pH ranges, including the non-acidic mine tailings of the Alberta oil sands.

The S_4I pathway, which describes the formation of tetrathionate intermediates via the oxidation of thiosulfate, is seen mainly in *Beta-* and *Gamma-proteobacteria,* specifically in obligate chemolithotrophic species including *Acidthiobacillus, Thermithiobacillus* and *Halothiobacillus* (Ghosh and Dam, 2009; Kelly et al., 1997; Pronk et al., 1990). Most commonly, the thiosulfate is oxidized to tetrathionate in the cells periplasm via enzymatic action of thiosulfate dehydrogenases, but no gene has been identified to code for this enzyme (Ghosh and Dam, 2009). Though, Muller et al. (2004)

found a novel thiosulfate:quione oxidoreductase enzyme in the membrane of a thermoacidophilic archeon *Acidivanus ambivalens,* that can also oxidize thiosulfate to tetrathionate.

New findings by Canfield et al. (2010), have found sulfur metabolism genes, including the s*ox* gene complex, in oxygen-minimal zone waters off the Chilean coast. Their results couple sulfur and nitrogen cycles, and although there is no *in situ* chemical expression of sulfur species here, the authors imply the sulfur cycling is occurring cryptically (Canfield et al., 2010). This work shows that the sulfur cycle is enormously widespread and is linked with many other elemental cycles, and more work needs to be done to understand these processes.

1.3 – Hydrogen Sulfide

Hydrogen sulfide ($H_2S_{(aq)}$) is most commonly the reported product of sulfate reduction coupled to the oxidation of organic matter by sulfate reducing bacteria (e.g Equation 7) (Holmer and Storkholm, 2001), but can also be a product of sulfur disproportionation metabolism (Equation 8) (Bottcher et al., 2001).

$$CH_3COO^- + H^+ + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (7)$$

$$4H_2O + 4S^0 \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \quad (8)$$

Hydrogen sulfide gas ($H_2S_{(g)}$) is an environmental issue due to its corrosive properties, odor, toxicity and its high chemical demand for oxygen (Grigoryan and Voordouw, 2008; Wang and Chapman, 1999). The solubility of $H_2S_{(g)}$ in fresh and saline waters is influenced by temperature and salinity. Douabul and Riley (1979) found that increasing

temperature and salinity of the water decreases the solubility of H_2S. The dissociation of $H_2S_{(aq)}$ is dependant on the pH of the system.

According to Henry's Law, the concentration of $H_2S_{(g)}$ in solution can be directly related to partial pressure in Equation 9 (Eby, 2004).

$$C_{H2S} = K_H P_{H2S} \quad (9)$$

Where C is the concentration of $H_2S_{(g)}$, K_H is the Henry's Law Constant (a function of temperature, mol L^{-1} bar^{-1}) and P is the partial pressure of $H_2S_{(g)}$ in the phase that the solution is in equilibrium with (in this case the atmosphere). According to the ideal gas law (Equation 10, where P is pressure, V is the volume of gas, R is the gas constant (0.08206 L amt K^{1-} mol^{-1}), n is the number of moles of gas, and T is the temperature in kelvins) dewatering activities in the composite tailings deposits, like the dewatering beneath the Sandhill Fen, can release $H_2S_{(g)}$ due to decreases in volume (since water is removed from the well), resulting in a decrease in partial pressure. These changes in volume and partial pressure will decrease the amount of gas that is able to be held in solution resulting in a release of $H_2S_{(g)}$. In confined anaerobic spaces, $H_2S_{(g)}$ can build up as it comes out of solution and then once exposed to oxygen will explode. This change of speciation from aqueous H_2S to $H_2S_{(g)}$ can be of environmental concern during reclamation of oil sand tailing activities when tailings are dewatered as $H_2S_{(g)}$ can build up in dewatering wells.

$$PV=nRT \quad (10)$$

Aqueous hydrogen sulfide ($H_2S_{(aq)}$) is a weak Lewis acid, only partially dissociating, and since it contains more than one proton, it has two dissociation constants (Eby, 2004). With a pKa_1 of 6.98 ± 0.03 at 25°C, H_2S dominates speciation when present

in acidic aqueous solutions, and HS^-, its dissociated form, dominates at alkaline pH values (Rickard and Luther, 2007). For example, in water with a pH value of 8, about 9% of the sulfide will be in the form H_2S, while the concentration of H_2S at pH 6 is approximately 91% (Wang and Chapman, 1999). Equation 11 shows the first dissociation step of hydrogen sulfide (Wang and Chapman, 1999). Equation 12 shows the final step in the full dissociation of H_2S (Morse et al., 1987). The pKa_2 is less constrained due to problems with polysulfide contamination during measurement, but is estimated to be 13.78 ± 0.74 (Morse et al., 1987) and greater than 18 (Rickard and Luther, 2007).

$$H_2S \rightarrow H^+ + HS^- \qquad pK_{a1} = 6.98 \pm 0.03 \qquad (11)$$

$$HS^- \rightarrow H^+ + S^{2-} \qquad pK_{a2} = 13.78 \pm 0.74 \qquad (12)$$

1.4 – Sulfate Reduction

Sulfate reduction is thought to be one of the oldest microbial metabolisms and is quantitatively the most important process in the oxidation of organic matter in anoxic sediments in lacustrine and marine systems (Finster, 2008; Sturman et al., 2008; Konhauser, 2007). Sulfate reducing bacteria (SRB) are able to gain energy through the coupling of sulfate reduction to the oxidation of H_2 and organic matter, including acetate, lactate, propanoate, and produce oxidized carbon species and sulfide (Sturman et al., 2008; Holmer and Storkholm, 2001). Sulfate reduction is estimated to account for over 50% of the organic carbon mineralized in marine sediments, which highlights the

importance of SRB and why they have been so widely studied (Muyzer and Stams, 2008).

Factors that control microbial sulfate reduction in aqueous systems include oxygen concentration, sulfate concentration, temperature and organic matter availability (Holmer and Storkholm, 2001). In environments high in organic carbon and sulfate, SRB will account for a large portion of microbial activity in reducing environments (Sturman et al., 2008). SRB are mostly restricted to the *Proteobacteria* clade, comprising over 20 distinct genera (Sturman et al., 2008). Not only are there numerous species of SRB but the environments where they are able to live are also wide ranging including: marine and freshwater systems, hydrothermal vents, AMD sites, methane zones and both high and low sulfate concentration environments (Leloup et al., 2009). Sulfate reduction is the most significant source of sulfides in sediments with temperatures less than 100 °C, and is only abiotically catalyzed by organic matter at temperatures greater than 150 °C (Li et al., 2006).

Historically, it was thought that SRBs played only a minor part in the global carbon cycle; however, this idea is changing as evidence, especially in marine environments, indicate SRBs play a dominant role in the anaerobic cycling of carbon (Muyzer and Stams, 2008). SRB have been reported to be able to use Fe^{3+} as an electron acceptor instead of sulfate, for example, when sulfate becomes limiting (Lovely et al., 2004; Li et al., 2006). The ability to metabolize ferric iron will also have impacts on iron cycling, but the extent to which SRBs are involved in Fe reduction is still unclear (Li et al., 2006). Sulfate reducers have also been reported to tolerate various oxygenated

environments, contrary to the historical view that they are strictly anaerobic (Finster and Kjelden, 2010; Morgensen et al., 2005).

Recently, greater SRB diversity has been shown due to the development of next-generation molecular tools and techniques (Leloup et al., 2009). For example, 16S rRNA sequencing, functional gene probes and fluorescent imaging techniques are now able to identify and classify SRB community members (Holmer and Storkholm, 2001). FISH probes that were once only used for identification are now being used to study larger questions about community structure, distribution and activity of SRB (Holmer and Storkholm, 2001). 16s rRNA based sequence analysis has identified seven phylogenic lineages that SRB can be grouped into, five of which are within the Bacteria domain, two in Archaea (Muyzer and Stams, 2008). Most of the known SRBs are in the *Delta-proteobacteria* group and some gram-positive species within the Clostridia (Muyzer and Stams, 2008). The detection of SRB can also be accomplished significantly using functional genes that code for the sulfate reduction pathways, like such genes as *dsrAB* (Wagner et al., 1998) or *aprBA* (Meyer and Kuever, 2007), that code for the dissimilatory sulphite reductase and the dissimilatory adenosine-5'-phophosulfate reductase respectively (Muyzer and Stams, 2008).

It was once thought that SRB were restricted to circum-neutral pH environments between 6 and 8 (Hao et al., 1996) but it is now well established that sulfate reduction can occur under lower pH conditions (pH < 5) (Koschorreck, 2008, Meier et al., 2012). Meier et al. (2012) showed sulfate reduction occurring soon after inoculation of enrichments with initial pH values of 5 and 6, and higher in enrichments of an initial pH 3 and 4 after a lag phase. Due to the consumption of protons, SRB could have a

competitive advantage at lower pHs compared to non-proton consuming processes (Koschorreck, 2008). The ability for microbial sulfate reduction to occur in low pH systems could have positive environmental implications with AMD, since sulfate reduction is a proton consuming process and could potentially neutralize these acidic environments as a type of bioremediation (Kakonsen and Puhakka, 2007). The ability to precipitate toxic heavy metals in AMD environments is also a useful secondary consequence of sulfate reduction as reductive precipitation of metal sulfides can be a good remediation technique (e.g. Dvorac et al., 1992; Kakonsen and Puhakka, 2007, Behum et al., 2011). The discovery of SRB in these low pH environments may be explained by microniches, small microbial community sites that differ from the bulk community (Koschorreck, 2008). Microniches have also been found to allow sulfate reduction and methanogenesis in aerobic sediments and wastewater biofilms (Jorgensen, 1977; Okabe et al., 1999;) and in acidic environments (Fortin et al., 1996; Koschorreck, 2008; Sanz et al., 2011).

Thermodynamically, sulfate reduction and methanogenesis are considered to be mutually exclusive reactions as microbial species responsible for sulfate reduction are thought to out-compete the methanogens for organic carbon (Mitterer, 2010). More recent work has shown that SRB and microbes associated with the oxidation and reduction of methane can coincide. Mitterer (2010) has shown that these two processes can occur simultaneously without the presence of a sulfate-methane transition zone. The author's explanation for this discovery is the non-competitive substrate use by the two communities (Mitterer, 2010). Methyl sulfides and methylamines are suggested to be these substrates (Mitterer, 2010). Similarly, Boetius et al. (2000) discovered a marine

microbial consortium at the Cascaida margin off the coast of Oregon. This consortium consists of a structure of Archaea and SRB, which symbiotically reverses the process of methanogenesis, and where sulfate is used as the terminal electron acceptor and methane is oxidized (Boetius et al., 2000). These new findings reveal that sulfate reduction can be found in even more environments than previously thought, and more work should be done to understand these interactions and dynamic communities.

1.5 – The Athabasca Oil Sands – Sulfur Oxidation meets Sulfate Reduction?

One of the largest examples of mining with circumneutral pH mine waste is the Athabasca oil sands. Further, the oil sands mine waste, while high in S containing compounds, also contains a variety of organics and Fe rich minerals. Thus there exists a substantial opportunity for microbial oxidative/disproportionating and reductive S pathways within oil sands waste. However, as yet little S based studies have been conducted in oil sands waste, especially those assessing potential microbial links to S cycling.

The Athabasca oil sand deposits cover over 100,000 km^2 of Northern Alberta (Golby et al., 2012), producing 20% of Canada's oil and an estimated 1.7 - 2.5 trillion barrels of bitumen (Fedorak et al., 2002; Holowenko et al., 2000; Penner and Foght, 2010). In turn, huge amounts of bitumen extraction waste is deposited into tailing deposits, like the Mildred Lake Settling Basin at Syncrude, which contains over 400 x 10^6 m^3 of fine tailings (Fedorak et al., 2002). The zero-discharge policy for all oil sand operators results in large amounts of waste and processed-water held on site, mostly in

these large tailings ponds (Holowenko et al., 2000), highlighting the major issue of tailings densification, which could take between 125-150 years (Eckert et al., 1996). Mature fine tailings ponds (MFT, now referred to as fluid fine tailings (FFT) by the oil sands industry) are a mixture of fine clay particles, processed oil sand, processed water, residual bitumen and organics, and naphthenic acids (Fedorak et al., 2002; Holowenko et al., 2000; Salloum et al., 2002). Figure 1.3 shows a schematic of the water-based bitumen extraction process beginning with oil sand and ending with composite tailings deposits. One kilogram of gypsum is typically added for each m^3 of MTF to help in densification (Chalaturnyk et al., 2002), water recycling and trafficability (Ramos-Padron et al., 2011). After the addition of gypsum ($CaSO_4 \cdot 2H_2O$) this mixture is referred to as composite tailings (CT), which releases water quickly and can reach up to 70% solids in a few hours (Salloum et al., 2002). CT deposits are dewatered to help work toward a dry landscape that will allow vegetation growth and reforestation (Fedorak et al., 2002; Salloum et al., 2002). The CT process is designed to hasten the settling, and prevent the segregation of materials in the tailings, but results in high concentrations of sulfate in pore waters. The gypsum addition provides the large source of oxidized sulfur (SO_4^{-2}) to the ponds, potentially stimulating sulfate-reducing bacteria (Holowenko et al., 2000).

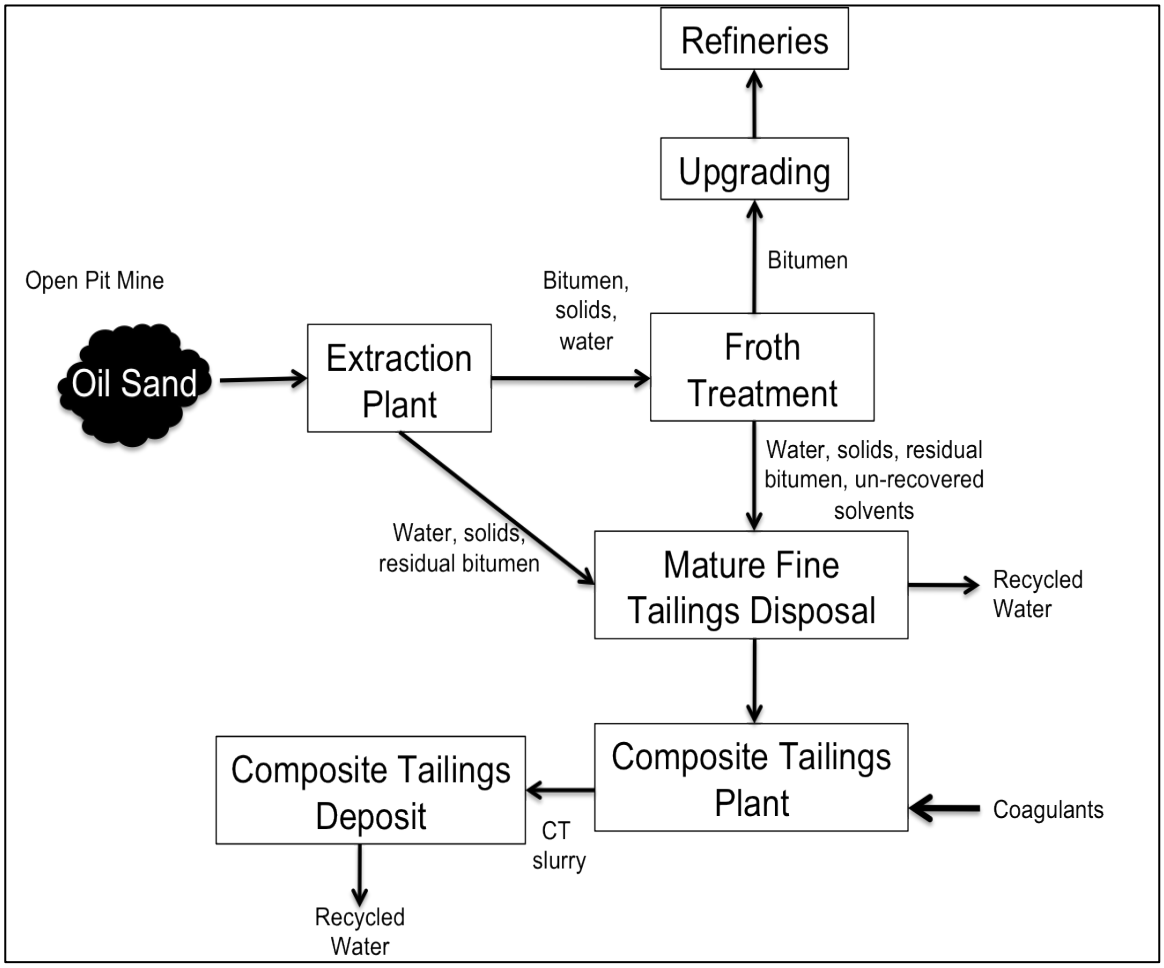


Figure 1.3: Water-based bitumen extraction from oil sand, resulting in composite tailings deposits. Modified from Masliyah et al. (2004) and Matthews et al. (2002).

Given that bacterial communities, specifically S-metabolizing microbes, have found to be diverse in acidic metal mine waste systems, as well as within non-mining systems of variable organic carbon, pH and O_2 conditions, it is highly likely that sulfur cycling occurs in mine systems with circumneutral pH values and could be associated with a diversity of both S pathways and microbes associated with those pathways, see Figure 1.4 for examples. Knowing if and how microbial communities in oil sand waste metabolize and cycle sulfur is crucial to the petroleum industry in Canada and world wide, particularly with regards to H_2S gas production.

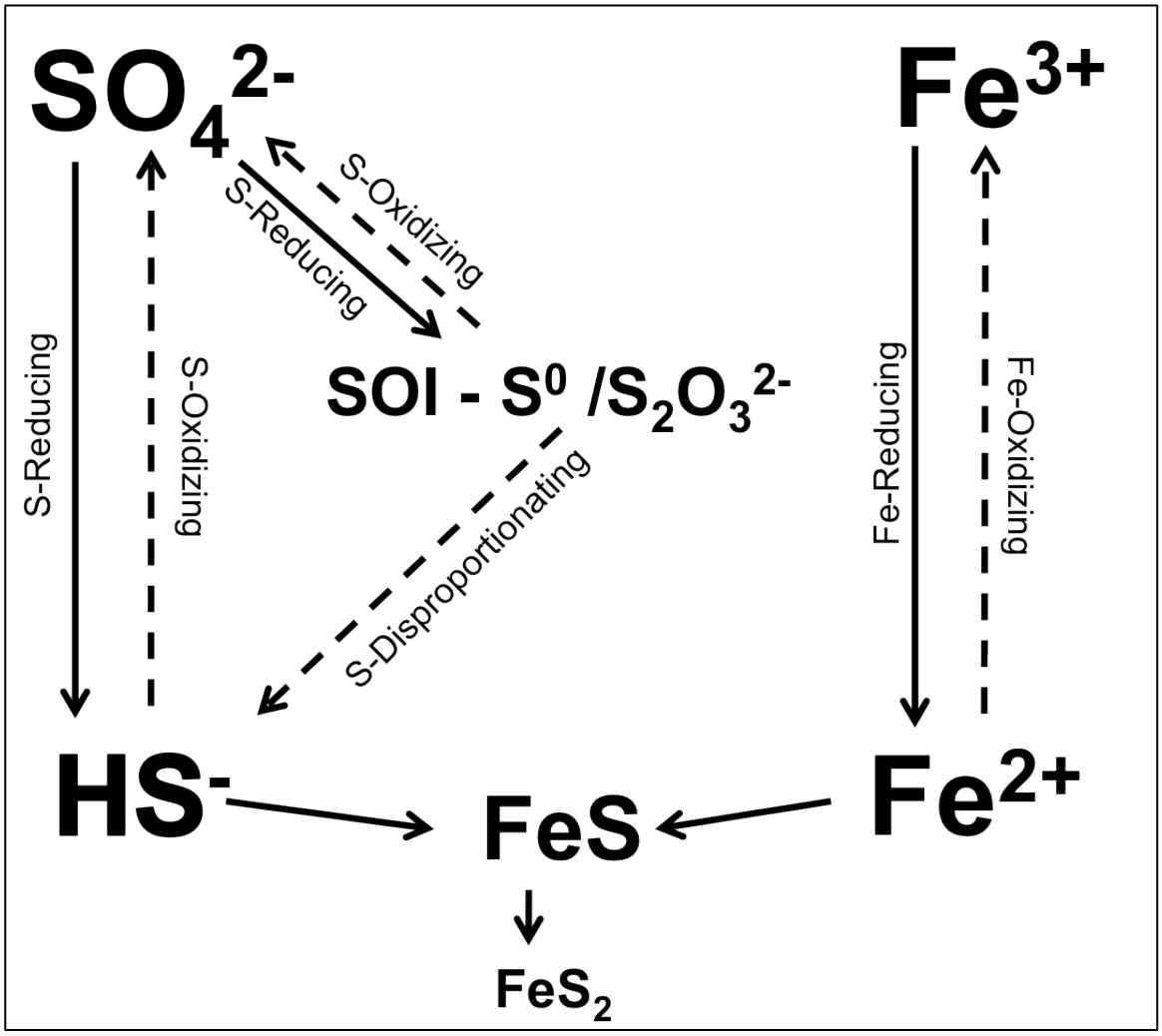


Figure 1.4: Potential microbial metabolisms that could be present in Athabasca oil sand composite tailings deposits. Sulfur-oxidizing and reducing and iron-oxidizing and reducing species could be present. There is potential for reduced species to precipitate as iron sulfides, or to remain in solution as aqueous sulfide and ferrous iron. Sulfur disproportionation could be cycling sulfur species.

1.5.1 – Microbial Presence in Alberta Oil Sand Tailings

Microbial activity of tailings in the Athabasca oil sands has been investigated since the late 1980s. These studies have been focused on and shown the occurrence and species diversity of microbial populations (Golby et al., 2012; Herman et al., 1994; Holowenko et al., 2000; Penner and Foght, 2010; Ramos-Padron et al., 2011; Salloum et al., 2002). Further, these studies have assessed methane gas production since this has been a key environmental issue, particularly in the Mildred Lake Settling Basin, and has been investigated for possible flocculation capabilities to assist in FFT densification (Bordenave et al., 2010; Fedorak et al., 2003; Fedorak et al., 2002). However, to date, CT deposit associated microbial studies have been fairly limited and none prior has coupled microbial characterization to sulfur geochemistry.

2.0 – Research Scope

Syncrude Canada Inc is currently completing production of a novel freshwater fen (the Sandhill Fen) reclamation project, in accordance to Alberta governmental regulations. The goal of this project is to reclaim composite tailings deposits to their natural landscape, prior to mining activities. To construct the fen, composite tailings are capped with a layer of processed oil sand, which separates the saline composite tailings from the freshwater fen being created above. The salinity of the composite tailings deposit water will keep the freshwater from the fen separated since fresh water is less dense and will float on top. As the CT consolidates, the salty water that seeps out of the deposit must be pumped out in order for the goal of dry landscape reclamation to be reached (compared to water capped tailings), as well as to recycle the water back into the bitumen extraction process. During construction of the Sandhill Fen, there was an episode of unanticipated H_2S gas release from one of the dewatering wells. This release was not predicted abiotically, which leads to the notion that the sulfide is likely microbially produced and may present itself to be a larger issue in future reclamation endeavors. Since the fen is being constructed with processed oil sand and is based on top of a composite tailings deposit, both of which are high in sulfur and iron compounds, it is likely that there is microbial cycling of S within the CT deposit, sand cap and overlying fen. It is important to Syncrude and other oil sand operators that the underlying sulfur dynamics in their waste products and reclamation initiatives are investigated and understood in order to safely and effectively reclaim lands disrupted by the strip mining of the Athabasca oil sands.

Therefore, the primary research questions and corresponding objectives and hypotheses of this field and experimental thesis are:

1. **Is H_2S generation widespread within the porewaters of the CT and sand cap of the developing Sandhill Fen reclamation project?**

Objectives:

i. Determine aqueous Fe^{2+}/Fe^{3+} and $SO_4^{2-}/\sum H_2S$ concentrations in the Sandhill Fen sand cap and CT porewaters over seasonal time scales.

Hypothesis:

a. Detectable hydrogen sulfide occurs within the CT and sand cap porewaters over spatial and temporal time scales.

ii. Characterize and assess the bioavailability of the sulfur and iron pools within the sand cap of Syncrude's novel freshwater fen reclamation project through operationally defined sequential extraction and experimental assessment of microbial Fe and S metabolism of CT.

Hypothesis:

a. CT components of gypsum ($CaSO_4$) and Fe rich clays provide bioaccessible pools of Fe and S. These bioaccessible pools of Fe and S will be metabolized by endemic microbes enriched within the sand cap as well as from sand cap and CT porewaters.

2. **Do microbial metabolisms capable of metabolizing Fe and S linked to H_2S generation occur within the CT and sand cap of the developing Sandhill Fen?**

Objectives:

i. Specific Fe and S metabolisms will be enriched from the Sandhill Fen porewaters and sand cap sediments on a seasonal basis to assess potential microbial metabolic processes occurring within the fen. Genetic characterizations will assess species diversity in the enrichments as fen construction progresses.

Hypotheses:

a. *There will be an increase the positive growth observed for all sediment and porewater Fe and S microbial enrichments from the Sandhill Fen corresponding with the progression of fen construction.*

b. *16S rRNA sequence analysis from Fe and S microbial enrichments of the Sump Vault will show change in species diversity on seasonal time scales, demonstrating increasing diversity with continuing fen construction.*

ii. Relate the metabolisms discovered by successful growth of microbial enrichments to potential sulfide gas generation or sequestration in CT and/or the sand cap in the form of FeS solids by means of fen replication microcosm experiments.

Hypotheses:

a. *The sterile experimental fen microcosm will show no aqueous sulfide formation and no formation of FeS minerals.*

b. *The endemic experimental fen microcosm will show sequestration of FeS minerals since SO_4^{2-} and Fe^{3+} introduced from the CT could support the metabolisms that reduce both of these terminal electron acceptors.*

c. *The sulfate-reducing bacterial treatment microcosm will show aqueous sulfide production.*

3. Will seasonality and ongoing fen construction impact H_2S generation?

Objectives:

i. Assess changes in sand cap and CT porewater Fe^{2+}/Fe^{3+} and $SO_4^{2-}/\sum H_2S$ concentrations, microbial functional enrichments and genetic diversity of enrichments over seasonal and fen development timescales.

Hypothesis:

a. *The continuing evolution and construction of the Sandhill Fen will affect H_2S generation within the porewaters.*

Overall, the information gained from the completion of this thesis will enhance the knowledge of microbial activity in oil sand composite tailings and subsequent reclamation, specifically in a constructed freshwater fen scenario, guiding future oil sand reclamation management strategies.

3.0 – Methodology

3.1 – Field Sampling

3.1.1 – Site Location and Description

Three field sampling campaigns were conducted between June 2010 and July 2011 (June 23-24th, 2010; August 31st-September 1st, 2010; July 24-25th, 2011) (Table 3.1). All sampling was conducted on the Sandhill Fen reclamation site at the Mildred Lake Settling Basin, Syncrude Canada Ltd., north of Fort McMurray, Alberta, Canada (Figure 3.1 – site location, Figure 3.2 – sampling site locations).

Table 3.1. Field Sampling Campaigns

Sampling Date	Well Water Samples	Surficial Sediment Samples
June 23rd-24th, 2010	Well 8C Well 5D Sump Vault	Upper Mid Lower
August 31st-Sept 2nd, 2010	Well 8C Well 5D Well 6A Sump Vault	Upper Mid Lower Lower *
July 24-25th, 2011	Well 8C Well 5D Well 6A Sump Vault	Upper Mid Lower **

The Sandhill Fen reclamation site is a novel reclamation project developed by Syncrude in which a freshwater fen is being constructed over top of a composite tailings deposit. Between the freshwater fen and the dewatered composite tailings is a 10m thick sand cap layer, which acts as an important barrier between the fresh water fen and the CT brine. Figure 3.3 shows the images of the fen over its construction and a schematic of the fen layers.

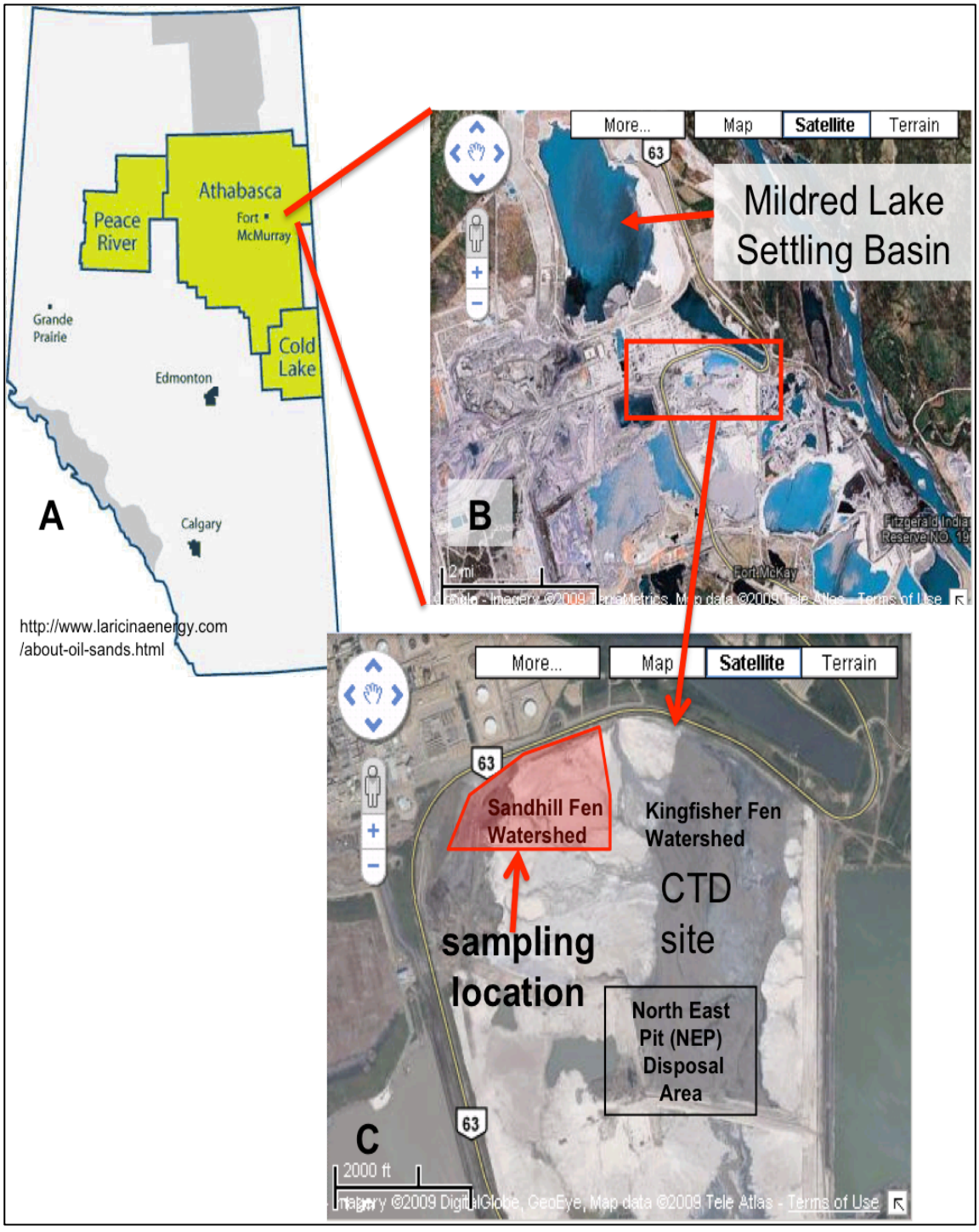


Figure 3.1: Site location at Syncrude Mildred Lake. A – Map of Alberta showing oil sand deposits. B – Satellite image of Mildred Lake Settling Basin at Syncrude. C – Location of Sandhill Fen reclamation project, adjacent to the Kingfisher fen watershed that has not currently being reclaimed. Satellite images from Google Earth.

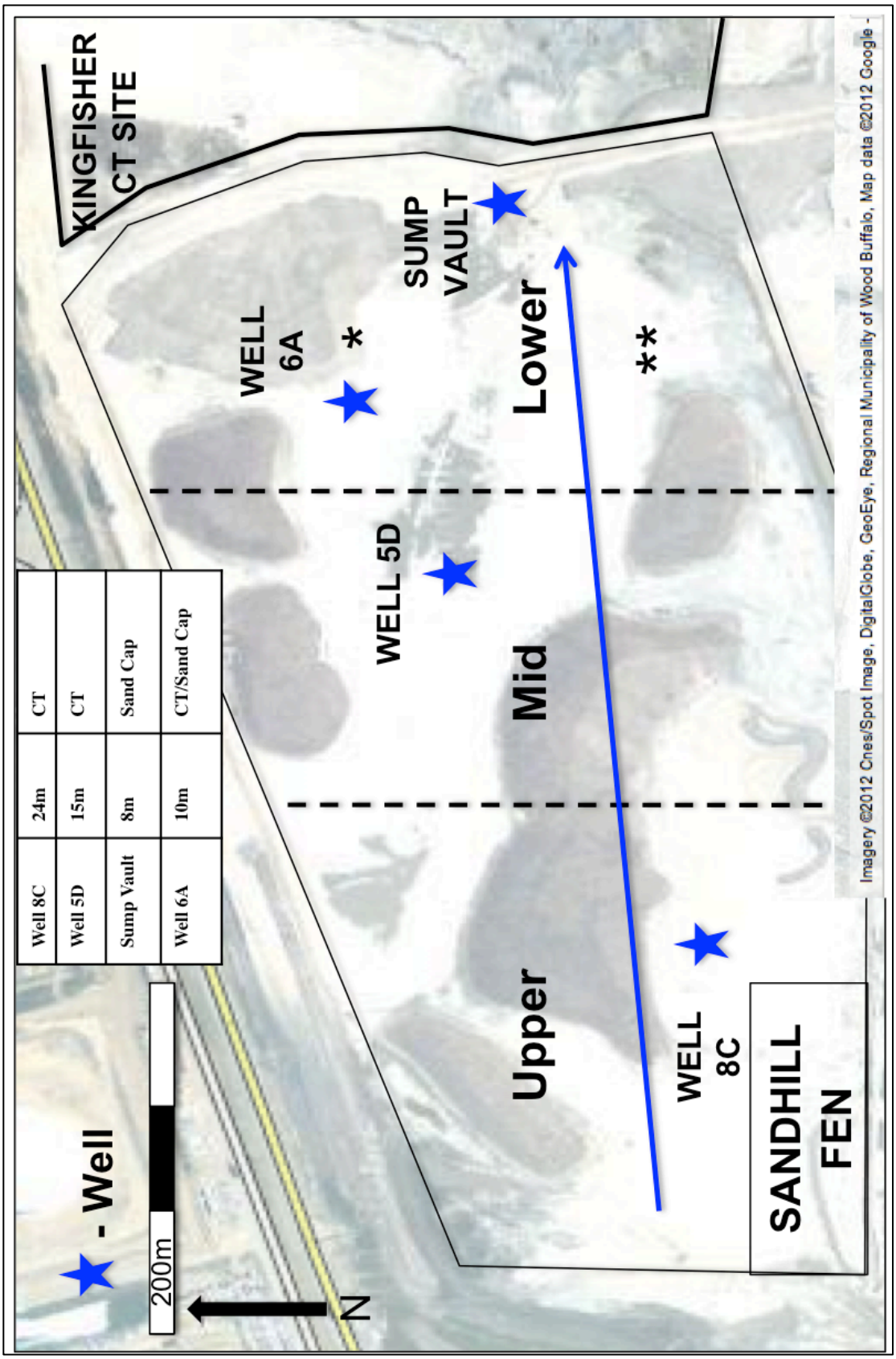
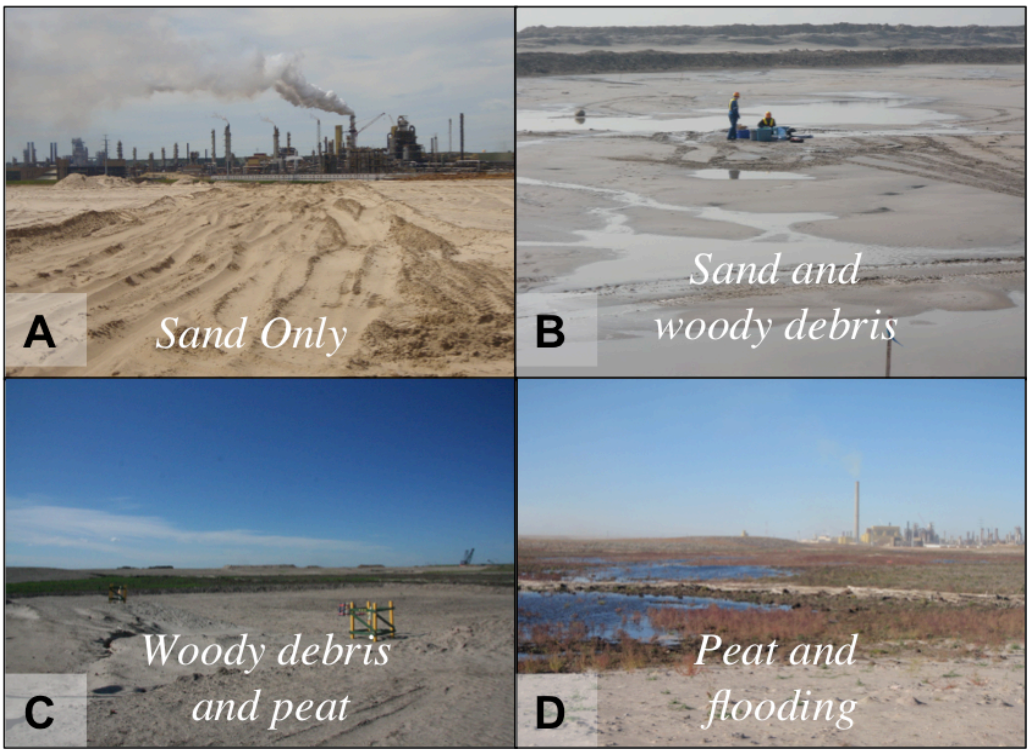


Figure 3.2: Sampling locations for all sampling campaigns on Sandhill Fen. Satellite image from Google Earth.



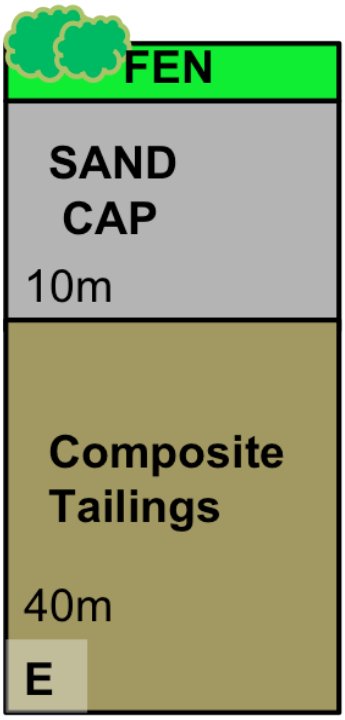


Figure 3.3: Photographs showing the evolution of Sandhill Fen. **A** – June 2010, *photo credit Lesley A. Warren*. **B** – September 2010, *photo credit Lesley A. Warren*. **C** – July 2011, *photo credit Tara Colendrander*. **D** – October 2011, *photo credit Kate Stephenson*. **E** – schematic of materials used in creating Sandhill Fen. The composite tailings are capped with processed oil sand and a freshwater fen.

3.1.2 – Well Water Sampling and Analysis

Each well was purged of water (~10 minutes, or more than three well volumes) using a foot-pump and plastic tubing. Water was extracted from the wells using this method or with plastic bailers. Well water was immediately measured for pH, temperature, dissolved oxygen, conductivity (Orion* 5-Star pH/RDO*/Conductivity portable multiparameter meter, Thermo Scientific). Samples to be used for microbial enrichments were immediately poured from the tubing into sterile 1L Nalgene bottles (either autoclaved at 121ºC for 30 minutes or sterilized using 70% (v/v) ethanol) with no headspace, sealed and stored at 4ºC until use in the laboratory. Samples to be analyzed for ferrous (Method 8146) and total iron (FerroVer Method 8008), sulfide (Method 8131) and sulfate (SulfaVer4 Method 8051) were pipetted directly from the bailers or tubing into 50mL falcon tubes containing the appropriate HACH reagents to prevent oxidation of reduced species and taken to the field laboratory station for immediate colorimetric analysis using a HACH spectrophotometer.

Well water for bulk DNA extraction and subsequent 16S rRNA analysis was immediately poured into sterile 4L carboys and filtered onsite, using 1L, 0.22μm Nalgene filter towers. Well water was filtered until clogged (approximately 1.5L) and substrates were visible on the filter paper. The filters were cut out using sterile scalpels, placed into sterile 50mL Falcon tubes and were stored on ice until the DNA extraction could be preformed approximately 6-10 hours later.

3.1.3 – Sand Cap Sampling

Sediment sampling was also completed during each sampling campaign. Sampling locations are indicated in Figure 3.2. Only surficial sand cap sediment samples could be taken and sample locations differed each time due to topographic changes on the fen and accessibility (i.e. were unable to walk on peat once it was added to the fen and when the fen had been flooded). If the ground was not trafficable by foot, samples were obtained with aide of an Argo (an amphibious vehicle). Sediment samples were taken approximately 10 - 40cm deep.

Samples were either hand cored using polycarbonate core tubes, sterilized with 70% (v/v) ethanol, or shoveled. Both cored and shoveled samples were allocated for microbial enrichment, sequential extraction of Fe and S, XRD, and 16S rRNA analyses. Sand samples were allocated and stored in sterile Whirl Pak bags and kept on ice. Samples for microbial enrichment and DNA extraction were stored at 4ºC, while samples for sequential extraction and XRD were stored at -20ºC until analysis.

3.2 – Sand Cap Sequential Extraction for Fe and S

All sand cap sediment samples underwent microwave digestion multi-step sequential metal extraction procedure to analyze Fe and S found within important, operationally defined, sediment phases. Field sand cap sediment samples were extracted using the Haack and Warren (2003) extraction scheme. Microcosm sediments were extracted using the modified Haack and Warren (2003) extraction scheme that is outlined in this section (3.2.).

Multi-step sequential metal extractions have most notably been used in trace element geochemistry to determine trace metal affinity for operationally defined sediment fractions (e.g. Haack and Warren, 2003; Tessier et al., 1979). In brief, the sediment sample is sequentially exposed to increasingly strong chemical reagents that release the metals bound to targeted phases within the sediment, providing details on the bioavailability, mobilization and transport of these metals (Tessier et al., 1979). The original sequential extraction scheme by Tessier et al. (1979) was modified by Haack and Warren (2003) (Table 3.2), who broke down the 'iron oxide reducible' fraction into two separate sediment compartments: amorphous iron oxyhydroxides and crystalline iron oxyhydroxides. The fractions determined by the Haack and Warren (2003) extraction scheme range from most loosely bound to residual: F1- exchangeable (loosely bound), F2 – acid soluble (e.g. carbonates), F3 – amorphous iron oxyhydroxides (easily reducible), F4 – crystalline iron oxyhydroxides (reducible), F5 – sulfides and organics (oxidizable), and F6 – residual (most tightly bound) (Table 3.2). Applying this method as a means to dissolve Fe and S phases rather than focusing on trace elements will show accessible pools or Fe and S in the sediments.

Table 3.2: Fe extraction scheme from Haack and Warren (2003) used for sand cap sediment samples

Sequence (Fraction)	**Reagents**	**Conditions**
F1: Exchangeable	1M sodium acetate, pH 8.2 (with HOAc)	1h shaking at room temperature
F2: Carbonates	1M sodium acetate, pH 5.0 with (HOAc)	Microwave program 1 (MP-1): Heat to 150 °C over 8 minutes. Maintain temperature for 5 minutes. 30 minute cool-down
F3: Reducible Amorphous Fe/Mn Oxyhydroxides	0.25M Hydroxylamine hydrochloride in 0.25M HCl	MP-1
F4: Reducible Crystalline Fe/Mn Oxyhydroxides	1.0M Hydroxylamine hydrochloride in 25% v/v acetic acid	MP-1
F5: Oxidizable Organics and Sulfides	3:2 ratio 30% H_2O_2: 0.02M HNO_3 + 1:4 ratio 3.2M NH_3OAc:MQWater	MP-1
F6: Residual Resistate Minerals	Concentrated HNO_3	Microwave Program 2 (MP-2): Heat to 180°C over 20 minutes. Maintain temperature for 10 minutes. 45 minute cool-down.

All field sediment analysis was done using approximately 1 gram of wet sediment and the extraction scheme by Haack and Warren (2003) (Table 3.2). The exchangeable and carbonate fraction values for the field sand cap samples were summed to create the easily soluble fraction (Figure 3.4) in order to compare between the field and experiment extraction results. . All samples were done in triplicate.

After microwave digestion or bench top shaking, the supernatants from each step or fraction are analyzed for the desired analyte, Fe_{Total} or SO_4^{2-} on the HACH spectrophotometer (FerroVer Method 8008 and SulfaVer4 Method 8051, respectively). The concentrations obtained from the HACH are normalized to mmol/g or μmol/g of dried sediment using wet-dry ratios and dilution factors.

By determining if there is biologically available Fe and S in the sand cap sediment samples we can infer that the microbes present in this environment could potentially be using this Fe and S in their metabolic processes, changing the associated sediment fractions of these elements and potentially being important in the production or sequestration of hydrogen sulfide.

3.2.1 – Modified Extraction Scheme for Iron for Microcosm Sediment Samples

The modified extraction scheme for Fe has the following sediment fractions: Step 1 – easily soluble, Step 2 – easily reducible, Step 3 – reducible, Step 4 – oxidizable I, Step 5 – oxidizable II, Step 6 – recalcitrant (Figure 3.4, Table 3.3). Microcosm sediment analysis was done using approximately 0.3-1 grams of sediment dried at 40ºC and the modified version of Haack and Warren (2003) sequential extraction scheme for iron (Table 3.3). A sulfur extraction method was developed for the microcosm sediments, but was not successful. Therefore there is only analysis for sediment iron done on the experimental microcosm t=0 and t=end sediments.

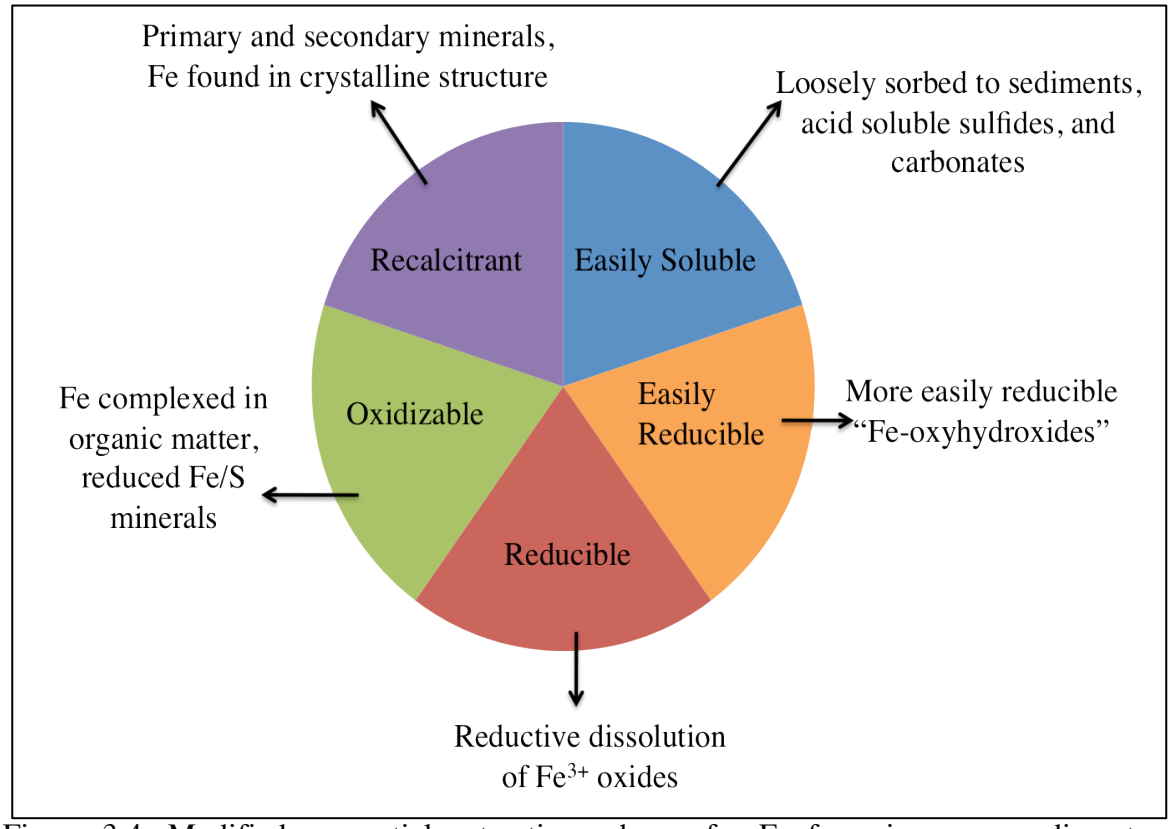


Figure 3.4: Modified sequential extraction scheme for Fe for microcosm sediments. Modified from Haack and Warren, (2003) and Tessier et al., (1979).

After digesting Step 4, 'oxidizable I', any Fe^{2+} that is present and is oxidized to Fe^{3+} may form Fe oxides that precipitate out. These Fe oxides need to be reduced once again in order for all the oxidizable Fe to be accounted for. Therefore, Step 5, 'oxidizable II', uses the same reducing reagent as Step 3. The oxidizable fraction of Fe is therefore the sum of Steps 4 and Steps 5.

Table 3.3: Modified Fe sequential extraction scheme.

Step (Fraction)	Reagent	Conditions
Step 1 – Easily Soluble	1M sodium acetate, pH 5.0 with (HOAc)	Microwave program 1 (MP-1): Heat to 150 °C over 8 minutes. Maintain temperature for 5 minutes. 30 minute cool-down
Step 2 – Easily Reducible	0.25M Hydroxylamine hydrochloride in 0.25M HCl	MP-1
Step 3 – Reducible	1.0M Hydroxylamine hydrochloride in 25% v/v acetic acid	MP-1
Step 4 – Oxidizable I	3:2 ratio 30% H_2O_2: 0.02M HNO_3 + 1:4 ratio 3.2M NH_3OAc:MQWater (F5 reagent, Haack and Warren (2003)) *Per reaction* - 20mL aliquot of F5 reagent, add 7mL of 30% H_2O_2 to make total concentration of H_2O_2 in reagent to 15%, add 2% v/v HNO_3 (0.54mL)	MP-1
Step 5 – Oxidizable II	Repeat step 3 - 1.0M Hydroxylamine hydrochloride in 25% v/v acetic acid	MP-1
Step 6 - Recalcitrant	Aqua Regia – 1:3 ratio HNO_3:HCl	Microwave Program 2 (MP-2): Heat to 180°C over 8 minutes. Maintain temperature for 15 minutes. 45 minute cool-down.

3.3 – X-Ray Diffraction Analysis

Sediment samples from each sampling campaign were dried at approximately 40ºC in crucibles or aluminum tins. Samples were powdered with a stirring rod and transferred into 15mL falcon tubes. The samples were analyzed using a high resolution Bruker D8 Advance Powder Diffractometer with a germanium monochromator at the

McMaster University X-Ray Diffraction Facility (McMaster University, Hamilton, ON, Canada). Samples were exposed to copper Kα1 radiation at 40kV and 40mA at a scan speed of 0.1 degrees 2θ per minute, with a step of 0.04 degrees and step time of 35 seconds. These data were analyzed with DIFFRAC PLUS Evaluation software.

3.4 – Environmental Microbial Enrichments

In order to explore S and Fe biogeochemical dynamics of environmental samples, integration of microbial biology and standard aqueous geochemistry in the laboratory is required. Batch enrichments were conducted to grow targeted bacterial metabolisms from water and sediment samples from the Sandhill Fen. Each liquid enrichment media is designed to select for specific Fe and S metabolisms and enhance the growth of those bacteria that use that metabolic pathway. Growing environmental microbial enrichments allows for the identification and characterization of which bacterial metabolisms could potentially be active in that environment, in this case the sand cap and porewaters of the developing Sandhill Fen, shedding light on potential microbial pathways and interactions. Enrichments were made for heterotrophic Fe(III)-reducing and S-reducing bacteria, as well as chemolithotrophic Fe(II)-oxidizing and S-oxidizing (acidophilic and neutrophilic) bacteria.

All samples collected, both well water and sediment, were enriched for each bacterial metabolism in order to determine potential metabolic communities at each sampling location. In general, 25mL of sample well water or 3-5 grams of sediment sample were added aseptically with in a Class II A/B3 Biological Safety Cabinet (BSC) (Model 1284, Forma Scientific Inc., Ohio, USA) to autoclaved 250mL Erlenmeyer flasks

and were capped loosely with a double layer of autoclaved tinfoil to allow gas exchange but prevent foreign bacteria from contaminating the sample. All liquid media was either filter-sterilized using a 0.22μm filter tower or autoclaved at 121ºC for 30 minutes. Approximately 100mL of liquid media was added to the initial enrichments. Subsequent enrichments were inoculated with approximately 20mL of previous enrichment and 100mL of fresh media. All enrichments were grown microaerophilically without agitation in the dark, with the exception of the S-reducers (grown under zero oxygen conditions in the anaerobic chamber). Positive growth and metabolism-specific details for each type of enrichment are outlined in the following sections: 3.3.1 – 3.3.5.

3.4.1 – Fe-Reducers

Heterotrophic, Fe(III)-reducing bacteria were enriched using the M1 media recipe in the procedure outlined by Kosta and Nealson (1998). Fe(III)-citrate was used as the reducible iron source (electron acceptor), and 10mM sodium acetate as the carbon source (electron donor). Positive growth for Fe-reducers is determined by a generation of ferrous Fe and accompanying colour change of the media, from orangey-brown to clear, as the citrate is being consumed and solid Fe precipitate settles at the bottom of the flask.

3.4.2 – Fe-Oxidizers

Chemolithotrophic, Fe(II)-oxidizing bacteria were grown using ATCC 2039 media. Solution A is pH adjusted using a bench top pH meter (Denver Instrument Company, Denver, CO, Model 215) with a 3M KCl High-performance pH/ATC glass-body electrode to 2.3 with 0.1N H_2SO_4. Solution B was pH adjusted to < 3 before the

$FeSO_4 \cdot 7H_2O$ was added to prevent the reduced Fe from abiotically oxidizing. After combining solutions A and B, the media was filter sterilized (<0.22μm). $FeSO_4 \cdot 7H_2O$ was used as the electron donor (i.e. Fe^{2+} source) for this media. Positive growth was observed by generation of Fe(III), an increase in turbidity, and colour change of parent media (clear to slightly orange or dark yellow).

3.4.3 – S-Reducers

Heterotrophic, S-reducing bacteria were grown in lactate (electron donor) media according to the procedure outlined by Kelly and Wood (1998). Media was autoclaved for 30 minutes at 121°C and subsequently bubbled with filtered 95% N_2; 5%H_2 gas for 45 minutes, then immediately placed in the sealed anaerobic chamber (5% H_2; 5% CO_2; 90% N_2; Forma Scientific, Model 1020). Samples were allocated ascetically to flasks in the BSC, capped and transferred into the anaerobic chamber where they were supplemented with the anaerobic media. This recipe uses sulfate as the terminal electron acceptor and lactate as its carbon source or electron donor. Positive growth for sulfate reducing bacteria is confirmed when the media turns black and black (FeS) precipitate is observed.

3.4.4 – Acidophilic S-Oxidizers

Chemolithotrophic, acidophilic, S-oxidizing bacteria were grown in modified ATCC 125 media, $(NH_4)_2SO_4$ – 1.5mM; $MgSO_4$ – 2.1mM; $CaCl_2 \cdot 2H_2O$ – 2.5mM; KH_2PO_4 – 22mM; $K_2S_4O_6$ – 16mM; $FeSO_4 \cdot 7H_2O$ – 26μM (Bernier and Warren, 2007). Media was filter sterilized (<0.22μm). After inoculation with environmental sample or transfer from a previous acidophilic S-oxidizing culture, two or three drops of

bromophenol blue pH indicator was added and the pH was adjusted using a bench top pH meter to pH 4. The pH probe was sterilized with 70% v/v ethanol before and after submersion into enrichment flasks and was rinsed in filter-sterilized (0.22μm) ultra pure water. Tetrathionate ($S_4O_6^{2-}$) is the sole terminal electron donor for this enrichment. Positive growth for these acidophilic S-oxidizers is denoted by the pH indicator colour change from grey-purple to yellow (pH < 3), identifying acid production related to the oxidation of sulfur.

3.4.5 – Neutrophilic S-Oxidizers

Chemolithotrophic, neutrophilic, S-Oxidizing bacteria were enriched with media following the procedures of Kelly and Wood (1998). Thiosulfate ($S_2O_3^{2-}$) was provided as the sole terminal electron donor for this reaction. After inoculation, phenol red pH indicator was added to establish positive growth. Growth was evident when the colour changed from pink to yellow (pH < 8).

3.5 – 16S rRNA Sequencing

Microbial enrichments allow us to explore potential metabolisms present within an environmental system in the laboratory setting. Combined with 16S rRNA sequencing, it is possible to analyze phylogenic and genetic diversity of targeted enrichments. Bacterial community characterization was done through polymerase chain reaction (PCR) (Spiegelman et al., 2005). Specifically selected bulk environmental DNA

(extracted during sampling campaigns) and microbial enrichments were analyzed using 16S rRNA gene sequencing.

Bulk genomic DNA was extracted using a Powersoil™ DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacture's standard protocols with slight modifications. These included: the DNA was combined onto one spin filter, the volume was eluted to 50μL and the spin filters were soaked for 5 minutes at 37ºC before the final centrifugation step (Norlund, 2011). Before DNA could be extracted from Sandhill Fen well water samples and microbial enrichments, the samples were filtered through Nalgene filter towers (<0.22μm) to concentrate the bacteria on the filter. The filter was cut out of the filter tower with a sterile scalpel and was cut into small pieces, which were allocated to the bead beating tubes. Sediment samples were simply aseptically added to the bead beating tubes. Three bead beating tubes were used for each sample to allow for sufficient DNA volumes.

The DNA for each sample was amplified using PCR (initial denaturation step of 3 min at 94°C, followed by 35 cycles of 94°C for 45s, 55°C for 45s, and 72°C for 1 min, with a final extension step of 72°C for 10 min. The reaction mixture contained final concentrations of 1x PCR buffer, 2 mM MgCl2, 1 μM of each primer, 200 μM dNTPs, and 5 U of Taq polymerase (Fermentas Canada Inc., Burlington, ON)). The 16S rRNA gene was targeted with primers 27F and 1492R. The PCR product was visualized through gel electrophoresis in a 1% gel made by combining 150ml of 1xTAE buffer (made of 40mM Tris acetat and 1mM EDTA), and 1.5g electrophoresis-grade agarose. The PCR product was compared to a 1kb DNA ladder (Gene Ruler, Fermentas) and visualized with a UV transilluminator. Desired bands were removed with a sterile scalpel

and the purified PCR product was eluted using a MinElute® kit (Qiagen Inc., Toronto, ON, Canada) according to the manufacturer-supplied protocols. 1-3μL of the eluted product was cloned using the pCR™ 4-TOPO® vector (TOPO TA cloning kit, Invitrogen Canada Inc, Burlington, ON, Canada) in a 20 minute reaction at room temperature. The cloned sample was transformed into *Escherichia coli (E.Coli)* MACH cells (Invitrogen) after a 20 minute incubation on ice by a 30 second heat shock at 42ºC in the Thermomixer. After heat shocking, the cells were placed on ice for 2 minutes before being incubated for 1 hour in 250μL of manufacturer-supplied S.O.C. growth medium at 37ºC on a rotating shaker. Fifty or 100μL of the incubated solution was aseptically plated using autoclaved glass beads on solid, carbenicillin-amended (100μg/mL) Luria-Bertani (LB) media plates. Plates were grown over night at 37ºC in the incubator. Individual colonies were aseptically picked from the plates using a 10μL sterile pipette tip and used to inoculate 2mL of carbenicillin-amended liquid LB media and incubated for an additional 4 hours on a rotating shaker. The plasmid DNA were spun down using a table top microcentrifuge (6800 x g for 3 minutes) the supernatant was decanted and the pelleted DNA subsequently purified using the QIAprep® Spin Miniprep Kit (Qiagen Inc., Toronto, ON, Canada) following the manufacturer's-supplied protocols. Sequencing was completed by adding 2μL of miniprepped product to 2μL M13R primer into 96-well plates and submitted to the McMaster Institute for Molecular Biology and Biotechnology (MOBIX Lab, McMaster University, Hamilton, ON, Canada) who sequenced the samples using ABI BigDye terminator chemistry (3730 DNA Analyzer, Applied Biosystems, Foster City, CA, USA). Sequences were analyzed against the Ribosomal Database Project (RDP version 10; (Cole et al., 2009)) and NCBI (US)

database using Basic Local Alignment Search Tool algorithm (BLAST) (Altschul et al., 1990).

3.6 – Microcosm Experiments

Microcosm experiments allow for the direct observation and measurement of H_2S aqueous production in the laboratory setting. Microbial $H_2S_{(aq)}$ production can be assessed and compared between treatments of abiotic and biotic microcosms, determining exactly if bacteria are producing $H_2S_{(aq)}$ in our constructed fen micro-system. In preliminary microcosm experiments, $H_2S_{(aq)}$ production was observed through colour change with HACH reagents, but was not able to be quantitatively recorded due to experimental design flaws. Changes in preliminary experiments to create new fen simulation microcosms are specifically outlined in Table 3.4.

3.6.1 – New Microcosm Experiment Set Up

New microcosms were set up using 5.4L plastic containers with sealable lids. Holes were drilled approximately 0.5 cm from the bottom of the container (4 on each side). The containers were acid wasted in 4% HCl for 2-4 hours, rinsed with MQ water 3 times and the holes were sealed with parafilm. After washing, the containers were sterilized with 70% v/v ethanol and dried aseptically in the biological safety cabinet (BSC). Plastic tubing was cut to appropriate length and sterilized with ethanol. To sample from within the microcosm, tubing was threaded through the drilled holes and attached to a sterile Slide-A-Lyzers® G2 Dialysis Cassette (Thermo Scientific) that were

cemented to the bottom of the container using silicon caulking. Slide-A-Lyzers® are 3mL volume cartridges that allow the diffusion of dissolved species through the membrane into the cassette. This allowed both for the sampling of porewater from within the microcosms without disturbing the sediment and also allows for multiple sampling sessions (since they can be refilled). The tubes are attached to the Slide-A-Lyzers® using 23.5 gage sterile needles and kept securely in place with silicon caulking. The free end of the tube was connected to 10mL sterile syringe that allowed sterile, degassed MQ water to be removed and added to the Slide-A-Lyzer® cassette.

Table 3.4: Changes in Microcosm Experiment Design.

Specific Aspect of Microcosm Experiment	OLD Microcosm Experiment Issue	Change for NEW Microcosm Experiment
Sample Extraction	- Large peepers of dialysis tubing were hard to extract from CT, lost peepers, water inside became dirty - Had to sacrifice a microcosm every sampling time	- Slide-A-Lyzers® (dialysis cartridges) - More ridged and allow for clean water extraction via tubing and syringes - Slide-A-Lyzers® can be refilled insitu, no sacrifice – keeps microcosm from being disturbed
Sample Volume	- Large volume but contaminated upon extraction	- Smaller volume in cartridge, but standard curves used to scale down volumes needed for measurement on HACH and more cartridges for analysis of other analytes
Microcosm Set Up	- Only CT used – not fully representative of fen system	- CT, sand and peat used, more representative of actual fen environment
Sampling Times	- Once a week – did not perform equilibrium test	- 3 or 4 days – equilibrium test preformed
Pore Water Analytes	- Only measured H_2S in peepers	- Measuring H_2S, Fe^{2+}, SO_4^{2-}, pH, temperature in dialysis cartridge
Sediment Analysis	- Fe and S via sequential extraction, did not see significant differences, problems with mass balance - Sample taken for 16S rRNA extraction for every microcosm	- New sequential extraction method of Fe - 16S rRNA sample taken at t=0 and t=end only for endemic species microcosm
Microbial Metabolisms	- Used environmental microbial enrichments - Unable to constrain which bacteria were performing each metabolisms due to large species diversity in the enrichments	- Comparing pure strains to endemic bacteria and a sterile system

After container set up, the entire container, including secured Slide-A-Lyzers® was placed under UV light in the BSC for 15 minutes to maintain a sterile microcosm system. Slide-A-Lyzer® membranes were hydrolyzed with sterile MQ water for one hour to allow membrane flexibility, preventing the cassettes from bursting when initially filled. Once the membranes were hydrolyzed, the microcosm container was moved into

the anaerobic chamber to fill the dialysis cassettes with degassed water and fill the containers with CT and sand. All three microcosm treatments were done in the anaerobic chamber to maintain the same construction conditions. The three treatments constructed for this investigation were; (1) sterile control, (2) endemic bacteria within unsterilized CT and sand, (3) addition of sulfate reducing bacteria to sterile CT and sand. CT, sand, Sump Vault water, and peat sampled in October 2011 were sterilized by gamma irradiation at the McMaster University Nuclear Reactor.

The microcosm sediments were added to the containers in layers to represent the construction of the Sandhill Fen. The bottom and thickest layer is the CT, followed by a smaller layer of sand and topped with a thin layer of peat. Sterile Sump Vault water was added to each substrate and mixed in thoroughly to create a saturated environment to allow for good flow into and out of the dialysis cassettes. The endemic treatment sediments were kept in the refrigerator (4ºC) until use in the microcosms. For the third treatment, SRBs were thoroughly mixed into the CT and the sand layers. 15mL of ATCC 700201 *Desulfovibrio gabonesis* pure strain heterotrophic sulfate reducers were added and mixed into the CT; 5mL were added and mixed to the sand. After adding the sediments in the anaerobic chamber, microcosms were removed and placed into the BSC, where the thin peat layer and more water was added to the top. Containers were sealed and placed on the bench top, covered in tin foil to prevent light from reaching the CT and sand layers, until sampling.

After 9 sampling periods, all three microcosm treatments were amended with a solution of 2mM acetic acid and 25mM sodium sulfate, to stimulate sulfate reduction by sulfate reducing bacteria. The solution was filtered sterilized in the anaerobic chamber

and added to the microcosms using a 1mL pipette. 10mL of solution was added to 10 random spots throughout the microcosms, including in between cassettes and the microcosms were left until the next sampling session. After sampling on day 48, the microcosms were amended once again with sulfate and carbon. A solution of 20mM carbon (acetate) and 20mM sulfate were added using the exact method as was used after sampling on day 28 to distribute the solution within the microcosm.

3.6.2 – Microcosm Sampling

All sampling was done in the anaerobic chamber to prevent reduced species from being oxidized. Table 3.5 details the samples taken for each sampling day of the microcosm experiments. An equilibration experiment was done using sulfate salts, water, Sandhill Fen sand and an experiment microcosm to assess when the water in the cassettes was at equilibrium with the pore waters surrounding it. At t= 0, 1, 2, 3, and 7 days, a cassette was emptied and water was measured for sulfate. It was determined that after 3 days, concentrations of sulfate were equal in the porewaters and the dialysis cassette, therefore approximately every 3 or 4 days the microcosms were sampled, unless otherwise indicated.

On a sampling day, the three microcosms were transported into the anaerobic chamber. Two or three of the four cassettes were emptied using 10mL syringes and stored in a 15mL falcon tube. Approximately 1-2.5mL were extracted from each cassette. In the falcon tube the pH was measured (Denver Instrument Company, Denver, CO, Model 215). 0.5mL of sample water was then allocated to pre-spiked falcon tubes containing 50μL of sulfide reagent one and topped up to a final volume of 1mL using

degassed MQ water. 0.5mL of sample were added to 50mL falcon tubes containing ferrous iron reagent and topped up to 25mL with degassed MQ water. Any remaining sample that was not used for reduced species analysis was used to measure sulfate on the HACH in the dialysis cassettes. 0.25mL of sample was added to a 15mL falcon tube containing sulfate reagent, and was filled to 10mL with degassed MQ water. After all sample was removed from dialysis cassette, it was immediately refilled with degassed MQ water to be sampled next time. Directly before removing samples from the anaerobic chamber, 50μL of sulfide reagent two was added to the falcon tubes allocated for sulfide, to prevent loss of colour due to delayed measurement of sample. Upon removal from chamber, sulfide values were immediately read on the spectrophotometer and ferrous iron on the HACH. A standard curve for sulfide concentrations was created in order to scale down the volume needed to measure for sulfide.

Table 3.5: Analyte measured according to sampling time.

Sampling Time	Sample	How anlayte was measured or what was analyzed
t = 0	SV water H_2S	HACH spectrometer
	SV water SO_4^{2-}	HACH spectrometer
	SV water ferrous Fe	HACH spectrometer
	SV water total Fe	HACH spectrometer
	Unsterilized (endemic) CT, sand and peat	16S rRNA sequencing, Fe/S extraction, XRD
	Sterilized CT, sand and peat	Fe/S extraction, XRD
	Sterilized CT, sand and peat with SRB	Fe/S extraction, XRD
t = n sampling day	Cassette H_2S	UV spectrophotometer
	Cassette pH	pH meter
	Cassette SO_4^{2-}	HACH spectrometer
	Cassette Fe^{2+}	HACH spectrometer
t = end	Cassette H_2S	UV spectrophotometer
	Cassette pH	pH meter
	Cassette SO_4^{2-}	HACH spectrometer
	Cassette Fe^{2+}	HACH spectrometer
	Unsterilized (endemic) CT, sand and peat	16S rRNA sequencing, Fe/S extraction, XRD
	Sterilized CT, sand and peat	Fe/S extraction, XRD
	Sterilized CT, sand and peat with SRB	Fe/S extraction, XRD

3.6.3 – Microcosm Sediment Analysis

3.6.3.1 – Epifluorescence Imaging

Live/dead staining (LIVE/DEAD® BacLight™ Bacterial Viavility Kit, Invitrogen Canada Inc., Burlington, Ontario, Canada) was used to confirm that the bacteria in the endemic and sulfate-reducing microcosm setups were in fact alive at the end of the experiment, to rule out the possibility that changes seen in the microcosm sediment and porewater characteristics were not purely abiotic. Approximately 0.5-0.9 g of sediment from the peat, sand, and CT from the endemic and sulfate-reducing microcosms was

suspended in 5mL of Milli-Q water. 10µL of the sediment/water slurry was added to 10µL of LIVE/DEAD® stain. The LIVE/DEAD® stain is a mixture of two nucleic acid stains, propidium iodide and SYTO®9. Propidium iodide stains only dead cells (fluoresce red), while SYTO®9 will stain all cells, both living and dead (fluoresce green), thus cells that only fluoresce in green will be living cells. Epifluorescence microscopy was completed using a Leica LEITZ DMRX epifluorescence microscope equipped with an HBO 100W mercury arc lamp (Leica Mircosystems Canada, Richmond Hill, Ontario, Canada).

3.6.3.2 – Microcosm X-Ray Diffraction Analysis

A sample of CT for each microcosm treatment was allocated for XRD characterization. This process was similar to the XRD characterization completed for the field sediment analysis (Section 3.3), except that with the suspected presence of FeS minerals and their likeliness to oxidize quickly, these samples were flash heated. The CT samples were dried by flash heating in a large crucible on a hot plate in a fume hood. If the sample is quickly heated, the water will evaporate, with the hope that the FeS minerals would become more crystalline in composition. To check if this method changes the structure of minerals, one sample of SRB CT t=end was dried in the anaerobic chamber and the same sample analyzed with the flash heated samples. The XRD analysis showed almost no difference in the sample that was flash heated compared to the sample dried in the anaerobic chamber (data not shown). Samples were flash heated due to time restrictions since drying sediment in the anaerobic chamber can be a slow process due to the high humidity in the chamber. After flash heating, the CT samples were immediately

sent for XRD analysis (see Section 3.3 for details on XRD instrument and analysis procedure).

3.7 – Statistical Analysis

All well water data values are displayed as mean values. The error bars represent one standard deviation. Mean Fe^{3+} values were obtained by subtracting measured Fe^{2+} from total Fe values obtained on the HACH spectrophotometer (Fe Total = $Fe^{3+} + Fe^{2+}$). Therefore, the Fe^{3+} error bars represent one standard deviation of the total iron values measured. The number of replicate analyses performed for each analyte of interest from the field well water samples ranged between 3 and 6. Microcosm hydrogen sulfide was analyzed in replicates of 3. Ferrous iron and sulfate were only measured one time due to inadequate amounts of sample for multiple replications.

4.0 – Results and Discussion

4.1 – Field Sampling

4.1.1 – Well Water

Field sampling of well water and surficial sand cap sediments was completed over three sampling campaigns in June 2010, September 2010, and July 2011. Table 4.1 summarizes the well water physiochemical properties of each well sampled during each sampling campaign. Figure 4.1 displays the aqueous iron and sulfur species concentrations measured from each well.

Associated with the underground drains in the Sandhill Fen site, water flows from northwest to southeast or from the upper regions of then fen to the lower regions of the fen towards the Sump Vault where it is drained (Figure 3.2). The wells sampled in this thesis occur along this spatial transect (Figure 3.2). Along the spatial transect there are also depth differences between wells which means that the upper two wells (Wells 8C and 5D, Figure 3.2) are located in actual CT, and the lower two wells (Sump Vault and Well 6A) are located in the overlying sand cap. Thus, our geochemical trends have to be considered in light of these spatial and depth dependent factors.

4.1.1.1 – Hydrogen Sulfide

Aqueous hydrogen sulfide ($H_2S_{(aq)}$) is detected in all wells sampled during each sampling campaign (Figure 4.1-A and Table 4.1) indicating widespread occurrence throughout CT and the sand cap in the Sandhill Fen. The pH values of these wells are between 7.41 and 8.8 (Table 4.1), which would indicate that HS^- is the major sulfide species present in these waters. In order to have build-up of $H_2S_{(aq)}$ in the porewaters of

the Sandhill Fen CT and sand cap, there must be micropockets or microenvironments with acidic pH values, since the bulk pH of the system is circumneutral.

Well 6A, although not measured in June 2010, appears to be a large input of $H_2S_{(aq)}$ to the fen porewaters – values measured in this well of 90.6µM (June 2010) and 69µM (July 2011) (Table 4.1) are the highest and second highest values measured for those sampling campaigns respectively. There are no wells that are fully situated in the CT at the Sump Vault or Well 6A, so we cannot say whether the CT at these physical sites are producing H_2S. There is an old tailings pipeline running underneath Well 6A that could be influencing the concentration of sulfide in the porewaters surrounding it. This input of $H_2S_{(aq)}$ at Well 6A is a likely source of H_2S concentrations at the Sump Vault, as the water is being drawn towards the Sump Vault to be extracted from the fen for dewatering purposes.

Somewhat surprisingly, the wells that are situated within the CT (i.e. Wells 8C and 5D, which are in the upper region of the fen (Figure 3.2)) have lower concentrations of $H_2S_{(aq)}$ than those in the overlying sand cap or at the sand cap/CT interface (i.e. Sump Vault and Well 6A, which are located in the lower region of the fen (Figure 3.2)). This finding could suggest that the $H_2S_{(aq)}$ is being generated within the CT and moving up through the fen towards the surface, or the $H_2S_{(aq)}$ is being generated within the sand cap and accumulating in the Sump Vault hoarding well or being generated in another location in the fen and then transported with water movement through the sand cap.

The widespread presence of $H_2S_{(aq)}$ within CT and the sand cap, suggests microbial sulfate reduction is occurring within the Sandhill Fen, since abiotic reduction of sulfate is uncommon at temperatures below 150ºC (Li et al., 2006; Rickard and Luther,

2007). The oxidation of S^{2-} to SO_4^{2-} is kinetically fast, however, the opposite reaction is irreversible at low temperatures, unless microorganisms capable of sulfate reduction are present (Rickard and Luther, 2007). Therefore, it is almost impossible for the $H_2S_{(aq)}$ concentrations measured in the porewaters of the Sandhill Fen to be abiotic, unless there are extremely powerful reductants present such as HI or acidified Cr^{2+} (Rickard and Luther, 2007). However, one possibility is that unstable sulfur phases exist in the CT generated by processing that would not occur within a non-anthropogenically generated "sediment", and these phases could result in reduced sulfur species. As mentioned previously, at pH values of 8, comparable to the Sandhill Fen porewaters, only about 9% of the aqueous hydrogen sulfide present will be in the form of H_2S (Wang and Chapman, 1999), which theoretically should all be microbially produced. The biogenic production of aqueous hydrogen sulfide and the increase of aqueous hydrogen sulfide concentrations along the transect towards the Sump Vault corresponds with the decreases in sulfate porewater concentrations from the upper regions of the fen to the lower regions (Table 4.1, Figure 4.1-B), highlighting the occurrence and importance of microbial sulfate reduction or sulfur disproportionation.

The stability of $H_2S_{(aq)}$ within the porewaters of the CT and sand cap is also an important dynamic to consider. The decreased in partial pressure caused by the decrease in volume associated with dewatering the composite tailings will allow $H_2S_{(g)}$ to come out of solution. Although $H_2S_{(g)}$ concentrations were not measured, the smell of rotten eggs associated with $H_2S_{(g)}$ at a number of these wells, most strongly at Well 6A and the Sump Vault where the $H_2S_{(aq)}$ concentrations are the highest, was noted during all sampling campaigns.

Table 4.1: Summary of well water data for each well and sampling campaign. Error is reported as one standard deviation. Hash mark indicates no data, nd indicates below detection limits.

	Well 8C			Well 5D			Sump Vault			Well 6A	
Water Parameter	Jun-10	Sep-10	Jul-11	Jun-10	Sep-10	Jul-11	Jun-10	Sep-10	Jul-11	Sep-10	Jul-11
Depth (m)	24	24	24	15	15	15	8	8	8	10	9.68
Temp (C)	12.7	16.1	12.6	12.6 - 14.3	11.8	8.8	19.4 - 22.2	11.5	12.7	12.9	7.3
pH	7.51	7.83	8.11	7.74	7.63	8.8	7.6 - 8	7.43	8.41	7.56	7.59
Conductivity (ms/cm)	-	-	5.36	-	-	4.34	-	-	3.99	-	3.61
Sulfide (µmol/L)	4.7 ± 1.46	4.9 ± 1.08	17.4 ± 2.4	1.76 ± 0.51	5.4 ± 0.58	3.4 ± 0.67	15.9 ± 0.008	82.7 ± 16.4	51 ± 3.12	90.6 ± 5.02	69.04 ± 62.14
Sulfate (mmol/L)	5.64 ± 0.3	3.99 ± 0.15	1.18 ± 0.15	2.3	2.15 ± 0.57	0.59 ± 0.08	5.3 ± 0.3	0.73 ± 0.21	1.1 ± 0.05	1.63 ± 0.33	1.15± 0.04
Fe^{2+} (µmol/L)	nd	1.91 ± 0.45	2.91 ± 0.45	3.3 ± 0.27	0.18 ± 0.18	2.98 ± 0.1	1.4 ± 0.1	4.88 ± 0.98	3.98 ± 0.18	nd	0.83 ± 0.37
Fe^{3+} (µmol/L)	25.7 ± 2.44	5.6 ± 0.36	13.3 ± 2.45	2.08 ± 0.36	0.12 ± 0.1	6.67	8.04 ± 041	nd	1.01 ± 0.9	0.41 ± 0.27	1.192 ± 0.88

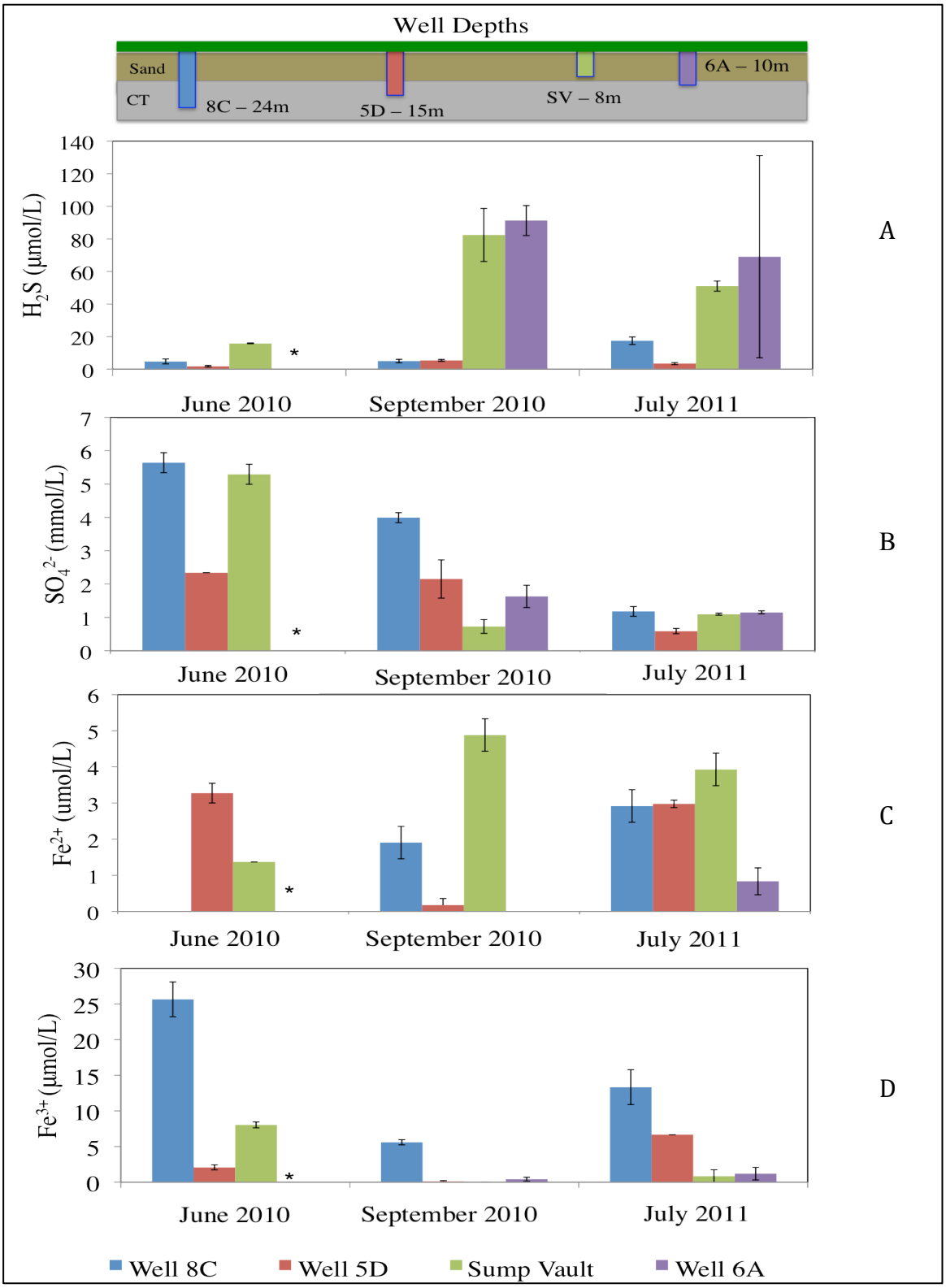


Figure 4.1: Aqueous Fe and S from well waters in Sandhill Fen. * indicates no sample for that time frame. Error bars represent one standard deviation.

4.1.1.2 – Sulfate

The sulfate concentrations vary between 0.3mM to 5.64mM within the well waters of the Sandhill Fen (Figure 4.1-B and Table 4.1). The highest sulfate concentrations were measured in June 2010, and decreased each sampling campaign for all wells, with the exception of Sump Vault in July 2011, where it increased to 1.1mM compared to 0.73mM in September 2010. These data are opposite of what was seen in the aqueous hydrogen sulfide data which showed increasing aqueous hydrogen sulfide concentrations from June 2010 to July 2011 (Figure 4.1-A), which is consistent with sulfate reduction or sulfur disproportionation in the porewaters.

The highest sulfate concentrations are measured in Well 8C (5.64mM and 3.99mM, Table 4.1), situated within the CT in the upper region of the fen. These higher values could be a result of heterogeneities within the CT and/or there is less microbial sulfate reduction occurring at this depth or in this region of the fen. The lower sulfate values in the wells located in the sand cap (i.e. Sump Vault and Well 6A) (Table 4.1, Figure 4.1-B) could be a result of sulfate reduction, based on the higher aqueous hydrogen sulfide values (Table 4.1, Figure 4.1-A) within these wells or the lack of sulfate present within the sand.

The differences in sulfate among sampling campaigns could also be a result of material differences associated with construction of the fen, especially in the wells located within the sand cap rather than in the CT. Between the sampling campaigns in June 2010 and September 2010, new sand was added to the fen as Syncrude began adding topographic details. Changes in sand content could affect sulfate concentrations within the fen porewaters. X-ray diffraction (XRD) characterization of surficial sand cap

sediment shows 1-3% of the bulk mineralogy as gypsum for the June 2010 samples (Table 4.2). There was no gypsum measured in the sand cap samples for September 2010 or July 2011. This measurable amount of gypsum is the most likely source of sulfate in the sand cap in June 2010 and would explain the highest porewater sulfate concentrations in the Sump Vault that is situated within that sand cap. However, the gypsum in the sand cap in June 2010, does not explain why the wells situated in the CT (Wells 8C and 5D) have higher values in June 2010 compared to September 2010 and July 2011, since all CT are augmented with gypsum. Possible explanations for the lower sulfate values in the CT porewater in September 2010 and July 2011 could be heterogeneities within the CT around each well and/or increases in sulfate reduction resulting in FeS mineral precipitation, since the aqueous hydrogen sulfide concentrations (Figure 4.1-A) do not show large increases in values for Wells 8C and 5D for those sampling periods.

4.1.1.3 – Ferrous and Ferric Iron

Fe^{2+} concentrations show no clear trends seasonally or spatially between each sampling campaign (Figure 4.1-C). Values range from below detection limits to 4.88μM (Table 4.1, Figure 4.1-C). Although, at specific sampling times, certain wells show a dominating redox species of iron. For example, at Well 8C in June 2010, there is no measurable Fe^{2+}, but 25.7μM Fe^{3+} (Table 4.1, Figure 4.1-C, Figure 4.1-D). This could indicate no measurable iron reduction in the porewaters around Well 8C. On the contrary, Sump Vault water in September 2010 shows the highest Fe^{2+} of all the wells for all sampling campaigns (Table 4.1, Figure 4.1-C), and the Fe^{3+} concentration is below detection limits (Table 4.1, Figure 4.1-D). This could indicate that there is iron reduction

occurring in the porewater at the Sump Vault during the September 2010 sampling campaign.

Contrary to aqueous hydrogen sulfide, Well 6A does not have a large influence on reduced iron concentrations. Well 6A has the lowest (below detection limits – September 2010) and second lowest (0.83μM – July 2011) Fe^{2+} concentrations during both sampling campaigns that it was measured respectively, suggesting lower overall concentrations of Fe^{2+} in the porewaters around Well 6A or potential sequestration of reduced iron in FeS minerals, since Well 6A has some the highest $H_2S_{(aq)}$ values (90.6μM and 69μM) along with Sump Vault (Table 4.1, Figure 4.1-A).

Ferric iron concentrations are consistently highest at Well 8C, the deepest well located in the CT ranging from 5.6μM to 25.7μM (Table 4.1, Figure 4.1-D). Well 8C also has the highest sulfate concentrations (Table 4.1, Figure 4.1-B). The lack of reduced iron species at Well 8C could suggest low relative intensity of bacterial reductive processes. Ferric iron and sulfate are both terminal electron acceptors and their reduction can be coupled to the oxidation of organic matter, suggesting that since they are still available to be consumed bacteria around Well 8C, there could be limited bacterial reduction processes occurring. Factors limiting microbial processes include nutrient limitation and/or organic substrate limitation (Vance and Chapin, 2001; Schimel and Weintraub, 2003), or the bacteria present are using a terminal electron acceptor that will provide more energy than iron or sulfate on the redox ladder (Froelich et al., 1978).

XRD results show an abundance of clay minerals that may contain Fe^{3+} including illite and kaolinite in all surficial sediment samples (Table 4.2, Table 4.3, Table 4.4). The presence of Fe^{3+} minerals and the measurement of Fe^{2+} suggest that microbial iron-

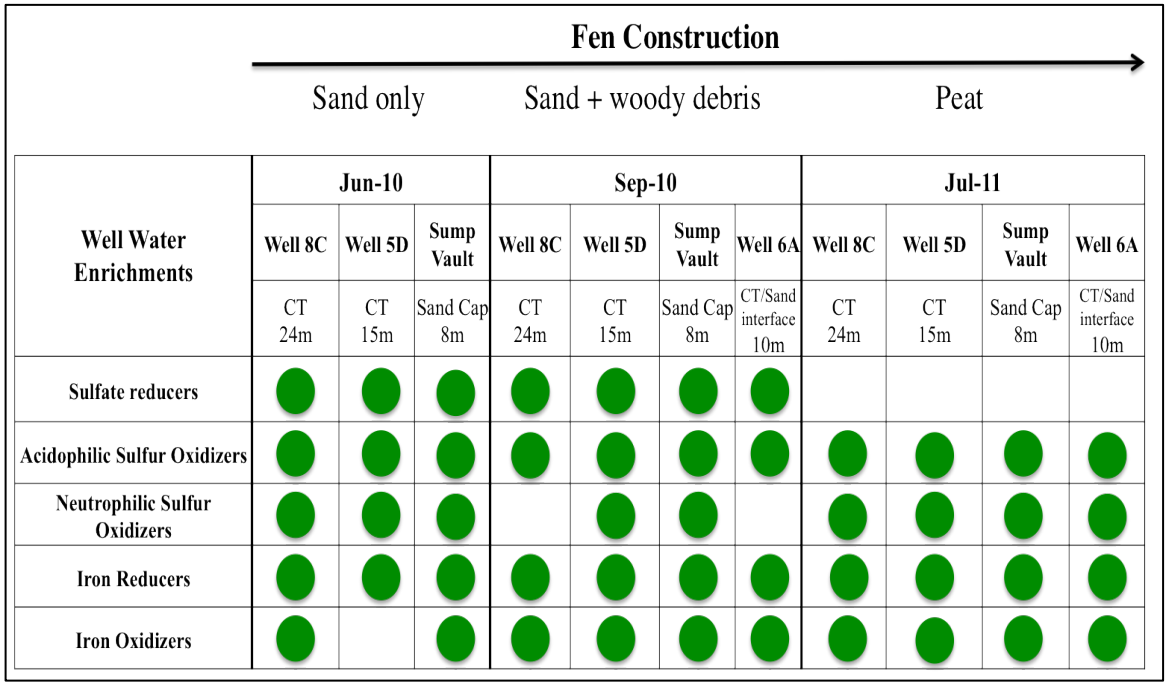
reduction is occurring within the fen porewaters. Magnetite, that can include of both Fe^{3+} and Fe^{2+} (Lovley and Phillips, 1988), only appears in the July 2011 in the mid sediment sample. The July 2011 mid sediment sample is most closely located to Well 5D (Figure 3.2). Although Well 5D has a Fe^{2+} concentration of 2.98μM in July 2011, it is not the highest concentration of ferrous iron in July 2011. The presence of magnetite could reflect precipitation of Fe^{2+} minerals by iron-reducing bacteria (Lovley et al., 1986; Lovley and Phillips, 1988).

4.1.1.4 – Water Environmental Microbial Enrichments

The extensive occurrence of reduced iron and sulfur species within the fen porewaters indicates microbial reduction. To examine the microbial metabolic capacity of the porewaters of the Sandhill Fen well water samples were enriched with media designed to promote the growth of specific microbial metabolisms. Positive growth of these enrichments for all well water samples can be seen in Figure 4.2. Positive growth of environmental microbial enrichments proves that these organisms are viable *in situ* and identifies their potential to occur in the environment, but does not necessarily mean they are always metabolically active.

Well water environmental enrichments were successfully grown for almost all microbial metabolisms for all sampling times, for each well location, excluding somewhat surprisingly the sulfate-reducers in July 2011. Acidophilic sulfur-oxidizers and iron-reducers are found at each well, for each sampling campaign. The ability to enrich for these metabolisms sampled at the locations and sampling periods in this thesis is consistent with the notion that microbial activity is playing a role in the oxidation and

reduction of iron and sulfur species in the Sandhill Fen and that S cycling with the production of aqueous H_2S is likely more broadly influenced beyond SRB activity.



Well Water Enrichments	Jun-10			Sep-10				Jul-11			
	Well 8C	Well 5D	Sump Vault	Well 8C	Well 5D	Sump Vault	Well 6A	Well 8C	Well 5D	Sump Vault	Well 6A
	CT 24m	CT 15m	Sand Cap 8m	CT 24m	CT 15m	Sand Cap 8m	CT/Sand interface 10m	CT 24m	CT 15m	Sand Cap 8m	CT/Sand interface 10m
Sulfate reducers	●	●	●	●	●	●	●				
Acidophilic Sulfur Oxidizers	●	●	●	●	●	●	●	●	●	●	●
Neutrophilic Sulfur Oxidizers	●	●	●		●	●		●	●	●	●
Iron Reducers	●	●	●	●	●	●	●	●	●	●	●
Iron Oxidizers	●		●	●	●	●	●	●	●	●	●

Figure 4.2: Positive growth chart of environmental microbial enrichments from well water samples. Green circles indicated positive growth, while a blank square indicates no growth.

The presence of acidophilic sulfur oxidizers in a circumneutral pH value environment could suggest microenvironments created by the bacteria themselves (Koschorreck, 2008). Their acidic microenvironments could be an important geochemical setting in which $H_2S_{(aq)}$ is the dominate sulfide species (Rickard and Luther, 2007). Microbial sulfate reduction can also occur at lower pH values (Fortin et al., 1996: Koschorreck, 2008) therefore these acidic microevironments might be a localized area of potential sulfur cycling.

The disappearance of positive growth for sulfate-reducing bacteria in July 2011 could just reflect an unsuccessful enrichment of the Sandhill Fen well waters during that

trip. The deposition of peat that was beginning to be laid down in July 2011 would provide a more liable source of organic carbon as well as more sulfate-reducing bacteria to the underlying sand cap and porewaters, which may have stimulated $H_2S_{(aq)}$ production. It is known that peat and wetlands contain large organic carbon stores (Fenner and Freeman, 2001; Bridgeham et al., 2006; Limpens et al., 2008), and it has been reported that sulfate-reducing bacteria are dominant in peat systems (Yavitt and Lang, 1990; Mandernack et al., 2000; Loy et al., 2004), which suggest that SRBs should be present in the surficial sand cap right below the peat in July 2011, further suggesting that we were merely unsuccessful at enriching for that metabolism.

The ability to enrich for both oxidizing and reducing Fe and S metabolisms in the same water samples could have great influence on Fe and S cycling within the fen if all aspects of their metabolic needs are present, like organic carbon and an abundance of reduced and oxidized Fe and S.

4.1.2 – Sand Cap

Aqueous porewater data indicates reduction of Fe^{3+} and SO_4^{2-} with widespread Fe^{2+} and $H_2S_{(aq)}$ present in the Sandhill Fen, with the highest concentrations of the reduced species in the sand cap wells in the lower region of the fen. This finding could suggest a localized source area, although $H_2S_{(aq)}$ is detected in the wells located in the CT in the upper region of the fen. XRD and sequential extraction data can point out iron and sulfur pools in the sand cap that could support microbial iron and sulfur reduction.

4.1.2.1 – Sand Cap Iron

Iron total was sequentially extracted from field sand cap samples on Upper, Mid and Lower samples on each sampling campaign (Figure 4.3). Total iron values ranged, excluding the recalcitrant fraction, from 10μmol/g to 132μmol/g.

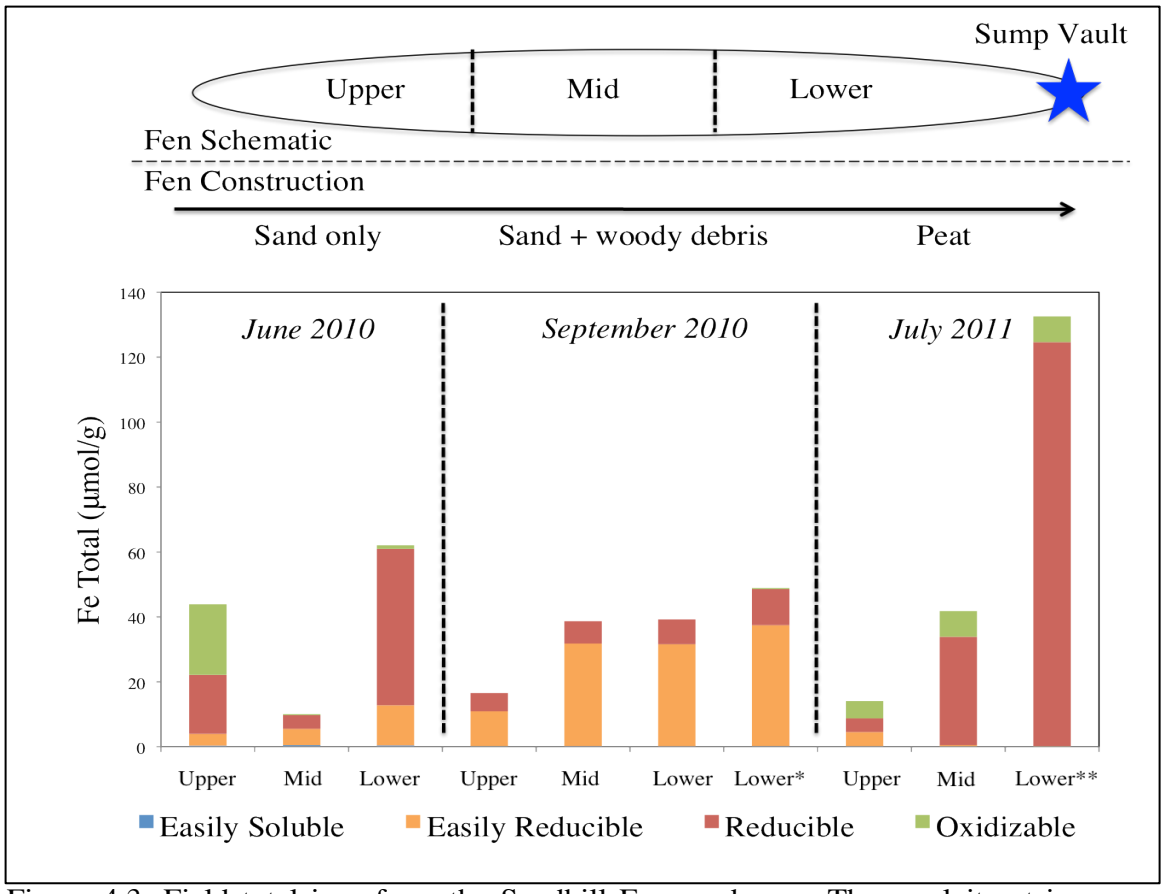


Figure 4.3: Field total iron from the Sandhill Fen sand cap. The recalcitrant iron was removed to allow easier comparisons to the more biologically available sediment compartments.

The dominating Fe containing operationally defined sediment fractions are the easily reducible and reducible fractions. The reagents used to extract these fractions cause reductive dissolution, and typically attack amorphous iron oxyhydroxides and more crystalline iron oxides respectively, which are widely abundant in sediments (Straub et

al., 2004). The easily reducible and reducible fractions are the most dominant Fe containing fractions in the Sandhill Fen sand cap (Figure 4.3).

X-ray diffraction analyses detects more crystalline minerals, and is unable to discern amongst specific amorphous minerals, where the iron could be more bioavailable. XRD results show clay minerals, such as illite and kaolinite in the total mineralogy ranging from 1 to 13% for all three sampling campaigns (Table 4.2, Table 4.3, Table 4.4). Illite and kaolinite are known Fe^{3+}-containing clays associated with oil sands processing and tailings (Kaminsky et al., 2009) and it has been previously stated that these clays contain iron oxides (Kaminsky et al., 2009; Penner and Fought, 2010). Mackinnon and Sethi (1993) reported that iron comprises 3% of the solids in mature fine tailings ponds at Syncrude and Suncor. The iron associated with these clays can be bioavailable and stimulate microbial reduction of iron (Kostka et al., 2002). The XRD did however show between 4 and 11% amorphous and organic materials (Table 4.2, Table 4.3, Table 4.4) amongst the three sampling campaigns, which could represent a more microbially accessible fraction. The September 2010 sediment sample showed the smallest amorphous percentages (3%, Table 4.3) of all three sediment samples, yet the sequential extraction data shows the iron is mostly associated with the easily reducible, more amorphous phases. This finding perhaps demonstrates that the iron from September 2010 is only bound to a small percentage of the sand cap sediments.

In all three sampling campaigns, the total sediment iron is the highest in the lower regions of the fen, towards the Sump Vault (June 2010 - 62μmol/g, September 2010 – 48.6μmol/g, July 2011 - 132μmol/g, Figure 4.3). Iron could be transported along the direction of ground water flow towards the lower region of the fen, increasing

concentrations at the Sump Vault and increasing the likelihood of iron reduction and oxidation processes occurring in this region.

The sand cap iron from June 2010 is dominated by the reducible and oxidizable sediment fractions (Figure 4.3). Moving from the upper region to the lower region of the fen, the more easily reducible fraction increases and oxidizable fraction decreases. The largest change in iron-associated sediment fractions occurs in September 2010 (Figure 4.3). The change of fen construction material may have provided more easily reducible iron to the fen. This easily reducible iron can be used by iron reducing bacteria and in turn stimulate iron oxidizing bacteria (see Section 4.1.3.1).

July 2011 iron-associated sediment fractions display the re-introduction of the oxidizable fraction, which represents reduced iron species and iron bound to organic matter (Tessier et al., 1979; El Bilali et al., 2002). XRD results show magnetite (a mixed Fe^{2+}/Fe^{3+}, mineral, Fe_3O_4) in the July 2011 Mid sediment sample (Table 4.4), potentially indicating that iron reduction is occurring, producing reduced iron minerals (Lovley et al., 1986). Although pyrite (FeS_2) was not picked up in the XRD analysis, the oxidizable fraction in July 2011 for all three sampling locations could include the reduced iron and sulfur mineral. The porewater data for July 2011 shows increased aqueous hydrogen sulfide and ferrous iron, which may be forming iron-sulfide minerals (El Bilali et al., 2002) another indication of microbial iron and sulfur reduction. July 2011 also demonstrates the disappearance of the easily reducible iron-associated sediment fraction, which again could indicate iron reduction as the bacteria may have consumed the more easily reducible iron in preference to the more crystalline iron, since more crystalline clays are reported to have slower rates of ferric iron reduction (Lovley, 1991). This

finding also supports porewater data from July 2011 (Figure 4.1, Table 4.1) that shows aqueous ferrous iron present in the wells within the sand cap and near the sand cap/CT interface (Sump Vault and Well 6A respectively).

Table 4.2 – XRD characterization of June 2010 sand cap sediments

	Upper	Mid	Lower
Quartz	70%	77%	67%
Albite/anorthite	1%	1%	1%
Microcline	6%	3%	3%
Illite/micas	4%	3%	4%
Gypsum	2%	1%	3%
Kaolinite	10%	8%	11%
Diaspore	1%	-	-
Organic	2%	2%	5%
Amorphous, ect.	4%	5%	6%

Table 4.3 – XRD characterization of September 2010 sand cap sediments

	Upper	Mid	Lower	Lower **
Quartz	91%	92%	95%	90%
Calcite	1%	~1%	-	-
Albite/anorthite	-	-	1%	1%
Microcline	1%	1%	1%	2%
Illite/micas	~1%	-	-	1%
Kaolinite	2%	2%	-	2%
Organic	1%	1%	-	~1%
Amorphous, ect.	3%	3%	~3%	3%

Table 4.4 – XRD characterization of July 2011 sand cap sediments			
	Upper	Mid	Lower *
Quartz	76%	49%	32%
Calcite	2%	5%	5%
Albite/anorthite	3%	5%	9%
Microcline	5%	5%	8%
Dolomite	-	5%	8%
Magnetite	-	3%	-
Illite/micas	3%	7%	13%
Montmorillonite	2%	9%	5%
Pyroxene	-	-	4%
Chlorite	3%	2%	5%
Kaolinite/serpentine	2%	5%	5%
Amorphous/organic, ect.	4%	5%	6%

4.1.2.2 – Sand Cap Sulfur

Operationally defined sulfur concentrations were sequentially extracted using the same extraction scheme as that for iron from field sand cap sediment samples on Upper, Mid and Lower samples on each sampling campaign (Figure 4.4). Total sulfur values (determined by the sum of the fraction concentrations) ranged from 11μmol/g (Lower** – July 2011) to 216μmol/g (Lower – June 2010) and there does not appear to be a trend either spatially or seasonally, only that June 2010 has the overall highest total sulfur concentrations.

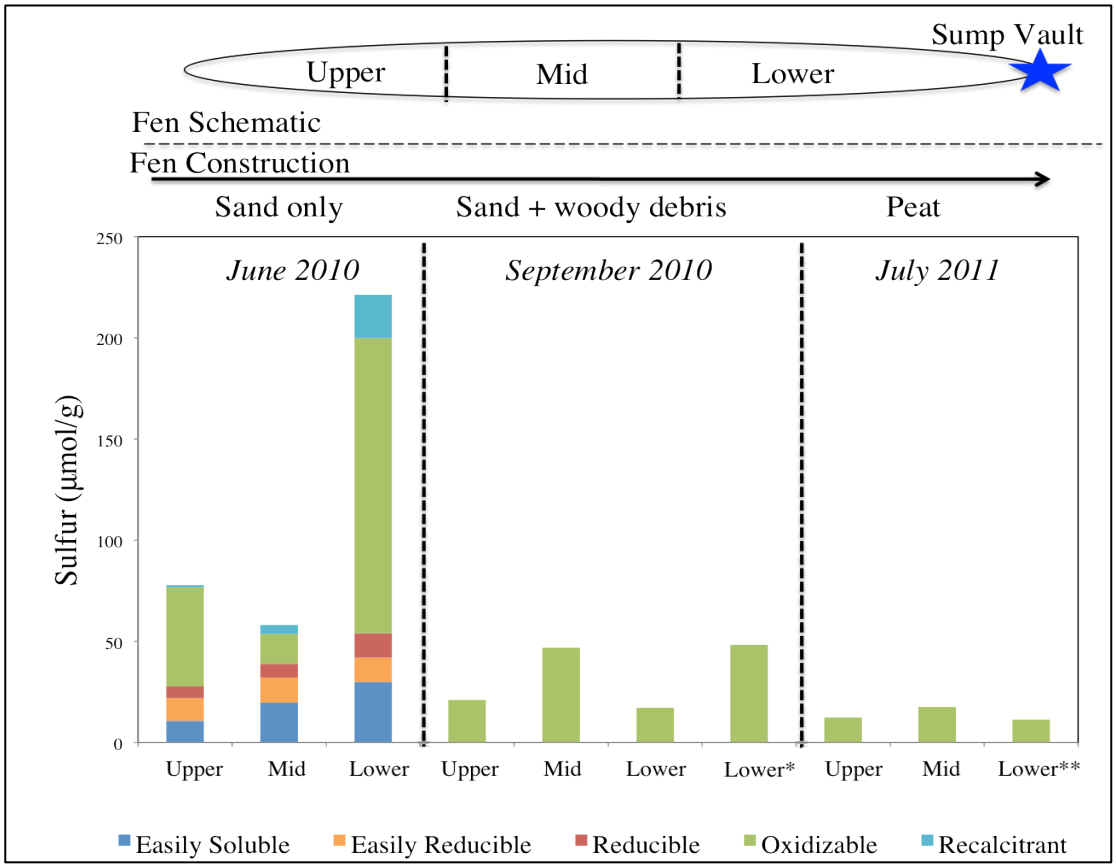


Figure 4.4: Total sulfur in sand cap sediments.

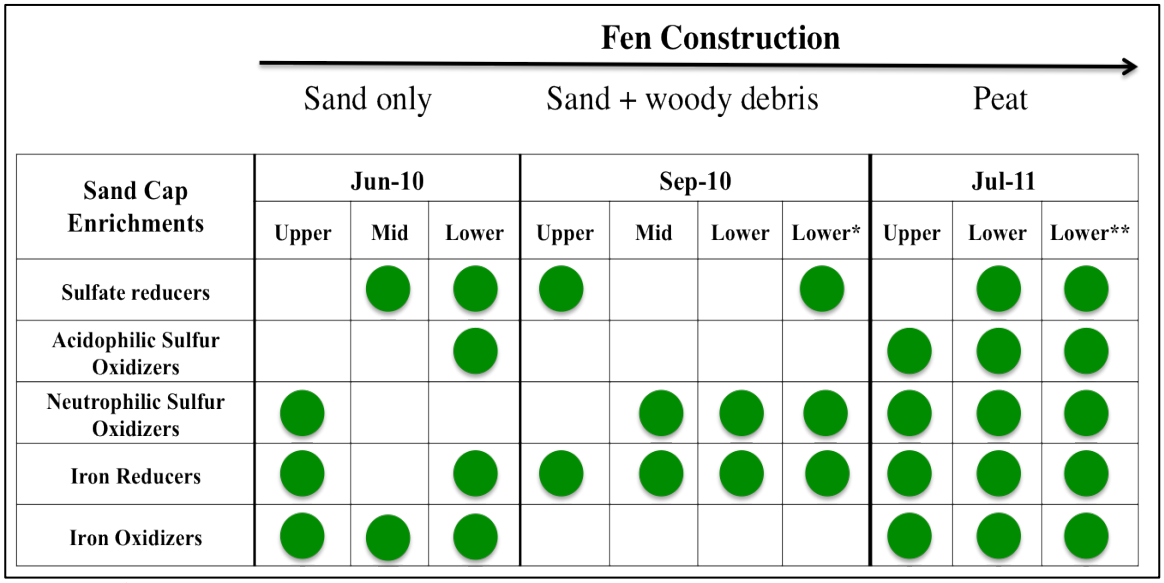
Sulfur pools are grouped into the same operationally defined fractions as the iron sediments. The most dominant sediment pool for sulfur in the sand cap for all each sample was the oxidizable sulfur, which is associated reduced sulfur in the form of sulfides and sulfur complexed with organic matter (El Bilali et al., 2002). The highest values for total sulfur were found in June 2010, and included sulfur from the reducible extraction fractions, representing oxidized sulfur species. XRD analysis indicates that June 2010 was the only sediment sampled that contained detectable amounts of gypsum ($CaSO_4$) (1-3%, Table 4.2). This source of sulfate likely accounts for the highest total sulfur concentrations in June 2010 amongst the three sampling campaigns.

The decrease in total sulfur values in the September 2010 and July 2011 sand cap samples (Figure 4.4) could be related to the new sand that was added, perhaps with less sulfur in the new sand cap materials. September 2010 and July 2011 sand cap sediment samples only show sulfur associated with the oxidizable sediment fraction (Figure 4.4). This oxidizable sulfur, most likely related to FeS minerals could represent FeS precipitates resulting from the increase in aqueous hydrogen sulfide and ferrous iron in the porewaters of the Sandhill Fen in September 2010 and July 2011 (Figure 4.1, Table 4.1), although no pyrite of sulfur minerals were found in the XRD results, which could be related to the heterogeneities in samples that were allocated for XRD and sequential extraction.

The sulfide species that most likely make up the oxidizable sulfur from the Sandhill Fen sand cap sediments might be the initial source of sulfur for sulfur-oxidizing bacteria present in the sand cap sediments and the well waters of the fen. Positive growth is observed for this metabolism and will be discussed in below.

4.1.2.3 – Sand Cap Microbial Enrichments

Positive growth for sand cap enrichments (determined by colour change of media and the formation of solid precipitates, see Section 3.4) is shown in Figure 4.5. There is less positive growth seen in the sand cap enrichments (fewer enrichments showed colour change and precipitation of solids) compared to the well water enrichments. This difference in growth between well water and sandy sediments could be the lack of nutrients available in the sand cap. The porewaters may contain more nutrients and organic carbon, therefore providing a better living environment for the bacteria to thrive.



Sand Cap Enrichments	Jun-10			Sep-10				Jul-11		
	Upper	Mid	Lower	Upper	Mid	Lower	Lower*	Upper	Lower	Lower**
Sulfate reducers		●	●	●			●		●	●
Acidophilic Sulfur Oxidizers			●					●	●	●
Neutrophilic Sulfur Oxidizers	●				●	●	●	●	●	●
Iron Reducers	●		●	●	●	●	●	●	●	●
Iron Oxidizers	●	●	●					●	●	●

Figure 4.5: Positive growth chart of environmental microbial enrichments from sand cap sediment samples. Green circles indicated positive growth, while a blank square indicates no growth.

There is a slight increase in positive growth for samples that are taken in the mid and lower regions of the fen. This trend may be related to the flow of water towards the lower regions of the fen towards the Sump Vault (Figure 3.2), or be due to material changes throughout the seasons. Higher total iron within the sand cap sediments is shown in the lower regions of the fen (Figure 4.3) and could be a source of energy for the iron-metabolizing bacteria. The positive growth of sulfate reducers towards the mid and lower regions of the fen corresponds with the increased $H_2S_{(aq)}$ at the Sump Vault and Well 6A, which showed the highest $H_2S_{(aq)}$ concentrations in the well waters (Figure 4.1).

In July 2011, excluding the upper region sulfate-reducing enrichment, all enrichments showed positive growth. This result is most likely due to the increase in organic carbon from the addition of the woody debris and the beginning of peat amendment to the fen. Similar to the well water enrichments, and perhaps even more important for the sand cap sediment, the addition of peat will increase organic carbon

content since wetlands and peatlands are high in organic carbon content (Fenner and Freeman, 2001) and could potentially introduce bacteria originally associated with that peat. When the fen is more fully grown, with the successful growth of peat and other plants, the sand cap would be expected to have even more diversity in microbial communities, and greater heterotrophic metabolisms associated with more reducing conditions including sulfate reduction and methanogenesis. Methanogenesis and methane-associated bacteria are common in wetlands and fens (Yavitt and Lang, 1990; Chanton et al., 1995, Loy et al., 2004)

4.2 – 16S rRNA Sequencing of Environmental Microbial Enrichments

The reported porewater, sequential extraction, and XRD data, and positive growth of environmental microbial enrichments suggest that microbial reduction and oxidation of iron and sulfur species are possible in the CT, surficial sand cap and their porewaters in the Sandhill Fen. Porewater Fe and S geochemistry and functional enrichment results suggest a trend of increasing heterotrophy as the fen developed over the sampling periods. 16S rRNA sequences were obtained from environmental microbial enrichments from the Sump Vault well during the sampling campaigns in June 2010, September 2010 and July 2011. Although there was positive growth recorded for the other wells and surficial sand samples from the Sandhill Fen, only the Sump Vault well water samples were submitted for 16S rRNA analysis because the Sump Vault had positive growth for the most metabolisms and is always accessible to sample, making

future sample comparisons possible. Therefore, the following sequence results will always be from the Sump Vault well water microbial enrichments.

A summary of putative function is reported for all sulfur and iron microbial enrichments. The putative functions reported describe the metabolic potential of bacteria that have been found in the current literature to the best of my knowledge.

4.2.1 – Iron Reducers

Iron-reducing enrichment sequences are shown in Figure 4.6, and their putative functions are displayed in Figure 4.7. Microbial diversity among the iron-reducers varies and there are noticeable shifts in putative function amongst the three sampling campaigns.

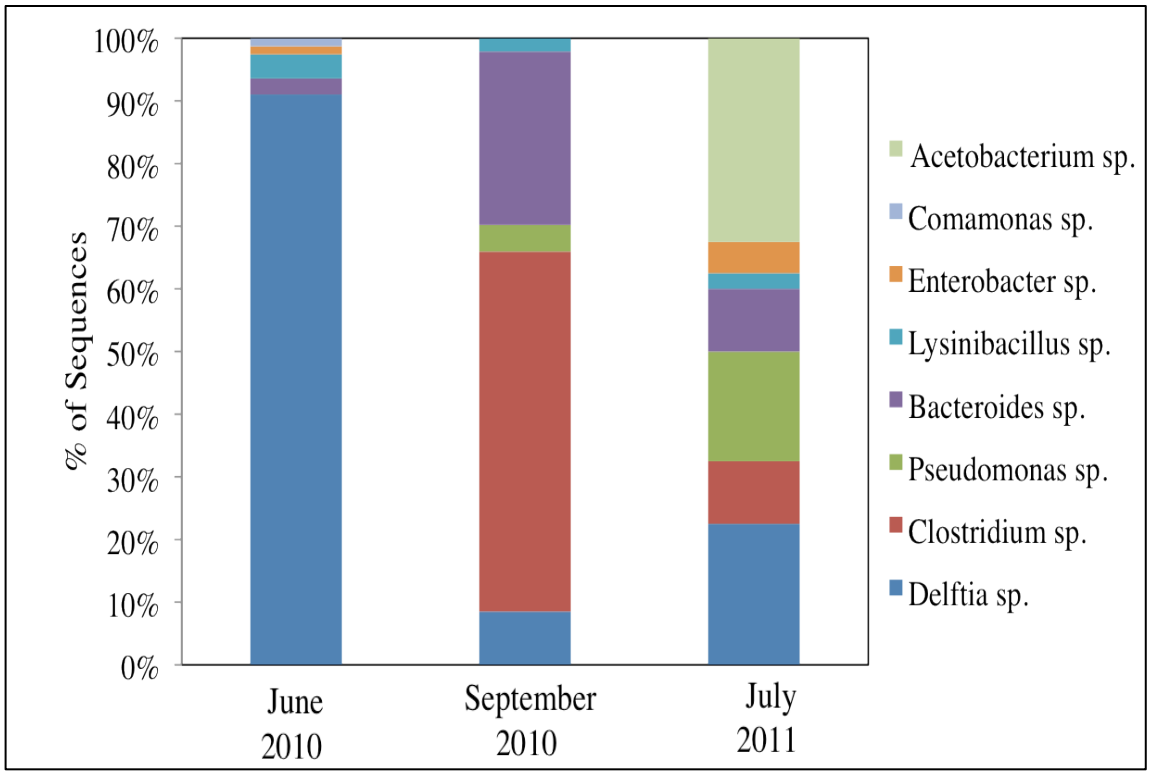


Figure 4.6: 16S rRNA sequences from iron-reducing enrichments.

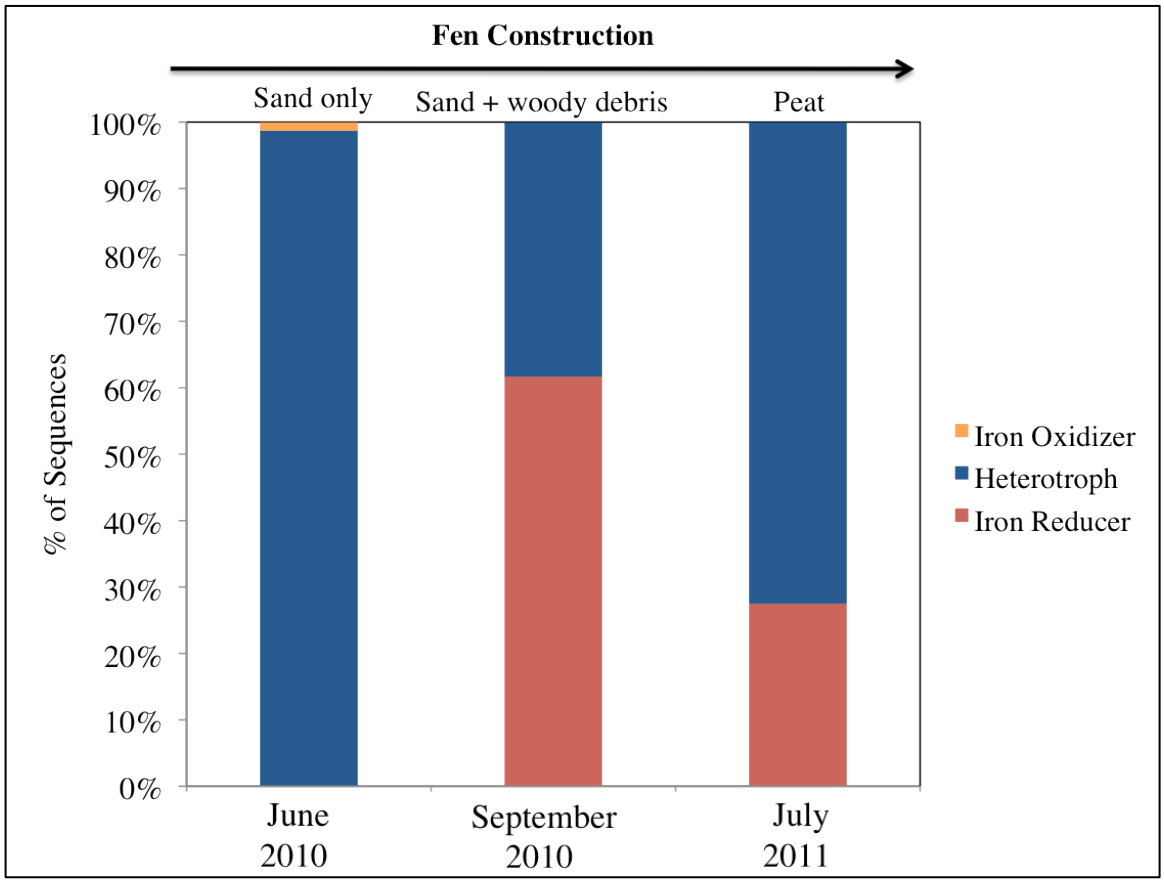


Figure 4.7: Iron-reducer putative function.

Well-known iron-reducers like *Geobacter* and *Shewanella* spp. were not present in the Sump Vault well water enrichments. The most likely iron-reducers in these enrichments are those from the genus of *Clostridium*, *Pseudomonas*, and *Bacteriodes*. *Clostridium* spp. have been shown to mostly reduce iron through fermentation (Lovley et al., 2004). Wang et al. (2009) found that *Clostridium* spp. are flexible in their use of electron acceptors, meaning there was no significant effect on their iron reduction capability when the Fe^{3+} source is more difficult to metabolize. *Clostridium* spp. have also been shown to be able to reduce ferric iron using smectite clays as the only electron acceptor (Kostka et al., 2002) and can also perform dissimilatory Fe(III) reduction

(Dobbin et al., 1999). Clays are an important iron input to the Sump Vault well water since clays are a large component in composite tailings.

Bacteriodes spp. have been detected from iron reducing enrichments (Lin et al., 2007). They are usually found within the human gut and in fecal matter (Yuan et al., 2011). It is not currently clear if the *Bacteriodes* spp. identified are in fact playing a part in the reduction of ferric iron, but it is possible they can utilize various substrates to produce the carbon molecules needed for dissimilatory ferric iron reduction (Wang et al., 2009).

Although *Clostridium* and *Bacteriodes* are known to be strict anaerobes (Wang et al., 2009), they may have created a microaerophilic or anaerobic microenvironment in the enrichment flasks in order to grow (since the iron-reducing enrichments are grown on the bench top), like sulfate-reducers found in oxidized marine sediments (Jorgensen, 1977). These microniches allow the bacteria to create an ideal environment around them, which differ from the bulk geochemical system (Fortin et al., 1996; Koschhorreck, 2008).

Pseudomonas spp. have been shown to aerobically reduce hydrous ferric iron-oxides (Hersman et al., 1996). Another study by Hersman et al. (2001) showed that a *Pseudomonas* sp. was able to grow in batch culture on ferrihydrite, goethite and hematite. These data suggest that Pseudomonas spp. could also be reducing iron in these enrichments

Most of the other species present in the Sump Vault iron-reducing enrichments are heterotrophs. *Delftia* spp. are aerobic chemo-organotrophs (Wen et al., 1999) and are present in all enrichments of iron-reducing bacteria for the Sump Vault well. They have not been reported to participate in iron reduction, but may have been using the acetate

that was supplemented into the iron reducing media. *Delftia* have been isolated from sediments, crude oil, activated sludge and clinical samples (Wen et al., 1999). It is most likely that the *Delftia* sp. are degrading hydrocarbons or other organic matter in the porewaters of the CT and in the Sump Vault well. *Acetobacterium* spp. have also not been found to reduce iron and are also found in oil-associated environments. *Acetobacterium* spp. are acetogens that oxidize hydrogen coupled to the reduction of carbon dioxide (Balch et al., 1977). They have been shown to be associated with oil in Alberta oil fields, using the oil phase as their source of hydrogen (Kryachko et al., 2012).

Figure 4.6 shows that the June 2010 enrichment does not show any known iron-reducers present. After the input of organic carbon from the addition of topography and the beginning of woody debris deposition to the developing fen, the iron-reducers are picked up in September 2010. This result could perhaps indicate carbon substrate limitations in June 2010, preventing the iron-reducers from performing their metabolic functions. This result is supported with the porewater data from the Sump Vault, which shows increasing ferrous iron concentrations as the fen develops, indicating ferric iron reduction (Figure 4.1, Table 4.1). July 2011 shows an increase in heterotrophic metabolic function, compared to those bacteria that are known to do iron reduction (Figure 4.7). This increase in heterotrophy could indicate that the iron-reducing bacteria and other heterotrophs are no longer starved for carbon, since the addition of carbon has been shown in increase reduction rates (Li et al, 2006) or that the deposition or peat and organic carbon have introduced heterotrophic bacteria to the Sandhill Fen porewaters.

The iron reducing enrichments at the Sump Vault well contain both known iron reducers and bacterial species that have not been shown to reduce iron. The iron reducers

include *Clostridium*, *Pseudomonas* and *Bacteriodes* spp. The differences in community members between sampling campaigns could reflect change in organic carbon availability and the introduction of new heterotrophic bacteria from the peat and woody debris.

4.2.2 – Iron Oxidizers

Similar to the iron-reducing enrichments, the iron-oxidizers vary in diversity amongst sampling campaigns, with the highest diversity occurring in July 2011 (Figure 4.8). The iron-oxidizing enrichment putative functions are shown in Figure 4.9. The most obvious iron-oxidizer in the enrichments is the *Alicyclobacillus* sp. *Alicyclobacillus* spp. have been shown to chemoautotrophically oxidize ferrous iron and sulfur species in various acidic environmental conditions including a gold mine in Montana (Yahya et al., 2008), a copper mine in China (Guo et al., 2009), solfataric soils (Jiang et al., 2008) and sulfidic mine waste dumps (Breuker et al., 2009). Although the Sump Vault has circumneutral pH values, *Alicyclobacillus* could create microenvironments or niches within the pore waters, surrounding themselves in more tolerable acidic conditions.

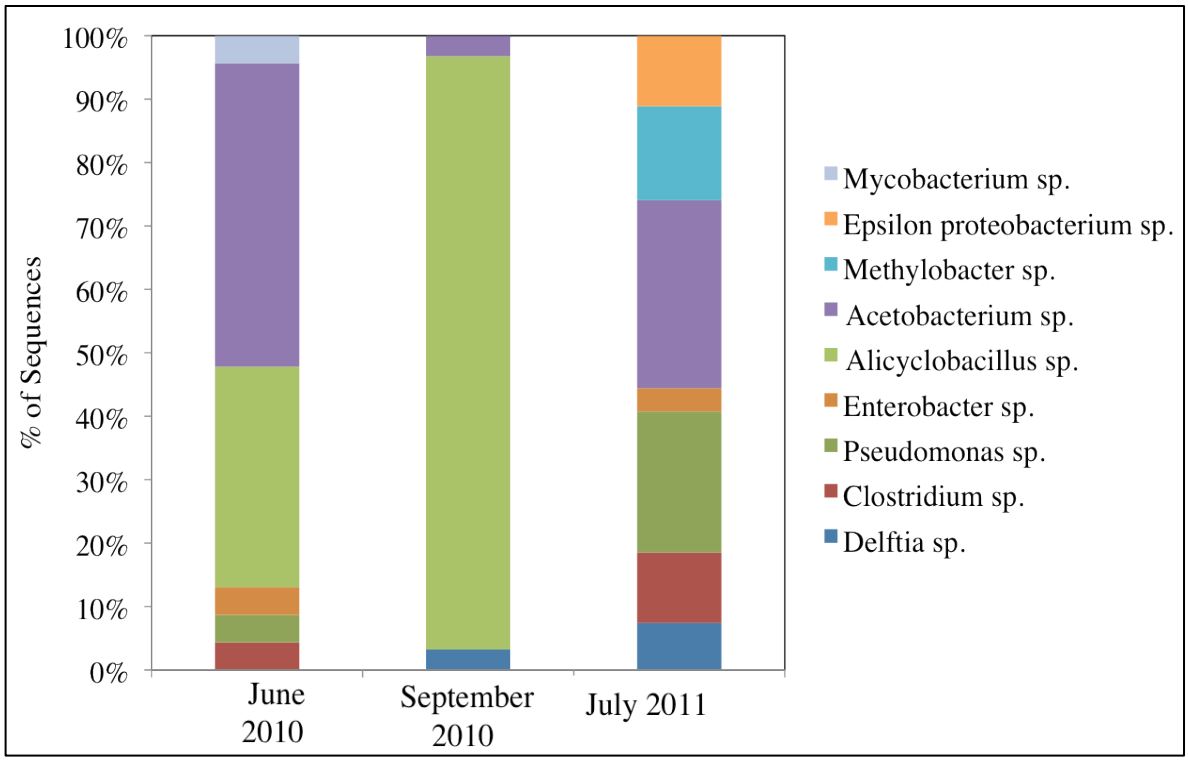


Figure 4.8: 16S rRNA sequences from iron-oxidizing enrichments.

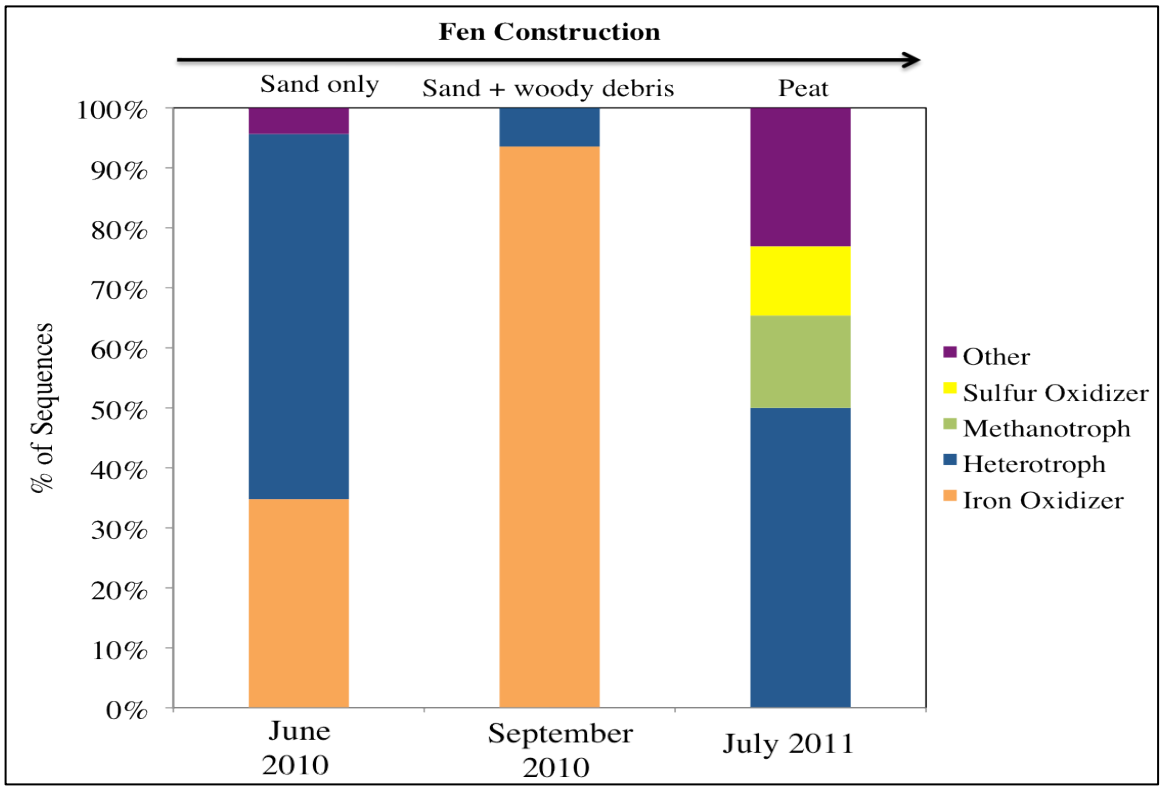


Figure 4.9: Iron-oxidizer enrichment putative functions.

Other species present in the iron-oxidizing enrichments are often involved with hydrocarbon-degradation or associations with oil fields, including *Mycobacterium* spp. (e.g. Chikere et al., 2009; Zeng et al., 2010), *Pseudomonas* spp. (e.g. Kanaly and Harayama, 2000; Chickere et al., 2009) and *Acetobacterium* spp. (e.g. Song et al., 2006; Kryachko et al., 2012). *Methylobacter* spp. are strict anaerobes that oxidize methane (Bowman et al., 1993). These species may be present in the iron-oxidizing enrichments presented above since there may be hydrocarbons and other oil waste materials in the initial Sump Vault water that inoculated the enrichments and may have been in the volume of enrichment that was used to inoculate the new flasks, since there is no carbon in the iron-oxidizing media (Wang et al., 2009), but it is unclear why there is methane present in the enrichment.

It is interesting to note, that in September 2010 the putative function of the enrichment community is dominated by iron-oxidizing metabolism (Figure 4.9). This increase in the percentage of iron-oxidizers corresponds with the increase in iron-reducing metabolisms also shown to increase in September 2010 (Figure 4.7). The increase in iron-reduction most likely stimulated by the increase in organic carbon addition to the fen increases the concentration of Fe^{2+} (Figure 4.1-C, Table 4.1). This ferrous iron increase could provide the ferrous iron that is oxidized by the iron-oxidizing bacteria, allowing for iron cycling occurring within the porewaters of the sand cap and perhaps the CT near the Sump Vault. The microbial coupling of iron reduction and iron oxidation or at least these processes occurring in close proximity has been discussed by Weiss et al. (2003), Straub et al. (2004), Weber et al. (2006), and Bruun et al. (2010). They suggest this coupling could take place in anaerobic environments or environments

shifting between oxic and anoxic, which could reflect the conditions in the porewaters of the developing fen.

The ability to successfully grow iron-oxidizers from all three sampling campaigns shows that the sand cap from which the Sump Vault is situated has a high diversity of both species and metabolisms and could be capable of sustaining this metabolism. The potential for iron cycling is high at the Sump Vault since there is available iron in the pore waters for both oxidation and reduction to occur (Figure 4.1-C, Figure 4.1-D, Table 4.1). It is interesting to find iron-oxidizers in mine wastes that are not acidic, since it is well known that they are abundantly found in acidic metal mine waste environments (Baker and Banfield, 2003; Druschel et al., 2004; Breuker et al., 2009). These results shed new light on the fact that iron cycling may occur at the microscale within microniches that differ from bulk alkaline conditions, such as the Athabasca oil sand tailings ponds and composite tailings deposits, where such acidophilic metabolisms have not been considered to date.

4.2.3 – Sulfate Reducers

Sulfate-reduction was detected both June and September 2010 in sulfate-reducing media inoculated with Sump Vault well water, but at the time of DNA extraction, the only sample that was still positively showing sulfate-reduction was June 2010. Sulfate reduction from Sump Vault water from July 2011 was not observed from inoculated enrichments from that sampling period. Hence, June 2010 is the only Sump Vault water sample that 16S rRNA sequencing could be completed on (Figure 4.10).

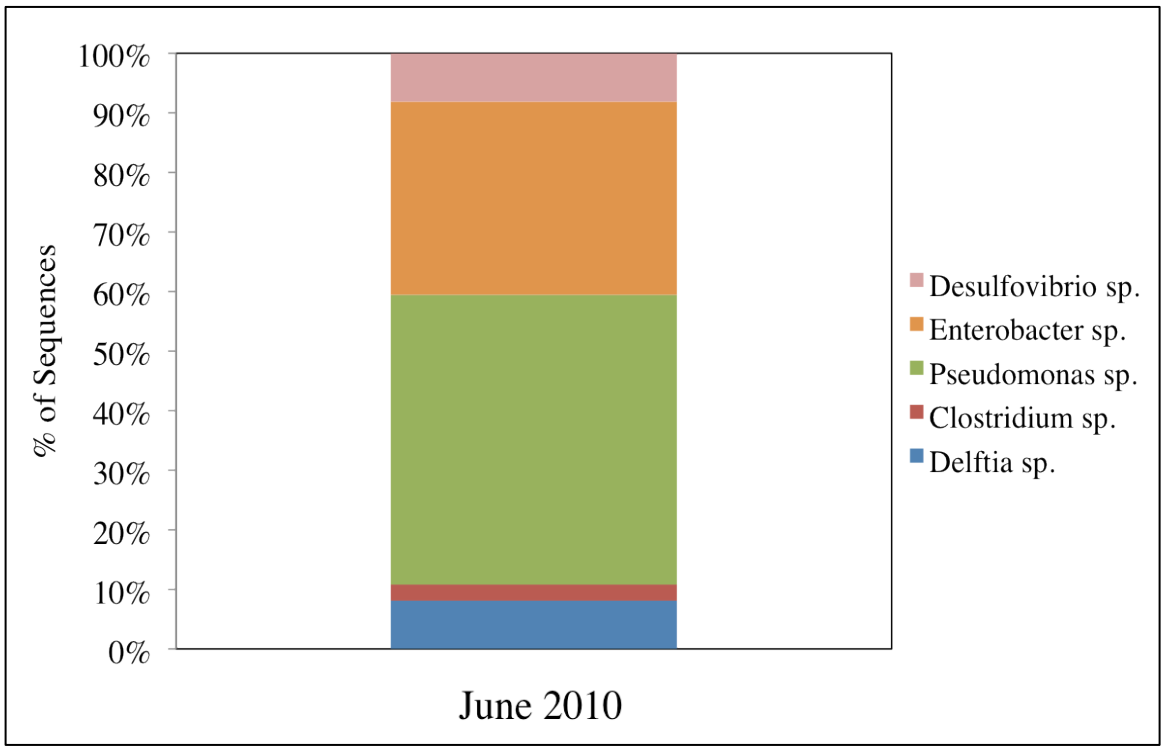


Figure 4.10: 16S rRNA sequences for sulfate-reducing enrichments.

The most obvious sulfate-reducer present in the sequence data is the *Desulfovibrio* sp. who is known as a classic sulfate-reducer (Hansen, 1994) and the majority of the sulfate-reducing bacteria in oil field environments are known to be from the genus *Desulfovibrio* (Agrawal et al., 2010). Several species of *Clostridium* are known to reduce forms of sulfur, including thiosulfate, and elemental sulfur (Mechichi et al., 2000; Hernandez-Eugenio et al., 2002; Sallam and Steinbuchel, 2009; Agrawal et al., 2010), and could be responsible for aqueous hydrogen sulfide production via the reduction of intermediates produced by *Desulfovibrio* sp. within the enrichment.

Enterobacter spp. are facultative anaerobes that are able to produce hydrogen gas (Nandi and Sengupta, 1998) and also associated with nitrogen-fixing roots of plants, fixing nitrogen to ammonia (Signh et al., 1983; Ronkko et al., 1993) and denitrification

(Paturea et al., 1999). *Enterobacter* species have only been recently shown be able to grown in media containing thiosulfate as the electron acceptor (Agrawal et al., 2010). The *Enterobacter* species present could in addition to the *Desulfovibrio* be contributing to the sulfur reduction at the Sump Vault.

Since there was only one successfully sequenced sulfate-reducing bacteria enrichment (Figure 4.10), a comparison of putative function amongst sampling campaigns is impossible. However, it can be expected that the introduction of organic carbon to the porewaters from the overlying peat and woody debris would increase sulfate reduction, as well as introduce new sulfate reducers to the sand cap. This is most likely the case, since $H_2S_{(aq)}$ concentrations increase each sampling campaign in the Sump Vault (Figure 4.1-A, Table 4.1). SRBs produce the aqueous hydrogen sulfide, but Fe/S-oxidizers that can clearly survive in the circumneutral pH conditions (Figure 4.3 and Figure 4.5) by creating microenvironments where the pH is lower, increases $H_2S_{(aq)}$ species concentration. This increase in acidic micropockets is the multifunctional process by which $H_2S_{(g)}$ is generated when the dewatering lowers the volume and partial pressure in the wells therefore releasing the gas out of solution.

Sulfate reduction at the Sump Vault well, is demonstrated in June 2010 and September 2010 (Figure 4.3), and known sulfate reducers are present in the June 2010 enrichment (Figure 4.9). Microbial sulfate reduction and sulfur disproportionation will play a large role in the cycling of iron and sulfur at the Sump Vault and in the porewaters of the CT beneath the fen.

4.2.4 – Neutrophilic Sulfur Oxidizers

Similar to the sulfate-reducing enrichments, only September 2010 and July 2011 neutrophilic sulfur-oxidizers were showing positive growth at the time of DNA extraction (Figure 4.11), but positive growth was initially observed for the June 2010 sampling campaign Sump Vault water (Figure 4.3). Putative metabolic function is displayed in Figure 4.12.

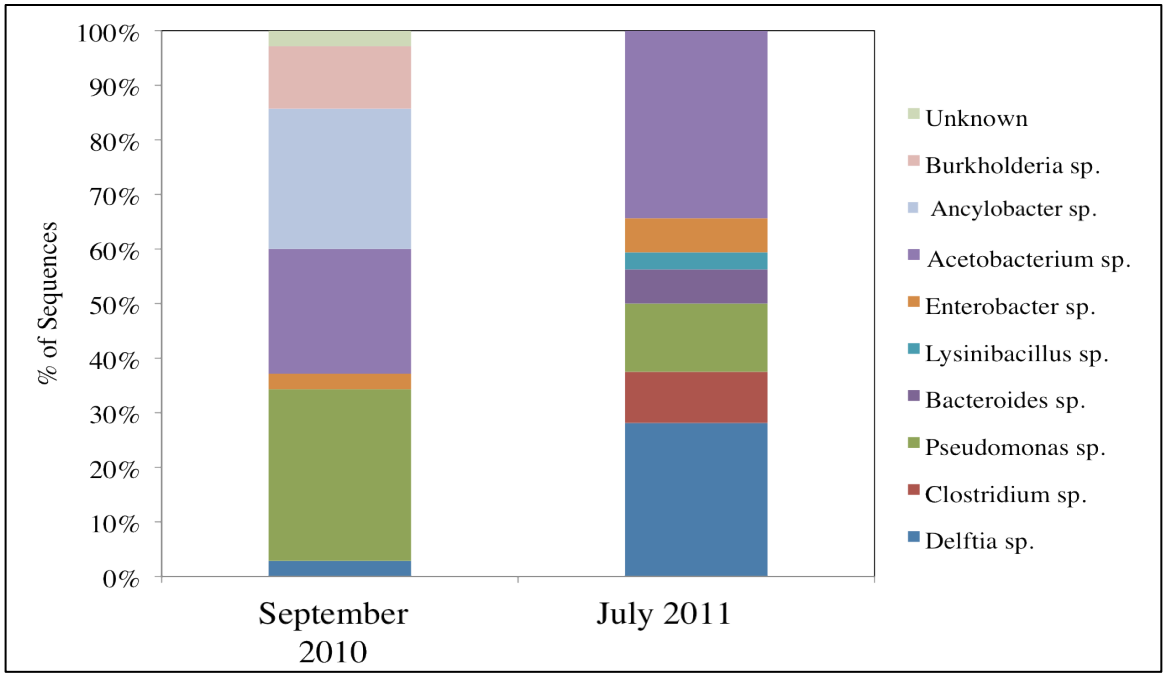


Figure 4.11: 16S rRNA sequences for neutrophilic sulfur-oxidizing enrichments.

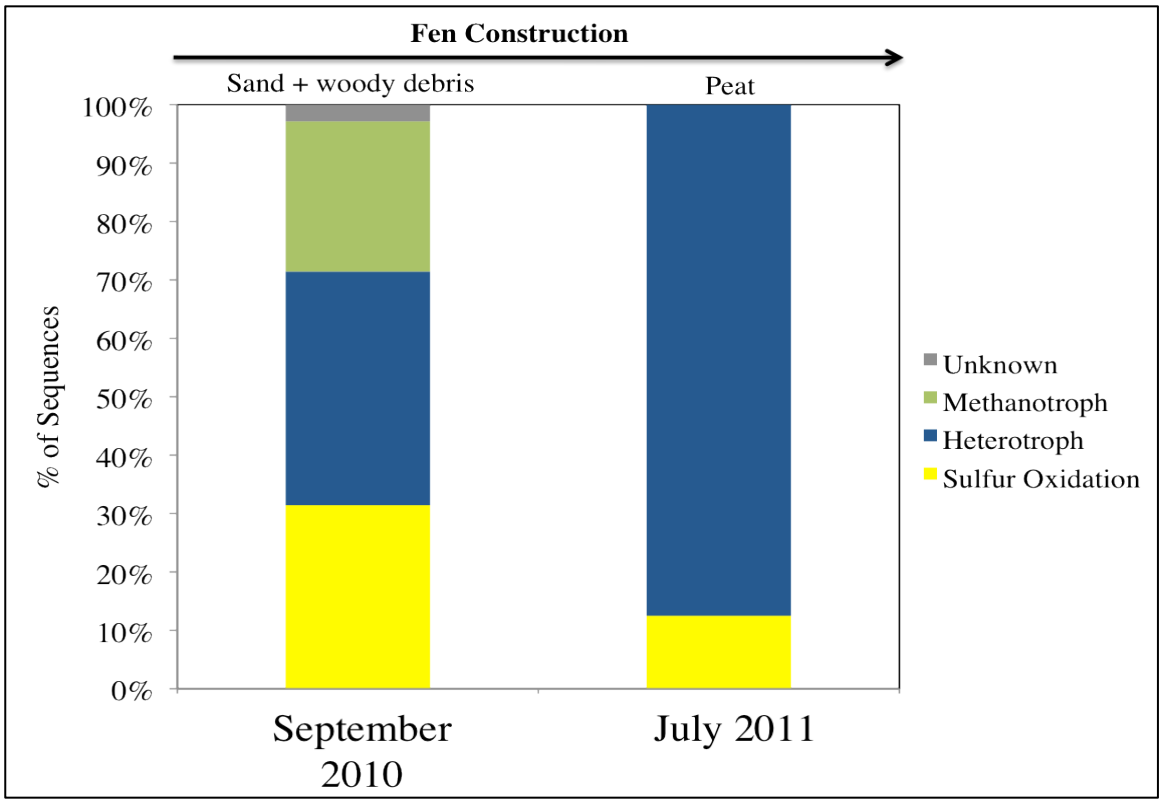


Figure 4.12: Neutrophilic sulfur-oxidizing enrichment putative functions.

Ancylobacter spp. appear for the first time in the all of the enrichments for Sump Vault well water in the neutrophilic sulfur-oxidizers. *Ancylobacter* spp. are known methylotrophs, who oxidize methane and other once carbon molecules as a carbon and energy source (Firsova et al., 2009). *Ancylobacter* spp. have recently been shown to be facultative, using other carbon molecules such as oxalate (Lang et al., 2008) and previously shown to degrade 1,2-dichloroethane (van de Wijngaard et al., 1992). Most recently, Firsova et al. (2009) discovered a new *Ancylobacter* sp. able to utilize dichloromethane as the sole carbon and thiosulfate as its energy source during aerobic facultative methylotrophy. This species was found in creeks, ponds, lakes and soil environment (Firsova et al., 2009). Although there have been several reports of aerobic methylotrophy within the *Ancylobacter* genus (e.g. Doronina et al., 2001; Firsova et al.,

2009) that does not explain why *Ancylobacter* spp are found within neutrophilic sulfur-oxidizing enrichments in which there is no carbon directly added to the media. There could possibly be carbon from the inoculation of the enrichments from the Sump Vault porewater. Wang et al. (2009) reported that residual organic matter from soil sediments added to microbial enrichments might have contained some organic carbon to support the growth of carbon utilizing metabolisms, but these residual carbon substrates would not likely be enough to support the growth of all of these carbon utilizing species.

Pseudomonas spp. have been observed to anaerobically oxidize thiosulfate to tetrathionate (Sorokin et al., 1999). *Pseudomonas* isolates were also able to anaerobically oxidize aqueous hydrogen sulfide, elemental sulfur and thiosulfate in culture media inoculated with artificial wetland sediment samples (Pacheco et al., 2008). The *Pseudomonas* sequences from the neutrophilic sulfur-oxidizing enrichments are a significant member of the enrichment community. They are potentially the most important species in these enrichments from Sump Vault water and *Pseudomonas'* potential participation in sulfur oxidation could play a large role in the sulfur cycling at the Sump Vault, especially since the pH values of the Sump Vault and the enrichment media are comparable.

Delftia spp. are organoheterotrophs, but one study by Graff and Stubner (2003) found isolates most closely related to *Delftia* spp when researching thiosulfate-oxidizing bacteria in Italian rice field soil. They concluded that they might represent new sulfur-oxidizers in rice field soil, but state that no species of *Delftia* have been known to oxidize sulfur (Graff and Stubner, 2003). It would be interesting to look deeper into the

metabolic potential of *Delftia* spp, since they are found in all sulfur and iron enrichments analyzed so far.

As was seen in the iron reducing and iron oxidizing putative function figures (Figure 4.7 and Figure 4.9), there is an increase in the number of heterotrophic metabolisms in the neutrophilic sulfur oxidizing enrichments (Figure 4.12). This increase in heterotrophy is again most likely related to the deposition of peat and peat-associated bacteria.

4.2.5 – Acidophilic Sulfur Oxidizers

The acidophilic sulfur-oxidizing enrichments from the Sump Vault well water by far show the most species diversity within individual enrichments, but also show the most differences amongst sampling periods, when compared to the other metabolisms (Figure 4.13). Figure 4.14 shows the putative function of the acidophilic sulfur-oxidizing enrichments.

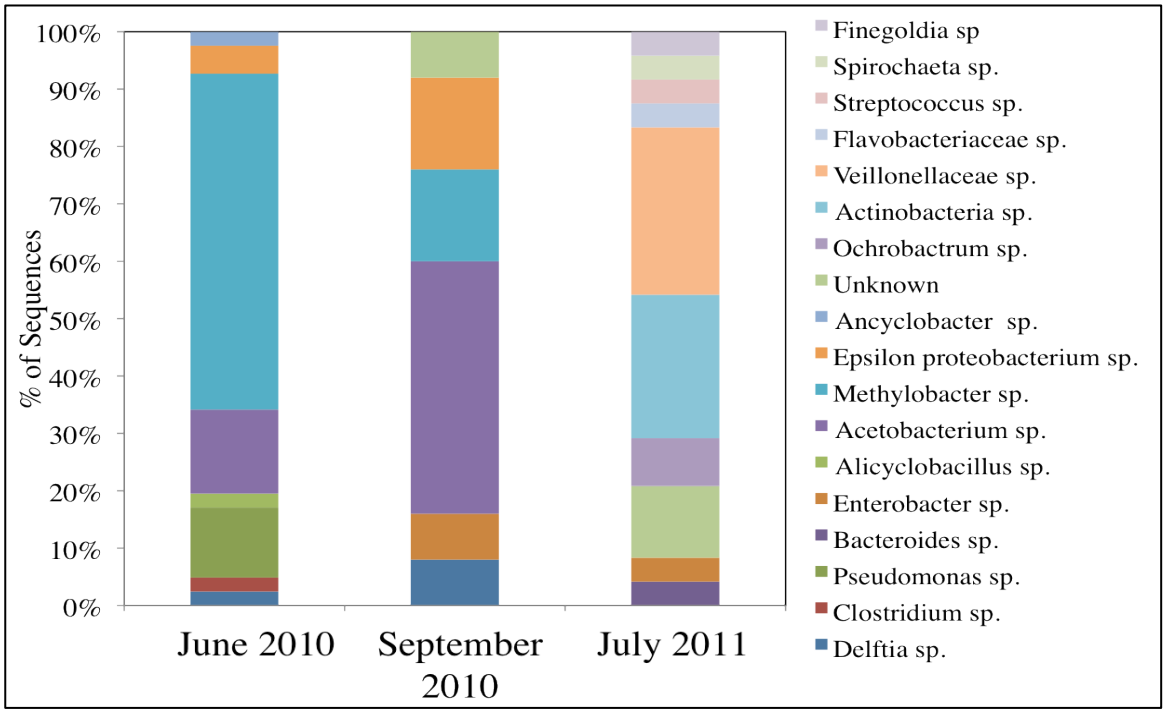


Figure 4.13: 16S rRNA sequences for acidophilic sulfur-oxidizing enrichments

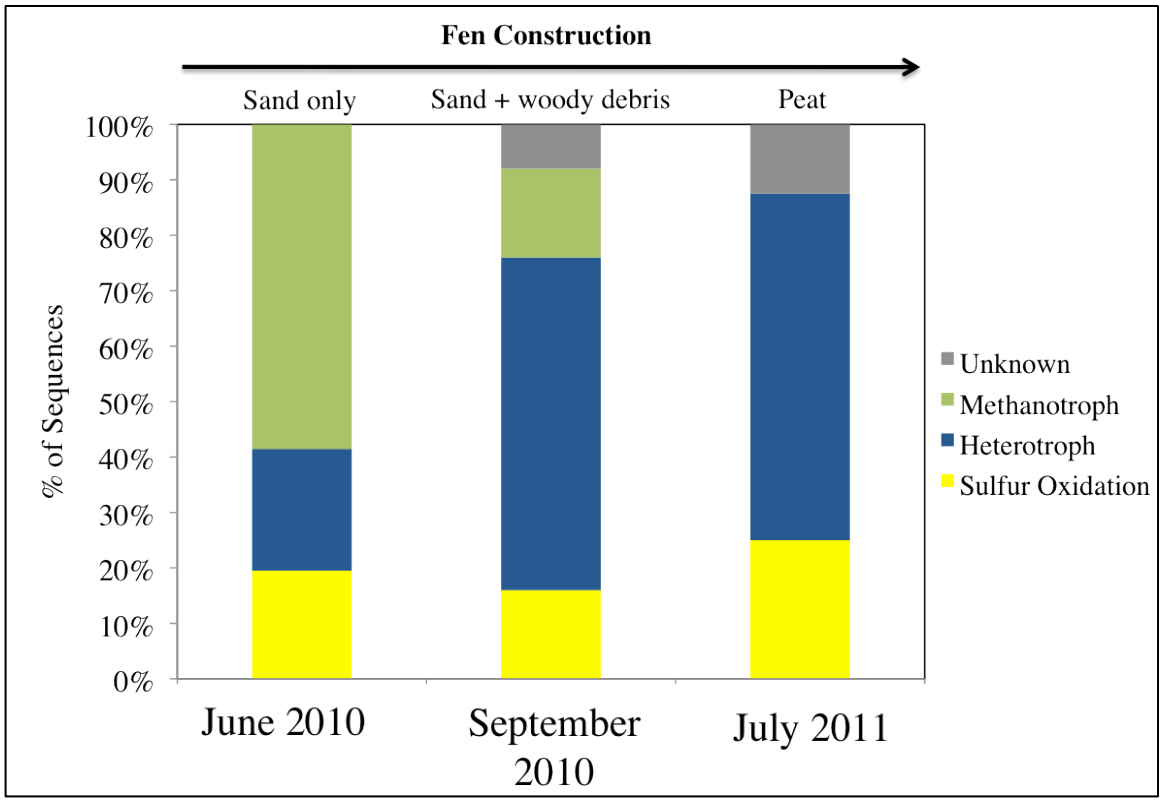


Figure 4.14: Acidophilic sulfur-oxidizing enrichment putative functions

The acidophilic sulfur-oxidizing Sump Vault water enrichment from July 2011 interestingly shows the highest species diversity. It also introduces many new species that were not detected in any other the other iron and sulfur metabolisms chosen. The *Clostridium*, *Delftia*, *Pseudomonas*, *Methylobacter*, and *Acetobacterium* spp. that were present in almost all of the iron and sulfur oxidizing and reducing enrichments have disappeared. July 2011 also has the highest percentage of unknown bacterial matches out of any enrichment.

The presence of large percentages of *Methylobacter* and *Acetobacterium* spp. is interesting since there is no carbon amendment in the acidophilic sulfur-oxidizing liquid media. But since there are residual hydrocarbons, naphthenic acids, and other products left over from the bitumen extraction (Fedorak et al., 2002; Holowenko et al., 2000; Salloum et al., 2002), it is likely that these bacteria are present in the oil sands composite tailings. The *Pseudomonas* spp. present in the June 2010 enrichment could potentially be sulfur-oxidizers similar to the *Pseudomonas* spp. described in Section 4.2.5.

Epsilon proteobacterium spp. are present in June and September 2010. It has been shown that *Epsilon proteobacterium* are dominant in Canadian oil sands reservoirs containing biodegraded oil (Hubert et al., 2012). They were shown to couple nitrate reduction to the oxidation of reduced organic sulfur compounds. *Epsilon proteobacterium* are known to oxidize sulfur in deep-sea environments including hydrothermal vents (Sievert et al., 2008) and crude-oil storage cavities (Kodama and Watanabe, 2004). These are the most obvious sulfur-oxidizers in the June and September 2010 enrichments if they were able to create anaerobic or microaerophilic microenvironments within the enrichment flasks.

In the July 2011 enrichment, the *Actinobacteria* spp., who are a huge portion of the enrichment community are the most likely to oxidize sulfur. Norris et al. (2011) demonstrated the acidophilic sulfur-oxidizing potential of several *Actinobacteria* spp. in ore leaching columns inoculated with enrichments from a geothermal site in Milos. *Actinobacteria* spp. have also been shown to oxidize thiosulfate in the rhizosphere of crop plants (Anandham et al., 2006).

Veillonellaceae bacteria are anaerobic or microaerophilic *Firmicutes*, found in rivers, lakes and most commonly in the intestines of vertebras (Rogosa, 1977; Manchandin et al., 2010). Since the *Veillonellaceae* family is mostly found within mammalian intestines and oral cavities, it is unclear why there are such a large number of sequences of *Veillonellaceae* in the acidophilic sulfur-oxidizing enrichments. It could be possible that there are unknown species within the family capable of more diverse metabolisms than is currently cited in the literature.

Other bacterial species that are present in the acidophilic sulfur-oxidizing enrichments from the Sump Vault include *Flavobacteriaceae*, *Orchrobactrum* and *Bacteriodes*. *Flavobacteriaceae* are mainly aerobic chemoorganictrophs and are common in oceans (Kirchman, 2002). The salinity of ocean water is comparable to the composition of CT, perhaps allowing them to habituate the pore water at Sump Vault. *Flavobacterium* have also been reported to potentially play a role in biodegradation of pollutants, including petroleum compounds (Abed et al., 2002). *Orchrobactrum* and *Bacteriodes* are most commonly found within clinical environments and the human body. Species of *Orchrobactrum* have been isolated from blood cultures (Holmes et al., 1988) and Bacteriodes are often found in human fecal matter and the colon (Kirchman, 2002).

The putative function of the acidophilic sulfur-oxidizing enrichments once again shows a shift to heterotrophy when the peat and woody debris are deposited on the fen (Figure 4.14). However, the relative percentage of potential sulfur-oxidizers stays the same amongst sampling campaign enrichments, suggesting that although there is an increase in heterotrophic metabolisms, there is still autotrophic sulfur-oxidation occurring. This could facilitate sulfur cycling due to the presence of reducing and oxidizing metabolisms potentially occurring in the same location.

4.2.6 – 16S rRNA and Putative Function Conclusions

The increase in reduced iron and sulfur species in the sand cap porewaters (Figure 4.1) indicates that reduction of oxidized iron and sulfur species increases towards the lower regions of the fen. Change in Fe and S-associated sediment fractions (shift to more easily reducible (amorphous iron oxides) in the September 2010 sand cap sample) and XRD (June 2010 gypsum and ubiquitous Fe(III)-associated clays) indicates bioaccessible pools of Fe and S, which can be utilized by bacterial species in iron and sulfur cycling (Table 4.2, Table 4.3, and Table 4.4, Figure 4.2, Figure 4.3) and XRD shows potential iron reduction with magnetite in July 2011 (Table 4.4) indicating microbial reduction of Fe. The ability to enrich for iron and sulfur metabolisms shows the potential for these metabolisms to be present in the CT and sand cap underneath the Sandhill Fen (Figure 4.2, Figure 4.5). The 16S rRNA sequencing analyzed on the Sump Vault well water enrichments proves that there are in fact microbial populations that are confirmed to metabolize iron and sulfur species and shows that the sand cap and CT porewaters have a large capacity for different microbial metabolisms that may not have initially been considered. Figure 4.15 summarizes the geochemical, microbial and putative function

trends seen in the porewaters and sediment sand cap samples in the Sandhill fen at the Sump Vault well over three sampling campaigns.

The Fe/S-oxidizing enrichments prove that these bacteria are clearly able to survive in the bulk circumneutral pH porewaters of the Sandhill Fen. The aqueous hydrogen sulfide that is generated by sulfate reduction and sulfur disproportionation can be concentrated in these micropockets or microenvironments. The subsequent dewatering of the CT can decrease the volume of water and release the aqueous hydrogen sulfide as sulfide gas. Therefore, the potential of more aqueous hydrogen sulfide in the porewaters can be related to the amount of acidic microenvironments created by the Fe/S-oxidizing bacteria, which reveals that the hydrogen sulfide generation within the CT and sand cap porewaters is multifaceted.

The shift in putative function of the environmental microbial enrichments from autotrophic to more heterotrophic metabolisms could be a result of increased labile organic carbon inputs from the woody debris and peat addition to the fen (Figure 4.16). It will be interesting to investigate further the geochemical trends and microbial population shifts as the fen continues to mature.

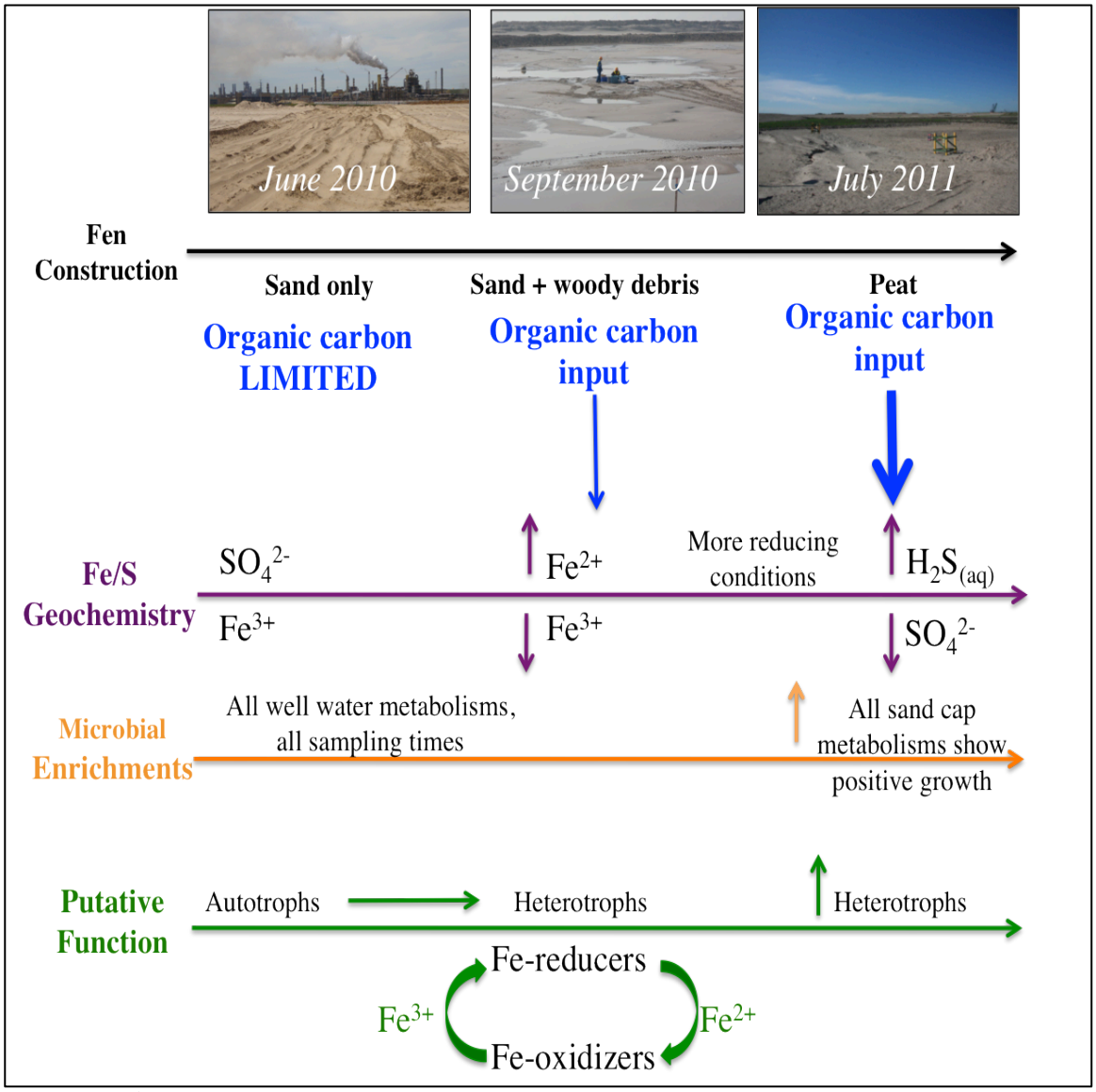


Figure 4.15: Summary of geochemical and microbial trends over the three discussed sampling campaigns at the Sandhill fen and the Sump Vault well.

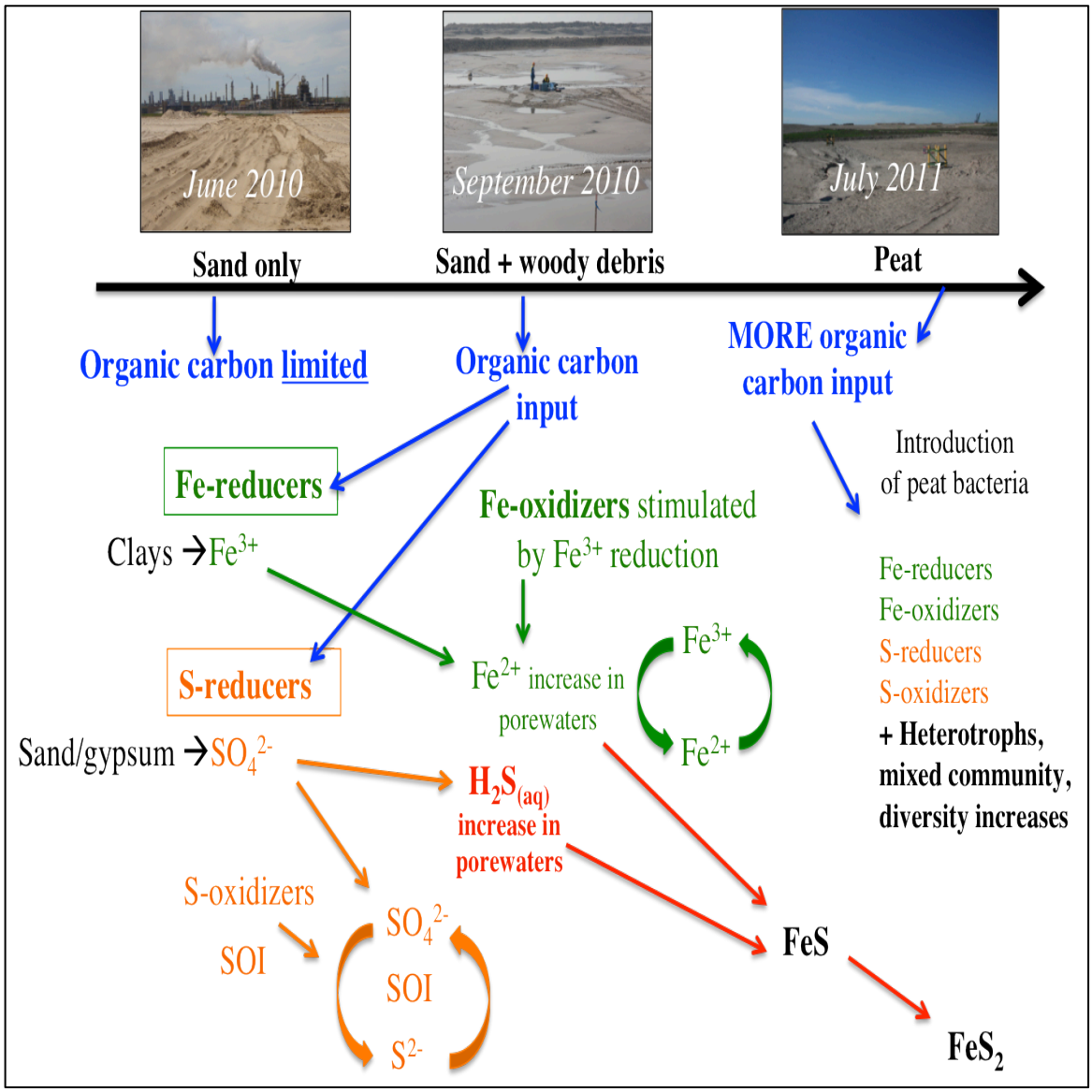


Figure 4.16: Summary of potential processes and pathways in the Sandhill fen porewaters and sand cap during fen construction over the three discussed sampling campaigns.

4.3 – Experimental Microcosms

Experimental microcosms were run to determine if aqueous hydrogen sulfide is produced microbially within a model fen system. Three microcosm treatments were set up and ran for approximately two months. A sterile control, a pure-strain sulfate-reducing bacteria and a non-sterilized endemic treatment were constructed and analyzed for porewater geochemistry, sediment Fe extraction and XRD analysis. The results of this experiment are discussed in Section 4.3.

4.3.1 – Microcosm Porewater

Each sampling time, water was extracted from one or two *in situ* cassettes from each microcosm set up and was analyzed for Fe^{2+}, SO_4^{2-}, $H_2S_{(aq)}$ and pH. These values are displayed in Table 4.5 and Figure 4.17.

Table 4.5: Microcosm porewater data. Hash line indicates no data.

Sterile Control				
Sampling Day	**pH**	**$H_2S_{(aq)}$ (µmol/L)**	**SO_4^{2-} (mmol/L)**	**Fe^{2+} (µmol/L)**
3	7.08	22.3	14.2	0
7	7.13	19.5	11.7	44.6
10	6.91	12.9	8.3	133.9
14	7.08	39.8	-	178.6
17	7.06	5.3	-	-
21	7.3	21.6	7.1	0
24	7.28	8.0	-	8.9
28	7.3	7.0	-	26.8
31	7.24	7.3	7.5	8.93
35	7.63	7.0	5.4	0
38	7.48	5.8	5.8	0
42	7.63	5.3	7.1	8.9
45	7.49	5.3	6.7	8.9
48	7.3	11.1	-	0
52	6.77	6.2	4.6	26.8
55	7.09	7.0	8.7	0
59	7.25	9.8	6.7	0
62	7.01	6.3	5.4	0
Endemic Microcosm				
Sampling Day	**pH**	**$H_2S_{(aq)}$ (µmol/L)**	**SO_4^{2-} (mmol/L)**	**Fe^{2+} (µmol/L)**
3	7.13	2.8	-	0
7	6.87	2.6	29.6	0
10	6.94	17.3	3.8	0
14	6.98	6.9	7.1	35.7
17	7.09	5.0	6.7	26.8
21	7.18	19.7	5.4	80.4
24	7.19	7.5	5.8	17.9
28	7.21	22.4	15	0
31	7.18	8.6	6.7	8.9
35	7.36	11.3	6.3	17.9
38	7.14	3.9	7.1	0
42	7.19	7.5	8.3	17.9
45	7.14	4.4	12.5	8.9
48	6.9	5.9	5.4	0
52	6.98	3.5	5.8	8.9
55	7.07	7.9	8.7	0
59	7.12	7.6	7.5	0
62	7.02	7.0	5.4	0
Sulfate-Reducing Bacteria Microcosm				
Sampling Day	**pH**	**$H_2S_{(aq)}$ (µmol/L)**	**SO_4^{2-} (mmol/L)**	**Fe^{2+} (µmol/L)**
3	6.8	12.2	13.3	125
7	6.54	21.9	9.2	223.2
10	7.09	17.3	6.7	0
14	7.15	22.3	7.1	17.9
17	7.21	8.0	14.6	62.5
21	7.14	18.0	8.7	142.9
24	7.17	5.8	5.8	35.7
28	7.23	17.4	18.3	44.6
31	7.18	13.8	9.2	80.4
35	7.23	8.3	7.9	8.9
38	7.12	8.7	6.7	8.9
42	7.1	7.5	9.2	17.9
45	7.12	8.9	7.9	17.9
48	6.87	7.3	6.2	0
52	7.08	5.9	4.6	0
55	7.1	11.1	6.2	0
59	7.09	10.0	6.7	0
62	6.84	10.5	6.2	0

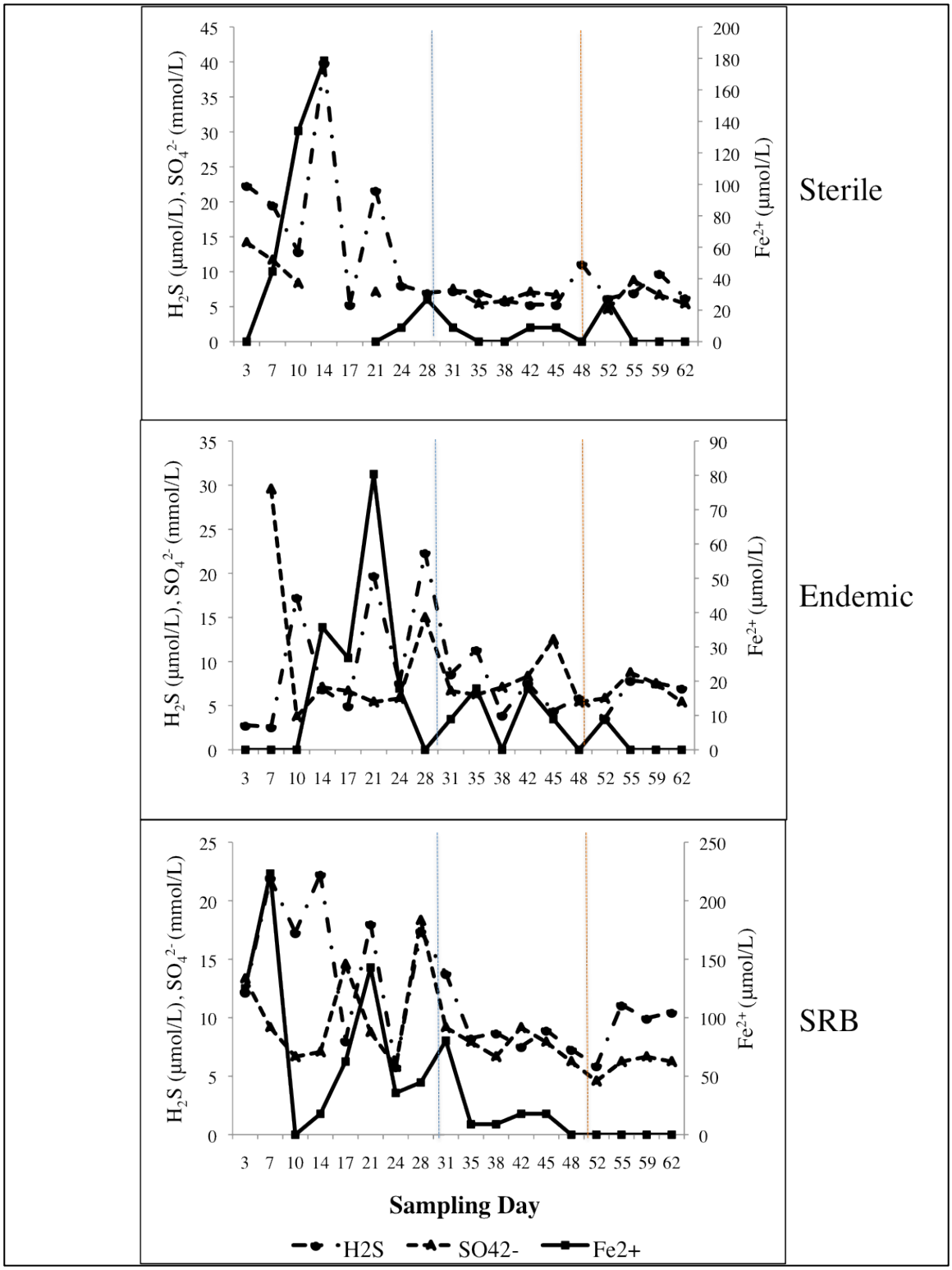


Figure 4.17: Microcosm cassette porewater data. Blue line indicates first carbon and sulfate amendment, the orange line represents the second carbon and sulfate amendment. Breaks in lines represent no data for that sampling time.

Porewater aqueous hydrogen sulfide concentrations in all three microcosms displayed in Figure 4.17 shows high variability in the first month of experimentation. This variability decreases to an almost constant concentration of between 5 and 10μmol/L during the second month of experimentation. Ferrous iron and sulfate concentrations follow the same pattern of variability and then display relatively constant concentrations in the second half of the experiment (Figure 4.17). The variability seen in all three microcosms for all three analytes could represent the microcosms trying to achieve some sort of equilibrium within the porewaters. The lower constant concentrations could also potentially indicate that FeS minerals were forming in the microcosms, lowering the porewater concentrations. The black precipitates shown in Figure 4.19 show evidence for these FeS minerals.

It is interesting to note that the sterile control microcosm produces aqueous hydrogen sulfide concentrations equal and sometimes greater than both the SRB treatment and endemic microcosm (Table 4.5, Figure 4.17). This result could be due to the sulfur geochemical instability of processed waste once the oil sand companies have deposited it. The CT and sand cap or not "normal sediments" but contain many unstable compounds from the bitumen extraction process, which could decompose once deposited.

Another potential cause of the leveling-out of all analytes could be that the microcosms became substrate limited (Vance and Chapin, 2001). To check if microcosms were substrate limited, they were amended with carbon and sulfate (blue and orange lines in Figure 4.16). However, an obvious large increase in aqueous hydrogen sulfide and ferrous iron was not observed in any of the microcosms post substrate amendment. There was a slight increase in aqueous hydrogen sulfide seen in the SRB

treatment over the last three sampling times. Aqueous hydrogen sulfide concentrations in the SRB microcosm may have continued to increase with time, but the experiment ended due to time constraints. Ferrous iron concentrations actually dropped to 0µM during the last 3 or 4 sampling times for all three microcosms (Figure 4.17).

4.3.2 – Microcosm Sediments

Sediment samples from all microcosms were collected at t=0 and t=end for Fe sequential extraction to determine if a change in reactive Fe pools was observed and XRD analysis to determine if there was formation of FeS minerals. The sequential extraction data for CT Fe is displayed in Figure 4.18. XRD results are shown in Table 4.6.

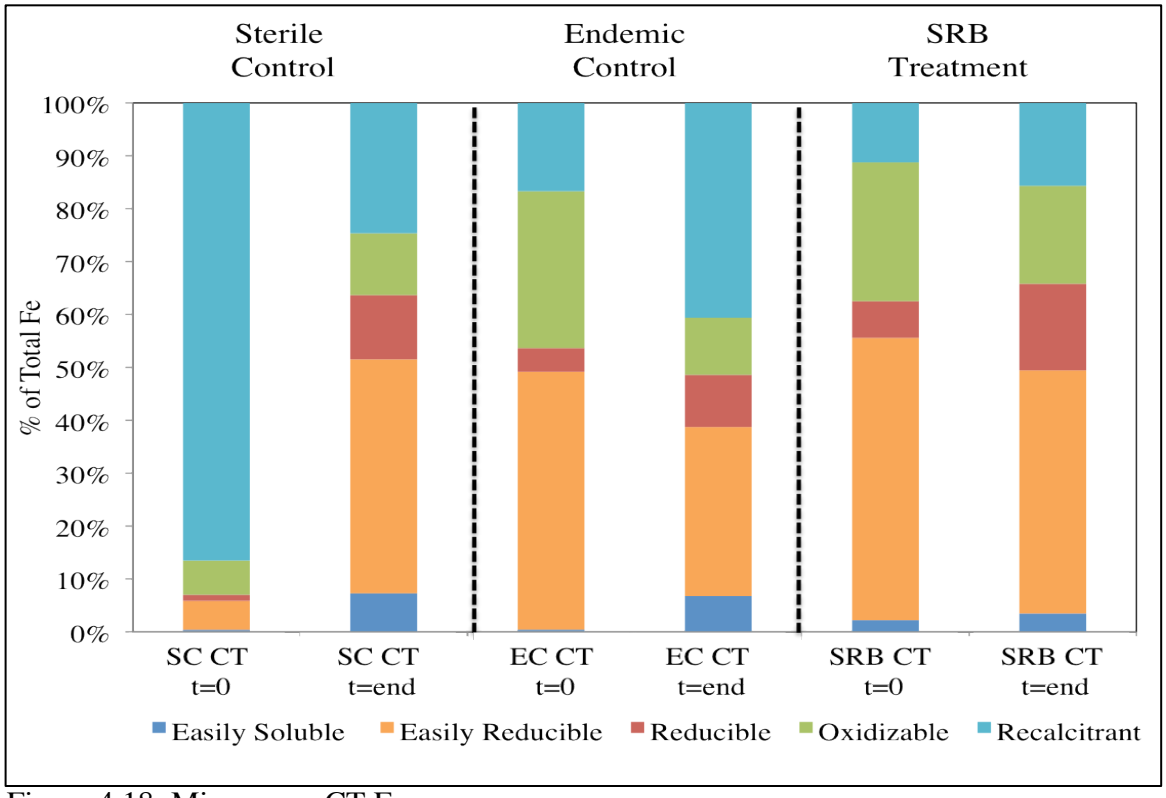


Figure 4.18: Microcosm CT Fe.

Due to the heterogeneity of composite tailings, it is difficult to compare normalized Fe concentrations amongst the three microcosm treatments, for example the large percentage of recalcitrant Fe is only shown in the t=0 sterile control CT, and not in any other CT microcosm sample (Figure 4.18). Instead, percentages of total iron in each operationally define sediment fraction are shown (Figure 4.18).

If FeS minerals were to be precipitated due to increased concentrations of ferrous iron and sulfide, (from microbial reduction) we would expect to see an increase in the percent of Fe associated with the oxidizable fraction. An increase in the Fe associated with the oxidizable fraction is only observed in the sterile control microcosms (Figure 4.18), but not in the endemic and SRB treatments, as would have been expected. This observation does not correlate with the t=end XRD data (Table 4.6) or the black sediments that appear in the microcosms after only a few weeks of sampling (Figure 4.18). XRD results conclude that pyrite (FeS_2) is present at t=end in all microcosms (Table 4.6). Kingfisher CT (the potential location of the next reclamation fen) (Figure 3.1) XRD percentages from July 2011 are shown as a comparison to the microcosm t=end XRD percentages to show there is no pyrite measurable in the CT prior to the experiment. The endemic microcosm treatment shows the highest pyrite percentage at 9%, the SRB treatment shows 4% and the sterile control shows the least amount of pyrite at 3% (Table 4.6). Although XRD data is not quantitative, it does indicate that reduced FeS minerals are forming within the CT of the microcosms. It is not surprising that the endemic treatment shows the highest percentages of pyrite since both microbial iron and sulfate reduction could be occurring, while in the SRB treatment, the only microbial reduction

occurring would be sulfate reduction. Magnetite is also present in all microcosms, indicating potential iron reduction within the microcosm CT layer (Lovley et al., 1986).

Table 4.6: Microcosm XRD

	Kingfisher CT July 2011	EC CT t=END	SC CT t=END	SRB CT t=END
Quartz	64%	~38%	40%	42%
Pyrite FeS_2	**-**	**9%**	**3%**	**4%**
Calcite	-	3%	2%	4%
Albite/anorthite	-	6%	8%	5%
Microcline	7%	5%	3%	6%
Dolomite	-	1%	4%	2%
Magnetite	-	2%	2%	2%
Illite/micas	6%	11%	~11%	10%
Montmorillonite	4%	2%	3%	1%
Pyroxene	2%	-	-	1%
Chlorite	2%	2%	3%	3%
Kaolinite/serpentine	10%	15%	16%	~15%
Amorphous/organic, ect.	5%	6%	5%	5%

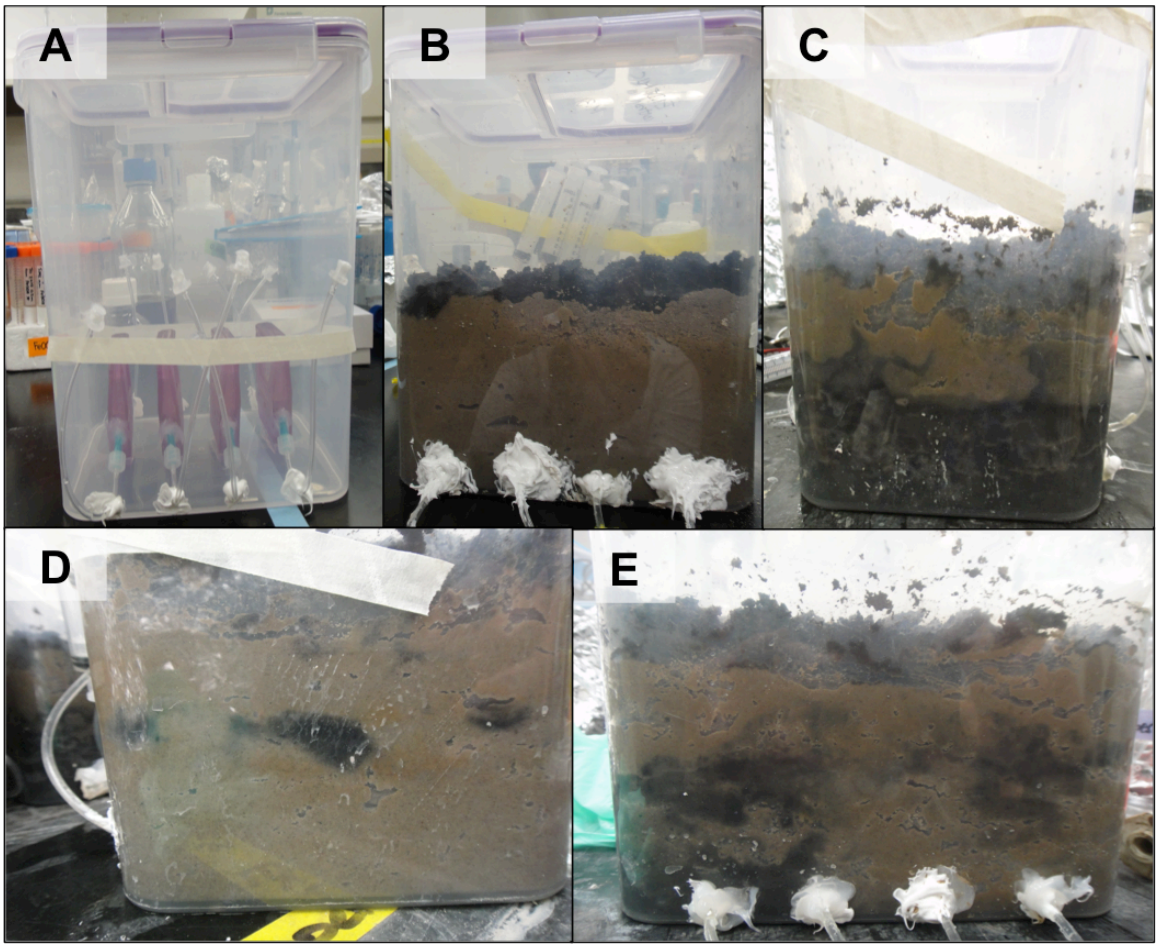


Figure 4.19: Photographs of microcosm set up and sediment at t=0 and t=end. A – Slide-A-Lyzers® are shown inside the microcosm before sediment is added. B – t=0 for all microcosm. C – Sterile control, t=end. D – Endemic treatment, t=end. E – SRB treatment, t=end. Black sediments are Fe-sulfides (Table 4.6).

It should also be noted that the presence of black precipitates was noted firstly and dominantly in the CT layer of the microcosms. This could suggest that aqueous hydrogen sulfide is being generated and then sequestered in the CT layer and not being released as sulfide gas in the sand and peat layers. Salloum et al. (2002) and Ramos-Padron et al. (2011) have also observed FeS precipitation in microcosm experiments with FFT and CT, suggesting that most aqueous hydrogen sulfide is in fact sequestered in the CT. Sequestration of aqueous hydrogen sulfide in the fen materials could be beneficial from tailings management perspective, since the sulfide is not being released as $H_2S_{(g)}$.

Salloum et al. (2002) suggest that H_2S gas release from composite tailings might only be an issue if aqueous hydrogen sulfide production exceeds the amount of metals available to form metal sulfides or if the pH of the whole system drops to acidic pH values.

To verify that there are living bacteria in the endemic and SRB microcosm treatments at t=end of the experiment, LIVE/DEAD® epifluorescence was preformed on CT, sand and peat samples (Figure 4.20). Imaging showed only a few bacterial cells in each layer, which suggests low biomass. This finding could reflect substrate limitations even though carbon and sulfate were added. In future experiments, carbon amendments should be in higher concentrations than used in this experiment. To investigate microbial metabolic impacts on sulfur and iron in the microcosms and be sure that iron and sulfur metabolizing bacteria were in fact present in the endemic microcosm, S-oxidizing, S-reducing, Fe-oxidizing and Fe-reducing microbial enrichments were established using t=end sand, CT, and peat as inoculants. S-reducing bacteria (pure strain) were re-grown from the SRB enrichment from the sand, CT, and peat as well. Positive growth for these t=end enrichments is displayed in Figure 4.21 and examples of positive growth are shown in Figure 4.22. The ability to re-grow the SRB in the SRB microcosm, successfully enrich for Fe and S metabolisms in the endemic treatment, and visualize live cells with epifluorescence imaging proves that there are bacteria living and potentially able to metabolize Fe and S in the microcosm experiments at the end. Proof of living bacteria able to reduce S and Fe suggest that they could be responsible in part for the FeS precipitation in the endemic and SRB microcosm treatments. Since the sterile control showed FeS precipitation, microbes may not be the only factor controlling FeS formation.

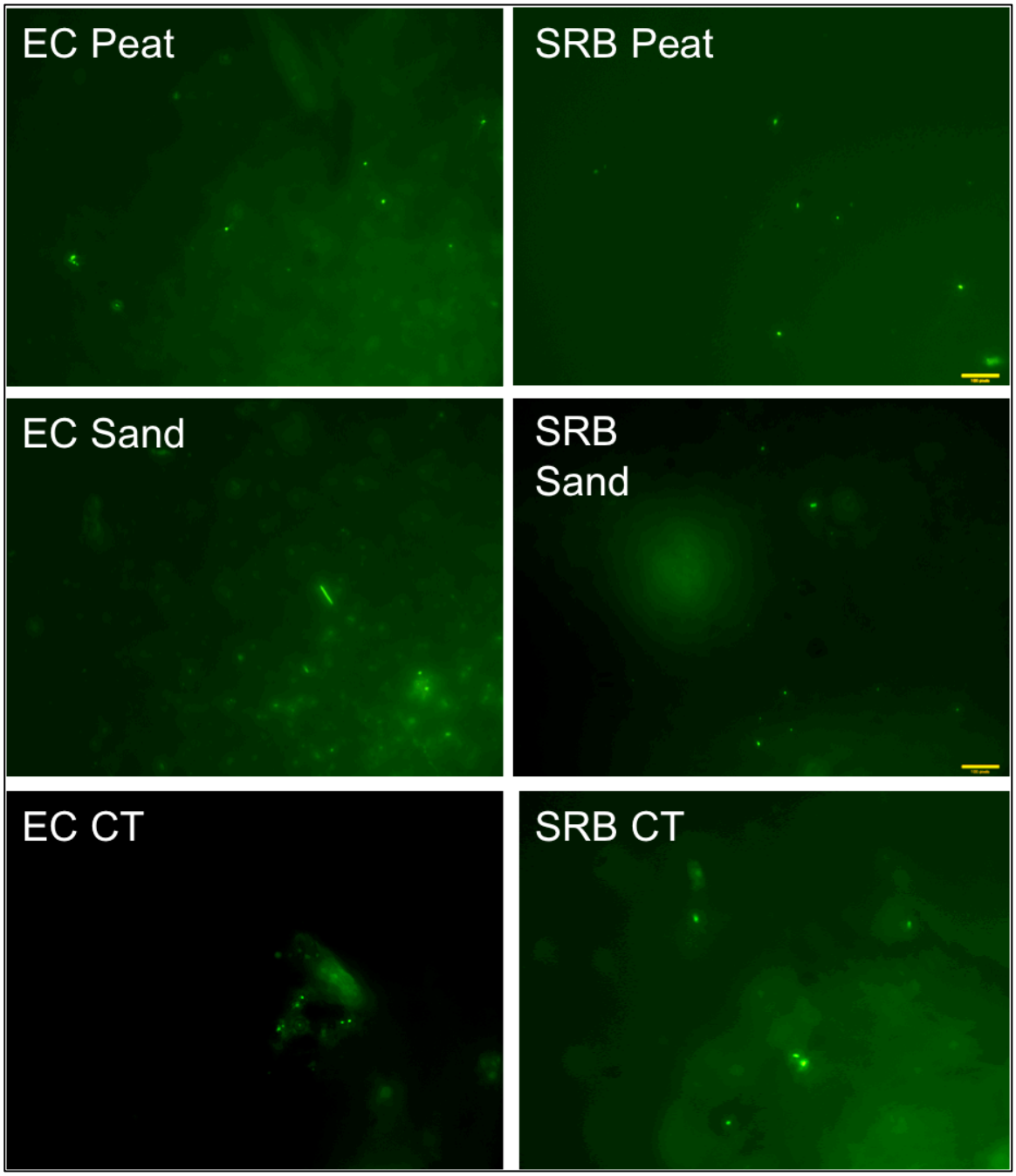


Figure 4.20: Epifluorescence imaging showing live cells at t=end of microcosm experiments.

Microbial Enrichment Regrowth of Microcosms t=55 days	Endemic Treatment			Pure Strain Sulfate Reducer Treatment		
	Peat	Sand	CT	Peat	Sand	CT
Sulfate reducers	●	●	●	●	●	●
Acidophilic Sulfur Oxidizers	●		●			
Neutrophilic Sulfur Oxidizers	●	●	●			
Iron Reducers	●	●	●			
Iron Oxidizers	●	●	●			

Figure 4.21: Re-growth of microbial enrichments from the endemic and SRB microcosms at t=end.

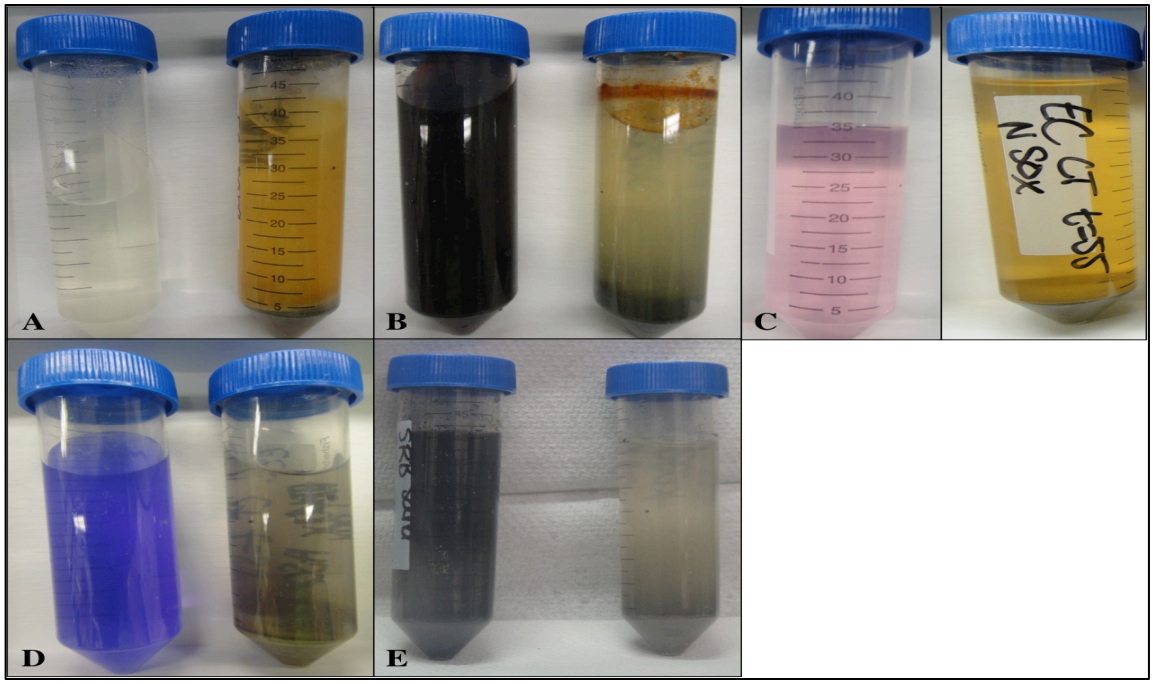


Figure 4.22: Positive growth of microbial enrichments at t=end of microcosm experiment. **A** – Fe-oxidizing enrichment. **B** – Fe-reducing enrichment. **C** – Neutrophilic S-oxidizing enrichment. **D** – Acidophilic S-oxidizing enrichment. **E** – Sulfate-reducing enrichment. The falcon tube on the left shows media with no growth, the tube on the right shows positive growth, except for E where the left shows positive growth and the right media with no growth.

4.3.3 – Microcosm Experiment Summary

The growth of microbial enrichments, post experiment (Figure 4.21, Figure 4.22), is analogous to the enrichments that were growth from field sediment and well waters (Figure 4.2, Figure 4.5). This finding confirms that the bacteria found in the Sandhill Fen sediments and porewaters have the capacity to form FeS solids, as those seen in the endemic microcosm (Figure 4.19). Additionally, the XRD data (Table 4.6) verifies the presence of microbially reduced Fe and S minerals, with higher percentages of pyrite generated in the endemic treatment compared to the sterile control. The ability to measure porewater $H_2S_{(aq)}$ concentrations, although only averaging around 10μM, (Table 4.5) also suggests that microbially produced aqueous hydrogen sulfide is possible in both the endemic and SRB treatments, and can again be related to the concentrations of $H_2S_{(aq)}$ measured in the well waters of the Sandhill Fen. It is unclear as to why $H_2S_{(aq)}$ is produced in the sterile control but most likely it is the instability of S species in the process material. The low porewater $H_2S_{(aq)}$ concentrations could be a result of the FeS minerals precipitating, was also reported by Salloum et al. (2002) and suggested by Ramos-Padron et al. (2011), who hypothesized that concentrations of sulfide found in metal sulfides can be ten folds higher than porewater sulfide concentrations.

These experimental microcosm experiments verify other findings that suggest aqueous hydrogen sulfide will be incorporated into the CT materials and are less likely to be released as H_2S gas. Increasing the liable carbon sources to the CT and sand porewaters with the continuing fen construction should be further investigated to more

fully understand how the addition of organic carbon might affect heterotrophic metabolisms like iron and sulfate reduction.

5.0 – Conclusions

The results of this thesis establish that $H_2S_{(aq)}$ generation is widespread throughout CT and sand cap materials at Syncrude's Sandhill Fen site. Further, porewater $H_2S_{(aq)}$ concentrations increased with development of the pilot reclamation fen most likely due to carbon stimulation of SO_4^{2-}-reducing bacteria, based on increasing aqueous hydrogen sulfide and decreasing sulfate measurements in the Sandhill Fen well waters. Diverse functional Fe and S metabolisms were enriched from every site for almost all sampling campaigns indicating that $H_2S_{(aq)}$ concentrations within CT would be affected by multiple metabolic pathways. Further, the widespread enrichment of typically acidophilic S and Fe oxidizing bacteria indicate that acidic microenvironments likely occur within CT and sand cap where $H_2S_{(aq)}$ species would dominate. Sulfate reducing bacteria and sulfur disproportionating bacteria produce $H_2S_{(aq)}$ which persists within acidic microenvironments generated by the Fe and S oxidizing bacteria. Dewatering activities drives this $H_2S_{(aq)}$ out of solution when the volume of water in composite tailings decreases, resulting in a partial pressures decrease in the wells. These results indicate that multiple metabolisms may play a role in the generation of $H_2S_{(aq)}$ and potential release of H_2S gas.

The genetic characterization of the Fe and S microbial enrichments identified diverse communities present within each functional enrichments and an overall shift to a greater proportion of heterotrophic species, consistent with the greater organic carbon concentrations associated with the developing fen. Microcosm results indicate that the solids of the CT itself are likely not geochemically stable with respect to S phases as $H_2S_{(aq)}$ generation occurred in sterile microcosm controls. Thus the processes contributing to $H_2S_{(aq)}$ generation are driven by both the geochemical instability of the CT as well as microbial S and Fe cycling within these materials. Reclamation fen activities are accelerating $H_2S_{(aq)}$ generation at least over the short term and shifting the microbial populations to a greater heterotrophic community. What the final long-term evolution of these materials will be with regards to S cycling will have to be determined in future investigations.

The creation of a fen that introduces organic carbon to a fairly organic carbon limited system can produce increased concentrations of $H_2S_{(aq)}$ as was seen in the porewaters of the CT and sand cap, but could also in itself increase H_2S production since fen systems create very reducing conditions. The need to further investigate the additions of carbon and the creation of a reducing and organic rich system on top of composite tailings is important for the safe reclamation of tailings deposits in the Athabasca oil sands. Microbial activity occurs in all oil sand process wastes and it is extremely important to understand what potential microbial dynamics are occurring within those wastes to properly and effectively manage those waste products.

The Sandhill Fen reclamation project aims to create an environment similar to the natural landscape that once was the North East Pit, but it should be continually monitored

and researched to more fully understand how the fen addition will impact the microbial populations in the fen and how in turn the microbial populations will impact the effectiveness of the fen reclamation initiative.

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