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Assessing the Biogeochemical Development of Oxygen and Sulfur in Oil Sands Fluid  
Fine Tailings in Batch Microcosms

by

Michael Chen

A Thesis  
Submitted to the Faculty of Graduate Studies  
through the Department of Environmental Sciences  
Great Lakes Institute for Environmental Research (GLIER)  
in Partial Fulfillment of the Requirements for the  
Degree of Master of Science at the  
University of Windsor

Windsor, Ontario, Canada

2012

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Assessing the Biogeochemical Development of Oxygen and Sulfur in Oil Sands Fluid  
Fine Tailings in Batch Microcosms

by

Michael Chen

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February 13, 2012

## DECLARATION OF CO-AUTHORSHIP/PREVIOUS PUBLICATION

### I. Co-Authorship Declaration

I hereby declare that the majority of this thesis is the product of my own work, but also incorporates material that is the result of joint research, as follows:

This thesis incorporates the outcome of joint research undertaken in collaboration with Dr. Ernest Chi Fru under the supervision of Dr. Christopher Weisener. The collaboration has been submitted for publication in peer reviewed journals, as follows:

Publication title/full citation	Publication status*
Chi Fru, E., Chen, M., Walshe, G., Penner, T., Weisener, C. 2012. Bioreactor studies predict whole microbial population dynamics in oil sands tailings ponds. Appl. Microbiol. Biotechnol.	<i>Submitted for publication to Applied Microbiology and Biotechnology</i>

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Chapter 3	Chen, M., Walshe, G. Chi Fru, E., Penner, T., Ciborowski, J.J.H., Weisener, C. 2012. Microcosm assessment of the biogeochemical evolution of sulfur and oxygen in oil sands fluid fine tailings.	<i>Submitted for publication to Science of the Total Environment</i>

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## ABSTRACT

Elevated concentrations of hydrogen sulfide produced by sulfate-reducing prokaryotes are highly reducing, and can impair function of higher trophic levels inside end-pit lakes in the Alberta oil sands region. Microcosms have previously simulated the microbial community structure of tailing ponds; they are used here as analogues of the sediment-water interface of end-pit lake environments to determine sulfide generation patterns and the behaviour of oxygen. In this study, sulfide generation was positively correlated with depth and biotic activity, with production fluxes of  $\sim 2 \times 10^3 \text{ nmol cm}^{-3} \text{ s}^{-1}$ . Oxygen consumption in the tailings is dependent on both biotic and abiotic processes. These results have implications for quantitatively estimating impacts of sulfide production and oxygen availability to biota, in addition to the biogeochemical cycles linked to their functional roles in tailings-affected ecosystems.

*To Planeteers far and wide. There is no fate but what we make.*



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Last but definitely not least, I thank my family and friends for simply being there for me, even if they did not completely understand what I was doing during my time away from home.

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## CHAPTER 1

### GENERAL INTRODUCTION

The Athabasca oil sands, located in north-eastern Alberta, Canada (Fig. 1.1), are one of the world's largest reserves of hydrocarbons, a heavily biodegraded petroleum known as bitumen (Holowenko et al. 2002). There is estimated to be approximately 1.5 trillion barrels (over  $238 \times 10^9 \text{ m}^3$ ) of recoverable bitumen combined with silica sands, clay minerals, and water. The oil sands industry presently produces approximately 1.31 million barrels (over  $200\,000 \text{ m}^3$ ) of bitumen daily, mainly through open-pit mining, a type of surface mining involving removal of material overlying the oil sands ore (e.g., forest cover, soil, rock, clay overburden) (Penner and Foght 2010). New projects are expected to increase the production of bitumen to 3 million barrels (over  $476\,000 \text{ m}^3$ ) per day by 2018 (Alberta Energy, <http://www.energy.gov.ab.ca/OurBusiness/oilsands.asp>). Processing oil sands ore to extract bitumen also produces a large amount of mine waste - approximately  $262\,000 \text{ m}^3$  of oil sands tailings per day. The tailings are a byproduct slurry consisting of water, sand, silt, clay, and residual hydrocarbons (Siddique et al. 2011). It is necessary to improve the understanding of how this material will behave to ensure environmental safety, as well as to enable sustainability of life in reclaimed landscapes.

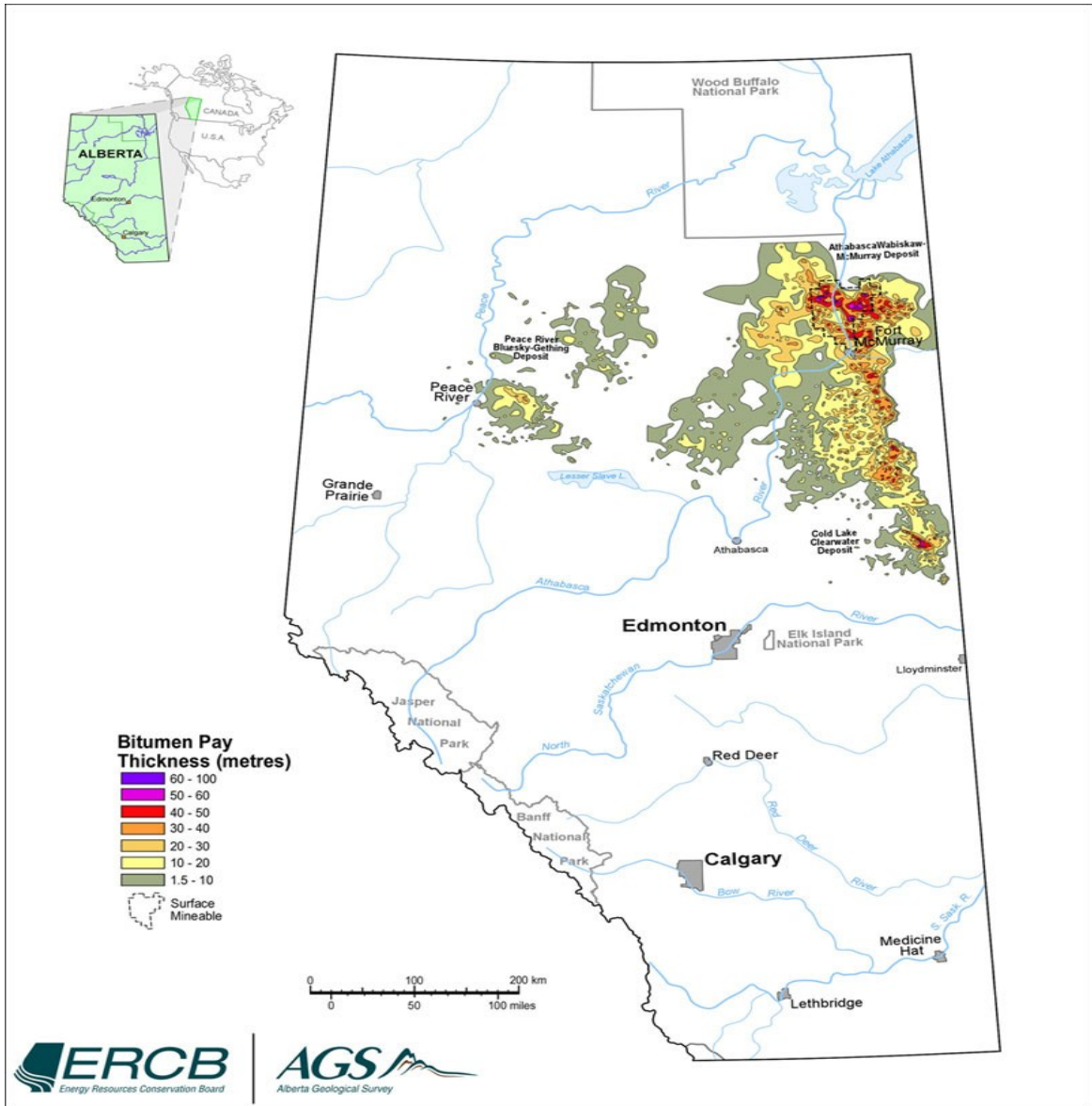


Figure 1.1 Location of Alberta oil sands deposits, which cover over 140 000 km<sup>2</sup>. Map taken from Alberta Geological Survey ([www.ags.gov.ab.ca](http://www.ags.gov.ab.ca)).

Bitumen is removed from the oil sands ore using the Clark Hot Water Extraction (CHWE) method. The extraction combines hot water separation and flotation; the process involves hot water (50 – 80°C), steam, and a conditioning agent (caustic soda, NaOH) to separate the bitumen from the oil loaded sand (The Fine Tailings Fundamentals Consortium (FTFC) 1995, Beier 2008). It requires approximately 2 m<sup>3</sup> of water to process each m<sup>3</sup> of oil sands ore, and it is estimated that each m<sup>3</sup> of water imported is recycled 18 times (FTFC 1995, Syncrude Canada Ltd. <http://www.syncrude.ca/users/folder.asp?FolderID=5911>). Depending on ore quality, bitumen recovery varies between 88 – 95% (Beier 2008). The oil sands mining companies must adhere to a strict policy prohibiting discharging of oil sands process material (OSPM) from their lease areas into the environment. Consequently, the oil sands process water (OSPW) and sediment (OSPS), which make up the OSPM, are contained on site. The current primary practice involves gravity settling and subsequent storage of the extraction waste in large basins, eventually to be reclaimed (Chalaturnyk et al. 2002, Holowenko et al. 2002). By 2011, over 170 km<sup>2</sup> of the oil sands region had been covered by tailing ponds, accumulating approximately 840 million m<sup>3</sup> of OSPM (Siddique et al. 2011).

During the first 3 to 4 years after deposition into the tailing ponds, the tailings settle relatively rapidly to form a denser slurry material called mature fine tailings (MFT; sometimes also referred to as, the more encompassing term, fluid fine tailings, FFT) (Leung et al. 2001, Siddique et al. 2011). Additional incremental settling also occurs over the longer term (i.e., decades of retention time), during the course of which mining

companies may perform various interventions; occasional water input (recharge) may occur or water output (discharge) may take place for use in landscape reconstruction or recovered for processing reuse instead. After 10 to 15 years, the densified FFT is proposed to be transferred to line a mined pit, which will be capped with several meters of fresh water, forming an end-pit lake (EPL), within which it is expected that a viable aquatic ecosystem will develop, able to support a microbial community and higher trophic levels of a food web (Zubot 2010) (Fig. 1.2).

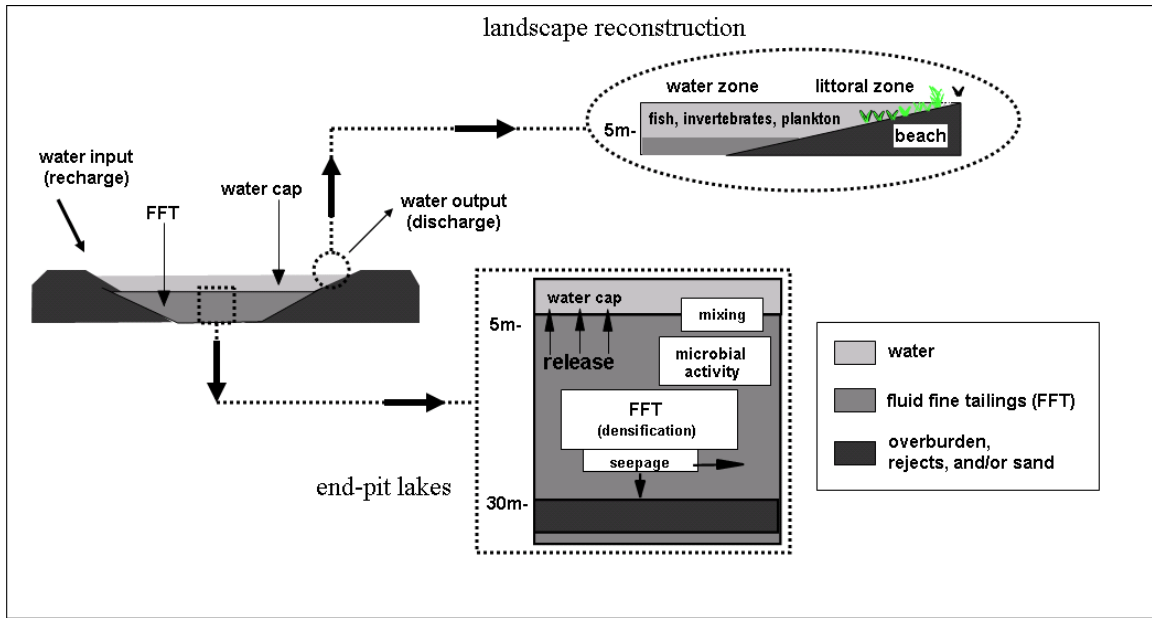
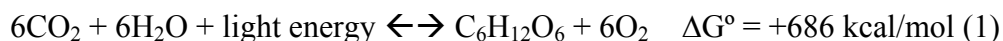


Figure 1.2 Illustration of end-pit lake concept (modified from Syncrude, 2010).

## 1.1 THE ROLE OF OXYGEN AND SULFUR IN END-PIT LAKES

Oxygen is an important part of many biogeochemical processes, and a vital product formed by photosynthesis. It is a major component that allows for the aerobic respiration of cellular organisms.



(Eby 2004)

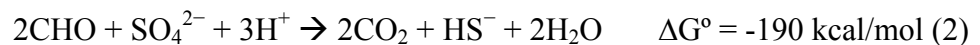
Photosynthesis is a biological process that transforms light energy into chemical energy. The energy is used to reduce carbon dioxide using the hydrogen from the water molecule to form a sugar, while the remaining oxygen is released as a gas; this generation of biomass is referred to as primary production. Biomass can be transferred through consumption, as by higher trophic level organisms (consumers), excretion, and/or breakdown (e.g., decay, decomposition), resulting in its cycling throughout the environment. Conversely, this process is reversed during cellular respiration, where sugar and oxygen are consumed while carbon dioxide and water are given off in order to provide energy (Eby 2004). Oxygen is a central contributor for both of these critical processes, and the amount of dissolved oxygen (DO) in an aquatic environment, such as an EPL, can regulate organism survival, community composition, and successional processes. The amount of DO that an aquatic organism needs depends on the species present; but temperature, water quality, and the state and condition (i.e., age, active/dormant) of the organism will also influence the demand. Typically, a DO concentration under 3 mg/L is too low for fish populations; 3 to 5 mg/L is between the

tolerant to stressful condition range, depending on the fish; 6 mg/L supports spawning, 7 mg/L supports growth and activity; and an excess of 9 mg/L supports abundant fish populations (Kalff 2003).

The net primary productivity of an aquatic system, similar to EPLs, can be determined by tracking the amount of DO in the system (Del Giorgio and Williams 2005, Gardner Costa et al. 2011). Other techniques that are used to observe primary productivity include using inorganic C<sup>14</sup> incorporated into organic matter, and also <sup>16</sup>O, <sup>18</sup>O and <sup>17</sup>O stable isotopes (Williams et al. 2002, Berman et al. 2004). Due to diurnal changes and varying depths of light penetration, some processes may take precedence over others (i.e., photosynthesis), depending on whether conditions are photic or aphotic. Complications for assessing DO production and demand can arise from factors such as stratification and seasonal effects. In addition to carbon dioxide and water, other essential cell constituents, such as nitrogen, phosphorus, sulfur, and iron, may be available or formed to become used as a substrate for respiration. Further losses of DO can also take place in the form of mineralization, a result of an oxidative breakdown (i.e., oxidation by fermenting, denitrifying, sulfate-reducing, and methane-producing bacteria) of organic matter, such as dead algal material or detritus (Jørgensen 1982). Net primary production can be obtained by subtracting respiration in darkness from the gross primary production (total oxygen production during light, or day and night, when referring to more practical system conditions); thus, net primary production can ultimately be calculated by subtracting the losses due to respiration and mineralization from the gross primary production over a set time period (Golterman 1975). This information can then be used to

determine whether the EPL is a net producer or net consumer of DO, and if the system is viable for the potential support of higher order organisms.

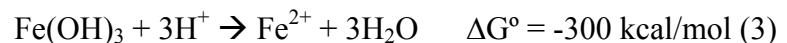
Whereas phytoplankton, epiphyton, and macrophytes are the major contributors to primary production in the water and on the substrate, sulfur-reducing bacteria (SRB) can be a source of primary production within sediments where anaerobic processes dominate. There are two broad conditions in which respiration takes place: aerobically (involving the presence of free oxygen in oxic environments) and anaerobically (involving the absence of free oxygen in anoxic environments). As long as oxygen is available, bacteria will use it (at least partially, due to its energetic favourability) as an electron acceptor for their respiration, but in the absence of oxygen, the system may instead use electron acceptors that are not as energetically favourable as oxygen. When oxygen is completely exhausted, sulfate ( $\text{SO}_4^{2-}$ ) can be used as a more favourable electron acceptor for respiration by strictly anaerobic SRB (Golterman 1975). The presence of sulfate in EPLs is derived from gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) (personal communication with Tara Penner, Syncrude). SRBs have different enzymes allowing them to derive energy for maintenance and growth from a lithotropic reaction (Voordouw 1994), producing hydrogen sulfide ( $\text{HS}^-$ ) – often used as a marker of SRB presence.





Hydrogen can be donated from organic compounds formed during anoxic decomposition of organic matter (van Dongen et al. 2007). Molecular hydrogen may also be found when metals interact with water, as in the case of many aquatic environments. Further input of nutrients into lakes, like organic matter (i.e., a carbon source) and metals (e.g., iron, magnesium), can come from allochthonous (e.g., runoff) and autochthonous (e.g., decay of algae) sources (Eby 2004).

Metal ions in the water may additionally react with  $\text{HS}^-$  to produce metal sulfides (i.e., iron reduction), such as iron (II) sulfide complexes (e.g. FeS, pyrite  $\text{FeS}_2$ , greigite  $\text{Fe}_3\text{S}_4$ ), which are insoluble. A black precipitate subsequently forms from iron (III) hydroxide ( $\text{Fe}(\text{OH})_3$ ) reduction, when  $\text{HS}^-$  becomes prevalent (Golterman 1975).

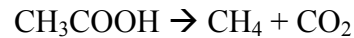


The formation of black iron (II) sulfide precipitate is considered to be an indicator of the presence of SRB (Fedorak et al. 1987, Holowenko et al. 2000). The reduction of  $\text{Fe}^{3+}$  by  $\text{HS}^-$  is not a stoichiometric reaction; it requires an approximately eightfold excess of  $\text{HS}^-$  to cause all iron to appear in solution (Golterman 1975), though the reasoning for this is unclear (Einsele 1936, Lee et al. 1977). The source of particulate iron existing in the water is from Earth's crust (~2% iron by weight) runoff (Lindsay 1979), and more likely to be present as iron (II) sulfide precipitate than other metals complexes, such as relatively more soluble manganese sulfide (Emerson et al. 1983, DiToro 2001).

There have also been studies showing hydrogen sulfide production during anoxic oxidation of methane (Goldhaber 2003, Burdige 2006), which is performed by a consortium of methanotrophic archaea and SRB (Hinrichs et al. 1999, Boetius et al. 2000, Thiel et al. 2001).



The methane is formed by archaea known as methanogens, which metabolize  $\text{CO}_2$  and/or acetic acid as the electron acceptor (DiToro 2001).



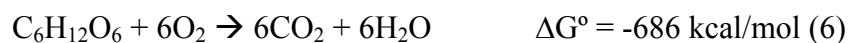
## 1.2 MICROBIAL PROCESSES IN END-PIT LAKES

Microbial communities are a fundamental component of aquatic ecosystems, and largely contribute to the succession and development of a lake system, such as an EPL. Microorganisms are primary consumers that are instrumental in the heterotrophic transformation of organic carbon, providing nutrients and energy to organisms at higher trophic levels (Sigeo 2005). It has become evermore apparent that these basal community structures are important and have significant impacts on the environment; microorganisms have received increasing attention for their roles in both freshwater and

marine systems over the past several decades (Pomeroy 1974, Legendre and Fèvre 1995, Bossio et al. 2006). Although practically invisible to the naked eye (by definition), microorganisms, such as viruses, bacteria, algae, protozoa, archaea, and fungi (Duarte et al. 1997, Ogrinc 2002, Batzer and Sharitz 2006), can have enormous effects on the viability and sustainability of an EPL system, through regulating oxidation of organic matter and nutrient flow. For this thesis, the terms “microorganisms” and “microbial processes” will refer to bacteria and archaea. The dominance of microorganisms in aquatic systems, similar to EPL systems, is well established (Landry 2002, Batzer and Sharitz 2006). Oxygen, sulfur, as well as other major elements supplied from primary producers, pass or cycle through the “microbial loop” and become incorporated into higher trophic levels (Berman et al. 1987, Pomeroy et al. 2007). However, the more complex concepts of food web structure are beyond the scope of this work.

There are two general conditions under which microbial processes may occur within FFT sediments of EPL systems: oxic and anoxic conditions. The EPL design is characterized by high maximum depth with a small surface area, resulting in limited light penetration (Castro and Moore 2000), and consequently precludes the possibility of biota supporting photosynthetic reactions at the FFT sediment-water interface. It is reasonable to speculate that oxygen concentrations found at the sediment-water interface of EPLs will be approximate to those of natural “deep” lake ecosystems. Engineering significance of these comparable systems will be further discussed in Chapter 4.

In oxic conditions, oxygen may be respired as carbon dioxide by the metabolism of carbon, which can be derived from multiple sources.



In terms of thermodynamics, aerobic respiration is a relatively energetically efficient process (Eby 2004), which is used by most aquatic organisms other than microorganisms (e.g., invertebrates, fish) (Barnes et al. 2001). In addition, low molecular weight, carbon-possessing molecules (e.g., methane) may be oxidized by various microorganisms (e.g., methanotrophic bacteria) (Madigan et al. 2002). In an EPL setting, oxygen is unlikely to be introduced into the system from autochthonous sources at the FFT sediment-water interface where photosynthesis is absent, and rather highly reducing conditions may be found. Wetzel (2001) proposes that permanent stratification (meromixis) of the EPL system prevents introduction of oxygen into the system from allochthonous sources (e.g. mixing of epilimnetic water with the atmosphere). Therefore, any DO available at the FFT sediment-water interface would, theoretically, come from residual molecules present during the construction period of the EPL. This assumption does not account for the complexities introduced by periodic (i.e. seasonal temperature) changes (e.g. turnover), which will be discussed in Chapter 4. DO concentrations regulate many functions in an aquatic environment by contributing to reduction-oxidation (redox) reactions, and as a requirement for many microorganisms to decompose organic matter (Dauer et al. 1992). For this reason, DO tends to become depleted over time at the FFT

sediment-water interface, after which anaerobic respiration can often become dominant in the anoxic strata (Wetzel 2001).

In anoxic conditions, which are unavoidable and characteristic of sediments of other aquatic systems (Glud 2008), especially when without photosynthesis (Sørensen et al. 2009), microorganisms must use electron acceptors other than oxygen in EPLs. Various microbial consortia are thought to be responsible for driving distinct redox gradient-dependent biogeochemical processes (Penner and Foght 2010, Ramos-Padrón et al. 2011). These complex systems include microbial communities that develop independently of a source of oxygen (Clemente and Fedorak 2005; Scott et al. 2005, Siddique et al. 2006, 2007, Penner and Foght 2010, Ramos-Padrón et al. 2011). If DO is depleted, then sulfate can be used as an electron acceptor. For example, the cause of natural remediation in aquatic ecosystems, similar to EPL systems, has often been linked to the activity of dissimilatory SRB (Castro and Moore 2000). SRB derive energy through sulfate reduction; organic compounds (i.e., a hydrogen source) are oxidized while sulfate is reduced to sulfide. Examples of SRB genera include but are not limited to *Archaeoglobus*, *Desulfomonas*, *Desulfotomaculum*, and *Desulfovibrio*. Sulfide will precipitate if sufficient reduced iron is present. The initially formed iron sulfide (FeS) will eventually become converted into disulfide (FeS<sub>2</sub>).

Enumerations of methanogens and SRB have been attempted in the past. The standard five-tube most probable number (MPN) assay method, outlined in Standard Methods (1985), was a popular technique employed to establish the numbers of

methanogens and SRB in OSPM. Methanogen MPNs were estimated by Jain et al. (1991), whom utilized methane presence in the headspace as a means of corroboration (Holowenko et al. 2000). SRB MPNs were determined as outlined by Fedorak et al. (1987), whom considered the formation of a black iron sulfide precipitate to be an indication of a positive result (Holowenko et al. 2000).

In the early 1990s, SRBs were detected by Sobolewski (1992) in the Mildred Lake Settling Basin (MLSB), the primary settling basin for Syncrude Canada Ltd., however, methanogen numbers were found to be below the analytical method detection limit (Holowenko et al. 2000). Over the course of 5 years, bubbles of gas were observed on the surface of MLSB. The samples collected in 1996 by Sobolewski (1997) yielded positive results for methanogens (Holowenko et al. 2000).

In 1997 and 1998, the MLSB, Base Mine Lake, and Demonstration Pond, all of which are located at the Syncrude Canada Ltd. lease site, were sampled by Holowenko et al. (2000). Also using MPN methods, they found that all sites contained SRB. The numbers of SRB were highest in locations in which sulfate consumption was occurring, but not necessarily where sulfate concentrations were highest, and lower where sulfate concentrations were depleted. Since sulfate serves as a terminal electron acceptor for SRB, it was expected that methanogen populations would increase and take the place of SRB once sulfate is depleted (Holowenko et al. 2000).

### 1.3 OIL SANDS RECLAMATION

Synchrude Canada Ltd. has proposed creating a permanent, self-sustaining FFT containment region by combining previously quarried areas, (i.e. West-In Pit mine, WIP) into a series of EPLs. A key factor in the implementation of a successful EPL design is to understand how the underlying sediment will develop over time. Sediment oxygen demand (SOD) is a major contributor to DO depletion. Biochemical processes occurring at the sediment-water interface can influence ecosystem function and sustainability. The biochemical reactions associated with natural sediment can be altered by the presence of OSPM, which can affect SOD and ecosystem viability by limiting oxygen concentrations in the overlying water (Truax et al. 1995). Microorganisms strongly facilitate and influence the rate and trajectory of biogeochemical development of tailing basins (Bordenave et al. 2010; Penner and Foght 2010; Ramos-Padrón et al. 2011) beyond the sediment-water interface. An understanding of the biotic and abiotic controls of SOD biogeochemical reactions is imperative in assessing and developing EPL remediation prediction models, if the goal is to establish productivity in these systems equivalent to that observed in comparable viable reservoir systems.

The changing biogeochemistry of FFT was studied using laboratory microcosm experiments to measure the redox chemistry at the FFT sediment-water interface. Details of the microcosm setup will be further discussed in Chapter 2. The tailings in the microcosms were designed to simulate the influence of the final layer of material deposited in WIP before it becomes an EPL. These layers were thought to have the greatest initial influence on the quality of the EPL development. The objective of this

research was to investigate the biotic and abiotic roles of developing microbial communities over time. Changes in the physicochemical properties and microbiological influences of the FFT were assessed in both oxic and anoxic environments. This project applied a novel combination of microelectrochemistry and DNA profiling (i.e., terminal restriction fragment length polymorphism, T-RFLP; with quantitative polymerase chain reaction, qPCR) to assess the changes of microbial assemblages at the FFT sediment-water interface (Chi Fru et al. 2012). The information collected from these experiments contributes toward the development of biologically based predictive models applicable to the sediments of EPL systems.

Processes occurring at the sediment-water interface can cause a significant decrease in the DO concentration by contributing to the overall SOD, which is defined as the rate at which DO is consumed by biotic and abiotic processes at the sediment-water interface. The SOD is usually expressed in terms of units of oxygen consumption per unit surface area per unit time (e.g.,  $\text{mg}/\text{m}^2/\text{d}$ ) (Murphy and Hicks 1986). Gardner et al. (2011) reported that SOD accounted for approximately 95% of the oxygen demand at the sediment-water interface of wetlands constructed with FFT, and roughly 90% of the oxygen demand in wetlands possessing native sediments.

SOD comprises two major components, the biological SOD (BSOD) and the chemical SOD (CSOD) (Wang and Reed 1984). The BSOD results from biotic respiration (multicellular benthic organisms and the microbially-mediated decomposition of organic matter). The CSOD arises from the consumption of oxygen during inorganic



abiotic reactions, such as the oxidation of molecules like iron compounds or ammonium. Gardner et al. (2011) recommended that CSOD required the most focus for successful reclamation.

The realization of the importance of microbial community's impact on the environment has led to some interesting new progress. Although one can compare microbial communities among areas (and find obvious differences in composition), relatively little is known about how nutrient fluxes and redox gradients that develop over time vary among environments that are dominated by these different communities (Bossio et al. 2006). Microorganisms are a major part of developing a viable aquatic ecosystem; these basal communities largely contribute to EPL reclamation efforts. Microbial assemblages at the sediment-water interface can influence the overlying water column, and eventually work their way up to higher levels of the ecology. Temporal comparisons must also be made to ensure continual sustainability of viable EPLs and future long-term reclamation projects.

The postulate was that biotic processes factor significantly in SOD budgets of tailings-affected sediments. Initially, there may be greater SOD in the tailings-affected sediments in which biotic activity occurs than if there is no biotic activity, but a shift towards anoxic reactions being favoured would occur when the amount of free oxygen decreases. It was expected that the redox gradients that developed over time under the various treatments would be principally driven by biotic mediators. Therefore, the microbiology of the tailings was expected to be the main determinant of SOD rates.

#### 1.4 THESIS GOALS AND OBJECTIVES

The aim of the project was to assess the biotic and abiotic causes of changes of FFT products over time; there was a need to investigate the underlying biotic or abiotic determinants of SOD, and to contrast the processes by which redox gradients develop in biotic and abiotic FFT material. It was expected that the biotic component would predominate over time, with microbial communities altering the chemistry at the sediment-water interface. This research investigation was carried out at the University of Windsor in close cooperation with the Cumulative Environmental Management Association (CEMA) of Wood Buffalo Municipal Region, and environmental research scientists from Syncrude Canada Ltd. and Suncor Energy Inc. responsible for management of oil sands process FFT. This research also addressed major concerns of CEMA and the industrial partners with regard to responsible management and long-term protection and sustainability of this region's water resources.

This thesis represents part of this collaborative effort toward developing a holistic understanding of biogeochemical conditions and their development in a system existing in a state of initial disequilibrium. More specifically, the goal was to evaluate a) biotic and abiotic conversion of DO (BSOD vs. CSOD) and sulfur compounds driven by redox mediated reactions; and b) the general physical changes of FFT over time, which affect the quality of a final EPL. Overall, this research provided important information on the chemical, physical, and biological processes in the FFT. Controlling microcosms allowed

for the testing of the postulate that FFT development is primarily driven by biotic factors rather than abiotic factors (Chapter 2, Chapter 3).

This thesis is composed of 4 chapters. The first chapter (General Introduction) reviews relevant background information. Chapters 2 and 3 focus mainly on the electrochemical data. Chapter 2 describes the short-term kinetics (0 to 2 months) of the microcosm study. Chapter 3 describes longer-term kinetics measured at 6, 9, and 12 months, and respective relationships with the short-term kinetics, with relation to oxygen and sulfur. Chapter 3 also addresses the relationships between the pore water and water column chemistry (cations/metals, anions, and carbon), as well as end point gas analysis. Chapter 4 discusses and reviews the findings of chapters 2 and 3.

This research will further our conceptual understanding of EPLs and will contribute to the evaluation of remediation designs supported from the data collected. Ultimately, these results describing the biotic and abiotic development of FFT products will help industry make responsible management decisions and create long-term protection strategies for the region's water resources (Chapter 4).

## CHAPTER 2

### USING MICROSENSOR TECHNOLOGY TO INVESTIGATE THE DEVELOPMENT OF BIOGEOCHEMICAL GRADIENTS IN OIL SANDS FLUID FINE TAILINGS

#### 2.1 INTRODUCTION

The realization of the importance of the microbial structure's impact on the environment is relatively recent, and has been receiving increasing attention since closer to the end of the 20<sup>th</sup> century (Legendre et al. 1995). Currently, studies focus on the nutrient cycling of carbon and nitrogen (Pomeroy 2001), but there is a need to understand rate changes of oxygen demand and the development of redox gradients of additional elements, such as sulfur, over extended time periods being mediated by biotic factors. Being able to comprehend the temporal implications of newly developing ecosystems helps improve the chances of establishing systems that are both viable and sustainable. Microorganisms are a major part of developing a viable and self-sustainable aquatic system, such as is desired for reclaimed end-pit lake (EPL) environments, and largely contribute towards making up the necessary basal community. Populations residing at the sediment-water interface (i.e., the boundary between the sediment and water compartments in EPLs) may influence other components of the lake system, such as the overlying water column, and eventually influence higher trophic levels of the food web – potentially affecting biota ranging from the cellular level life forms (such as the microbial communities) to higher order organisms (such as fish and waterfowl) (Chapter 1).

It is expected that the biotic component may be an important factor in sediment oxygen demand (SOD) response in the lake systems containing oil sand tailings-affected

sediments. Initially, there can be greater SOD in the tailings-affected sediments possessing an active biotic component; this would result in a shift towards anoxic reactions as the amount of available dissolved oxygen (DO) decreases. Over time, established redox gradients are expected to become established under the various microcosm treatments, and these gradients will be driven primarily by biotic mediators. Therefore, the microbiology of the tailings is expected to influence initial SOD responses.

Determining the short-term, initial, introductory kinetics of fluid fine tailing (FFT - the residual sediment produced during bitumen extraction, which are transferred to on-site tailings ponds for retention, where sand quickly settles out and the resulting clay suspension is allowed to dewater through density-dependent settling) development is an essential part of understanding the elaborate potential impacts that FFT will have on both the physical environment and living organisms that reside in a sustainable aquatic ecosystem, such as reclaimed EPLs. This information will ultimately be useful in elucidating the effects of FFT and reclamation treatment efforts in aquatic food web dynamics over longer time periods as these systems develop into the future (Chapter 1).

This chapter investigates the short-term behaviour of FFT oxygen and sulfur, specifically at the sediment-water interface in simulated EPL environments, using a microcosm experiment. The oxygen and sulfur consumption/production rates of the FFT at the sediment-water interface in these microcosms were determined with the aid of microsensor technology. This study assessed the biotic and abiotic processes that contribute to the development of key elemental cycles (e.g., Fe, N, C) that may perturb or

stabilize these reclaimed environments, in relation to changes observed in oxygen and sulfur concentrations. Tracking the short-term production of hazardous molecules such as  $\text{HS}^-$  will be an essential component to predicting how these elemental cycles become established, as referred to in Chapter 1. Faunal abundance and biomass diversity in aquatic ecosystems correlates positively with SOD (Tahey et al. 1994). SOD is comprised of two components, the biological sediment oxygen demand (BSOD) and chemical sediment oxygen demand (CSOD) (Wang and Reed 1984, Gelda et al. 1995). CSOD dominates epibenthic processes found in young marshes created with oil sands process material (OSPM) (Gardner Costa et al. 2011).

### *2.1.1 SEDIMENT OXYGEN DEMAND*

It is important to understand both biotic and abiotic factors when interpreting oxygen concentrations in EPL sediments. Examination of the contributors that actively (i.e., consumption or production) or passively (i.e., diffusion) remove oxygen at the sediment-water interface can aid interpretation of whether the water column is suitable to sustain particular specific species. SOD is the rate at which DO is consumed by biotic and abiotic reaction processes at the sediment-water interface; and although these reactions may occur over a very small component of a lake system, the reactions contribute greatly to the overall governing of sustainable productivity in EPLs. Reactions that reduce the DO concentration at the FFT sediment-water interface will contribute to the overall SOD (Murphy and Hicks 1986), which greatly influences oxygen depletion in most water bodies (Truax et al. 1995), and is especially important in shallow waters (Sweerts et al. 1991). The BSOD involves processes such as respiration as well as the

decomposition of organic matter; both of which may be attributed to microbial communities. The CSOD represents abiotic processes that arise from the consumption of oxygen during inorganic chemical reactions, such as the oxidation of iron or ammonium (Wang and Reed 1984).

This chapter addresses the change in oxygen and sulfur concentrations in FFT to help assess their potential behaviour in EPL settings during initial introductory periods using microcosms, which have previously been shown to simulate *in situ* tailing ponds and microbial community structures (Chi Fru et al. 2012). Specifically, the experiment resolves the question of whether these processes are predominantly regulated by biotic or by abiotic activity, with respect to oxygen and sulfur concentrations, over a short-term period. This study determines the processes governing SOD function in the FFT, and if these processes directly influence DO concentrations in the water. Chemical gradients across the sediment-water interface are measured in a series of microcosms. The experiment ultimately aims to determine whether the biotic or abiotic oxygen consumption processes dominate at this boundary, and to quantitatively estimate FFT effects on oxygen demand. Similarly, changes in sulfide generation at this FFT sediment-water interface are documented over the same short-term time period.

It was expected that the microcosms containing a biologically active FFT component would exhibit greater oxygen demand than microcosms with FFT from which the biotic component had been inactivated (see section 2.2 Materials and Methods for sterilization process details). The principal BSOD would result from the biotic respiration

associated with bacterial decay processes at the FFT sediment–water interface, together with the release of chemically reduced compounds, such as iron complexes, sulfide, methane, and ammonia. These soluble compounds would exert a rapid kinetic oxygen demand as the reduced compounds undergo oxidation.

### *2.1.2 RATIONALE*

The presence of the biotic component in FFT material was expected to cause more rapid changes in oxygen and sulfur than would be apparent in the abiotic microcosms (i.e., those subjected to gamma irradiation treatment explained in the following section). The biotic component (i.e., microbial community) is responsible for the reducing nature of the FFT, and will cause oxygen concentrations to decrease, while increasing sulfide production is expected.

## 2.2 MATERIALS AND METHODS

### *2.2.1 STUDY SAMPLE COLLECTION AND MAINTENANCE*

FFT and oil sands process water (OSPW) were obtained from Syncrude Canada Ltd., in the Athabasca oil sands region near Fort McMurray (56.66° N 111.21° W), Alberta (Fig. 2.1, Fig. 3.1). Several mining and refinery companies are excavating oil sands using open-pit mining operations in this area (Fedorak et al. 2002), and the mined area is anticipated to continue growing, eventually exceeding 1406 km<sup>2</sup> by 2023 (Williams 2003, Cooper 2004). Approximately 200 L of newly retrieved (i.e., fresh) FFT and 150 L of OSPW were obtained from Syncrude’s West In-Pit (WIP) tailings facility



(57.02° N, -111.62° W). The FFT samples were composed of 1 to 2 wt% bitumen, less than 0.1 wt% naphtha, 30 to 60 wt% clay, and water.



**Figure 2.1 Map showing the location of the Athabasca oil sands deposit near Fort McMurray, Alberta (taken from Google Images).**

Upon arrival, samples were stored at 4°C in a walk in-freezer room dedicated for environmental samples located at the Great Lakes Institute for Environmental Research (GLIER), Windsor, Ontario, Canada. Aliquots of FFT (100 L) and OSPW (75 L) were sterilized using gamma irradiation at 28 kGy over 24 hrs, at the McMaster Nuclear Reactor (MNR), Hamilton, Ontario Canada, utilizing a Gamma Irradiation Hotcell, 10 000 Currie Co-60 Gamma Source, to eliminate biological activity. These materials were used in abiotic control treatments.

### *2.2.2 MICROCOSM SETUP DESCRIPTION*

A short-term study was conducted in the lab to monitor redox gradient changes and chemical flux in the microcosms. The short-term study monitored the initial stages of effects from the tailings treatment, and led into the monitoring of continued changes with aging over a prolonged period of time discussed in Chapter 3.

To distinguish the biotic from abiotic processes in OSPM, one half of the microcosms contained raw, unsterilized FFT and OSPW samples, and the other half contained sterilized samples.

Oxic environments were also compared with anoxic environments in the study. O-ring-sealed microcosm covers were used to create a closed system, forming a simulated anoxic environment within some units. Microcosm environments that did not receive O-ring fitted covers were exposed to laboratory atmospheric conditions, and represented oxic environments. Each microcosm unit was constructed from ethanol-sterilized 4-quart

(3.8 L) Camwear® Round plastic containers (Cambro Manufacturing Company, Huntington Beach, CA). Containers had a top diameter of 21 cm and height (including cover) of 22 cm. Half of the containers (2 of the 4, used for the short-term study) that were designed to simulate an anoxic aquatic environment were fitted with O-ring airtight seal covers retrofitted with two push-plug valves, which could be used for headspace gas sampling (Chapter 3) and purging if required (Fig. 2.2). These containers (16 in total, one short-term microcosm sampling session, described further in the following section; plus three long-term sessions described in Chapter 3) were modified at the Technical Support Centre of the University of Windsor and sterilized using ethanol again before adding the FFT and OSPW for the experiment. To ensure that anoxic conditions were maintained throughout the experimental period, the seals for each anoxic microcosm were checked for leaks and were kept in an anoxic gas chamber (i.e., under nitrogen and hydrogen gas mixture).



**Figure 2.2 Illustration of microcosm container design with unmodified (left) cover for oxic conditions, and modified (right) cover for simulated anoxic conditions.**

The FFT was thoroughly mixed using a power portable drill with a sterilized Teflon-coated stirring paddle. A 2000 g aliquot of FFT was added to each microcosm and overlaid with 1000 g of OSPW. Anoxic microcosms were then flush-cycled with ultra pure nitrogen gas for 2 mins, and cycled additionally when set inside the anoxic gas (95% nitrogen, 5% hydrogen mixture) chamber. Oxidic microcosms were incubated on shelves with unmodified lids (without O-ring seals) in laboratory atmospheric conditions. All microcosms remained in a consistently low light surrounding environment (under a black canvas in a basement room) at ~22°C.

### *2.2.3 EXPERIMENTAL DESIGN*

The short-term experiment was conducted over the course of 8 weeks, starting on June 5, 2010. Microelectrode sensors were used to collect pH, Eh, oxygen, and sulfide data during the initial 6 weeks; cores and water samples from both the FFT and overlying OSPW were collected after weeks 0, 5, and 8. The results of the core and water analyses are discussed further in Chapter 3. Four sets of microcosms indicative of four unique environmental conditions were arranged for this experiment representative of the important introductory developing time interval (Fig. 2.3). A separate microcosm was created for both oxidic and anoxic conditions as well as biotic and abiotic FFT. This microcosm experiment setup for the introductory period comprised four individual units set up in a controlled environment (laboratory at GLIER, University of Windsor in Windsor, Ontario).

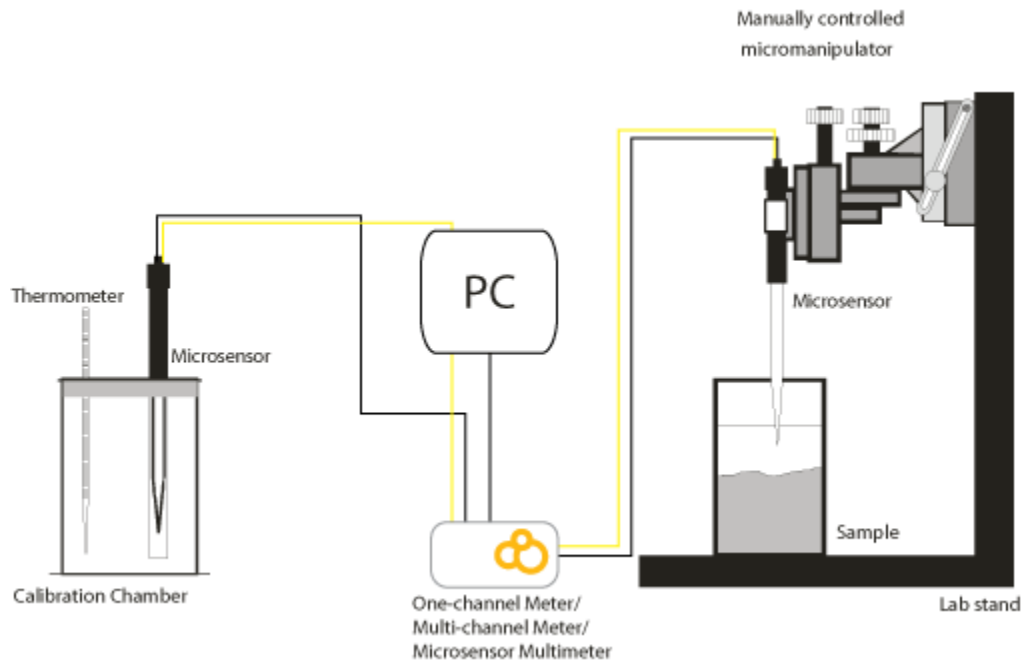
		<b>atmospheric environmental treatment</b>	
		oxic (no seal)	anoxic (o-ring seal)
<b><math>\gamma</math> irradiation treatment of fluid fine tailing (FFT) and oil sands process water (OSPW)</b>	steriled (abiotic)	oxic abiotic	anoxic abiotic
	unsteriled (biotic)	oxic biotic	anoxic biotic

Figure 2.3 Microcosm classification scheme.

#### *2.2.4 MICROELECTROCHEMISTRY MEASUREMENT PROTOCOL*

Microelectrode sensor-based methods were used throughout the experiment for the environmental monitoring of the microcosms over the initial short-term introductory period (Fig. 2.4). Several microsensors developed by Unisense Science (Aarhus, Denmark) were used to measure pH (Revsbech and Jørgensen 1986), oxidation-reduction potential (i.e., redox or Eh), oxygen concentrations (Revsbech 1989), and sulfide concentrations (Kühl et al. 1998). Each microsensor was calibrated according to the procedures described by Mohanakrishnan et al. (2009) and Revsbech and Jørgensen (1986) before each experimental time period. The chemical microenvironment of a simulated EPL system can be described in detail using these microsensors, and it is possible to obtain measurements down to a micrometer scale with this technology. The microsensors constructed for our experiment had diameters ranging between 100 and 500  $\mu\text{m}$ . Each sensor is characterized by its linear responses to its respective substrate (Damgaard et al. 1995).





**Figure 2.4 Manual profiling setup (note: the multimeter may be interchanged with a picoammeter and A/D converter) (Unisense Science, 2011).**

All microelectrode sensor measurement data were recorded manually in addition to the automatic data collection featured in the Unisense SensorTrace software. The current signal of each microsensors is measured by a specialized high quality picoammeter.

During the short-term study, the signal from the amperometric microsensors required adjustable polarization through the Unisense PA2000 picoammeter and external A/D-converter unit. The potentiometric microsensors currents were amplified using the Unisense pH/mV meter, a high-impedance millivoltmeter.

Because of the fine scale microsensors tips and dramatic gradients found in the microenvironments of the microcosms, it is possible that a few micrometers displacement of the microsensors would cause a change in the signal received in the SensorTrace program. To achieve precise control over microsensors depth when taking profiles, the sensors were attached to a Unisense LS18 lab stand and MM-33 micromanipulator. No coarse lateral adjustments were made during measurements to prevent disturbance on the microcosm environments and damage to the microsensors.

Each microcosm was set up on the lab stand with the microsensors initially mounted and positioned in the water column. This configuration permitted individual measurements to be made both in the OSPW and into the FFT, providing a comprehensive depth profile across the sediment-water interface. The sensor signal was allowed to stabilize for 5-10 mins before the first measurement was recorded.

Subsequently, the sensor was lowered toward the FFT-OSPW interface by 1 mm intervals, allowing 1-3 mins for signal stabilization at each point before recording, depending on the response of the microsensor. The depth at which the sensor reached the FFT-OSPW interface was recorded. This depth was also roughly verified by visually checking the distance which the sensor penetrated the FFT; the verification was performed by measuring the amount of FFT that had attached to the microsensor tip after completing the entire profile.

#### *2.2.4.1 PH MEASUREMENTS*

The Unisense pH sensor is a potentiometric sensor that requires an established reference potential. These sensors permit very high spatial resolution measurements, but require an external reference electrode (a Radiometer-Analytical Ag/AgCl electrode with gel-stabilized electrolyte).

Sensors with a 100  $\mu\text{m}$  diameter tip were used. A three-point calibration (buffer solutions with pH of 4, 7, and 10) was used to cover the range of measurements that were to be made. The corresponding signals from the SensorTrace program were recorded and then inspected to verify that the pH values responded linearly between the points with a slope between -50 and -70 mV/pH-unit. A linear conversion calculation was then used to estimate pH values from the software's mV readings.

#### *2.2.4.2 EH MEASUREMENTS*

Similar to the Unisense pH microsensor, the Eh sensor is also a potentiometric sensor with a 100  $\mu\text{m}$  diameter tip that requires an external reference electrode.

A two-point calibration was sufficient for this microsensor. Quinhydrone redox buffer solution was prepared by mixing 100 mL of pH 4 and pH 7 buffer solutions with 1 g of quinhydrone each in order to obtain oxidation-reduction potentials of approximately 462 mV and 285 mV, respectively. The corresponding signals from the SensorTrace program were recorded and then verified that the difference between the potentials obtained were between 170 and 185 mV.

#### *2.2.4.3 OXYGEN MEASUREMENTS*

The Unisense oxygen sensors are amperometric Clark-type sensors that possess a guard cathode and internal reference electrode. Oxygen diffuses through a silicone membrane tip to a reducing cathode that is polarized against an internal Ag/AgCl anode. The tip diameters for the sensors used in this experiment were 100  $\mu\text{m}$ . A two-point calibration was used – an atmospheric reading and a zero reading. The atmospheric reading was obtained using an aerated calibration solution, which was prepared by oxygenating 500 mL of deionized water for 20 mins through vigorous bubbling using a RENA® Air 50 Pump and sterile air diffuser. The zero reading was obtained by preparing an anoxic solution of sodium ascorbate and NaOH, both to final concentrations of 0.1 M (approximately 2 g of sodium ascorbate into 100 mL of 0.1 NaOH). The oxygen

concentration of each solution was verified by taking an additional measurement using a Thermo Orion polarographic DO probe before calibrating the microsensor.

#### *2.2.4.4 HYDROGEN SULFIDE MEASUREMENTS*

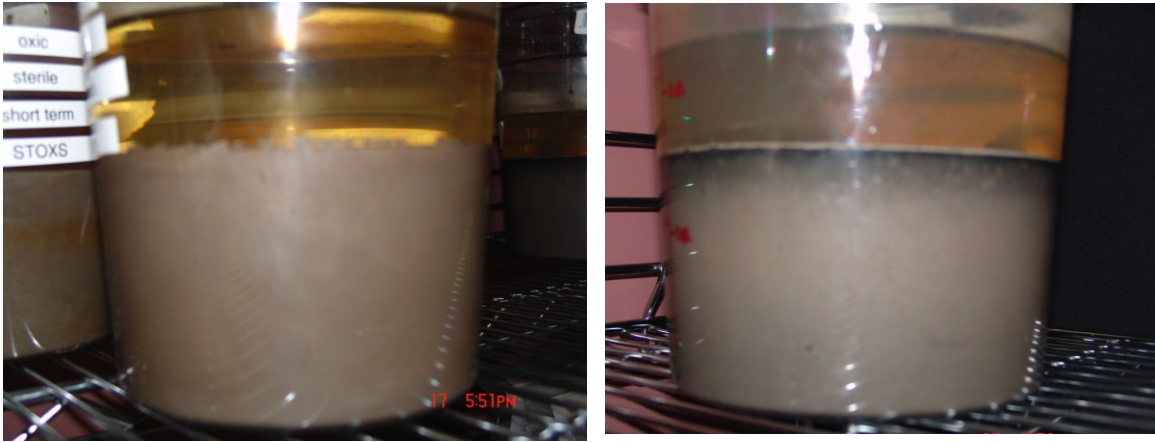
The Unisense hydrogen sulfide microsensor is an amperometric sensor with a guard cathode and internal reference electrode. This experiment used microsensors with 500  $\mu\text{m}$  diameter tips. In the aquatic environment of the microcosms,  $\text{HS}^-$  ions are oxidized by ferricyanide to produce sulfur and ferrocyanide. The signal for this particular sensor is obtained when ferrocyanide is reoxidized at the anode in the sensor tip (Jeroschewski et al. 1996). A five-point calibration curve was used for this microsensor. A stock solution of  $\text{S}^{2-}$  (500  $\mu\text{mol/L}$  total sulfide) was prepared by dissolving 0.12 g of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  in 1 L of nitrogen flushed deionized water in a closed container. A calibration buffer was also prepared by deoxygenating 500 mL of pH 2 buffer solution using nitrogen flushing for 15 mins. The stock solution was serially diluted to make the calibration standards.

### 2.3 RESULTS AND DISCUSSION

#### *2.3.1 INITIAL DEVELOPMENT OF MICROCOSMS*

Microcosms were inspected daily to monitor visible changes in the water column and the FFT material. By the end of 6 weeks, a clear zonation pattern was evident in all biotic microcosms (first noticeable observations qualified starting at week 3). A consistent dark, black band was detected just below ( $< 2$  mm) the FFT-OSPW interface. These bands occurred regardless of whether the microcosms were oxic or anoxic (Fig

2.5). Such banding was not observed in any of the abiotic microcosms; this was an indication of the development of a sulfidic zone, primarily driven by biotic activity, most notably by potential sulfur-reducing bacteria (SRB) communities (Golterman 1975, Fedorak et al. 1987, Holowenko et al. 2000). Application of sulfide microsensors confirmed the presence of sulfide in this zone. SRB normally only function in anoxic conditions, but the presence of SRB in the oxic microcosms will be addressed later in the chapter. Intriguingly, a sulfidic band was also reported in *in situ* FFT tailing retention ponds, similarly at the FFT-OSPW interface (Ramos-Padrón et al. 2011, Chi Fru et al. 2012). Furthermore, the water column in the biotic microcosms, regardless of having oxic or anoxic microcosm environment, became increasingly turbid, compared to the abiotic microcosms, which relatively clear throughout the study period. This turbidity has been thought to be linked to microbial growth in these systems (Chi Fru et al. 2012).

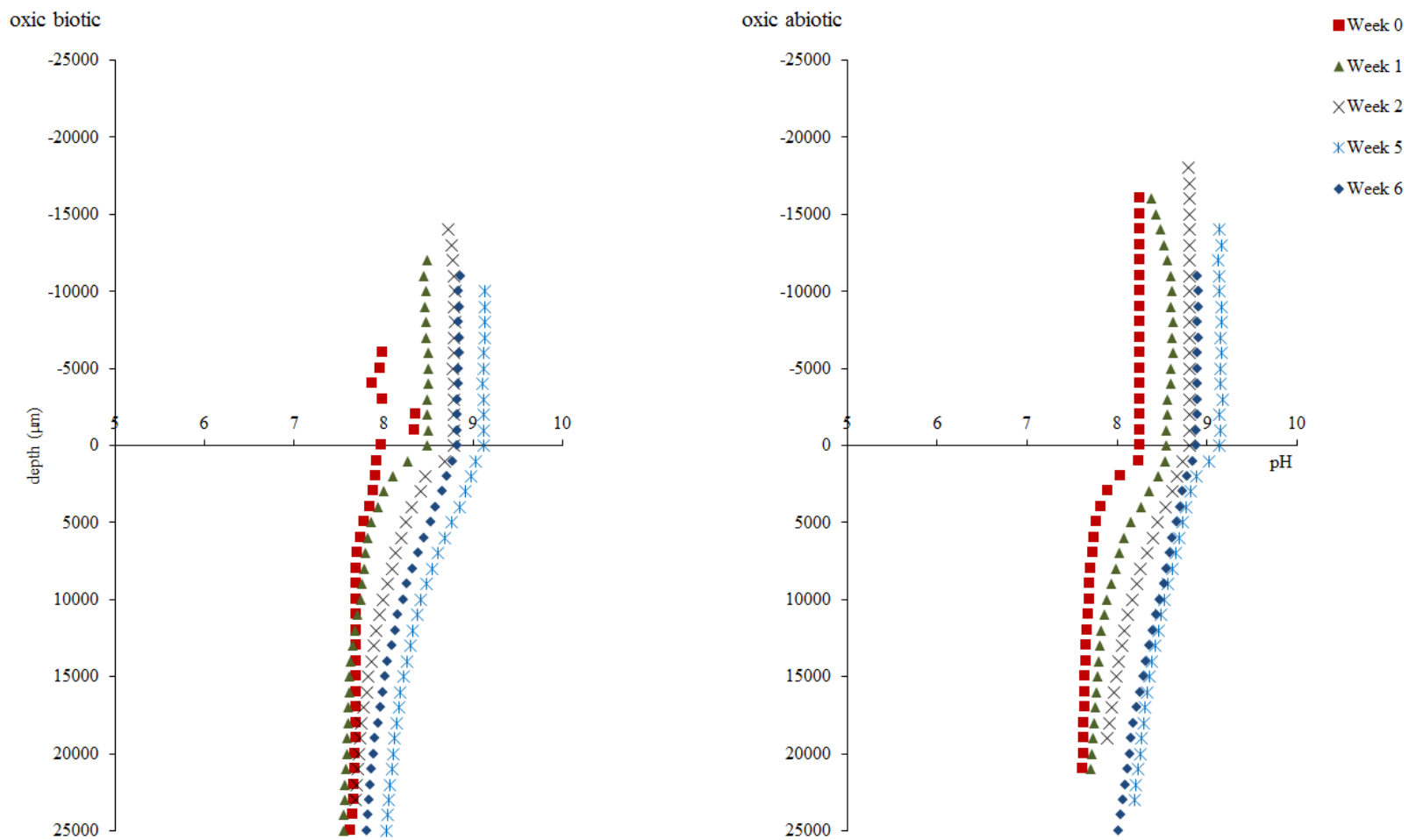


**Figure 2.5 Comparison of oxic abiotic (left) and biotic (right) microcosms after 6 weeks. Note the distinct black band at the FFT-OSPW interface, evidence of sulfide development during week 6.**

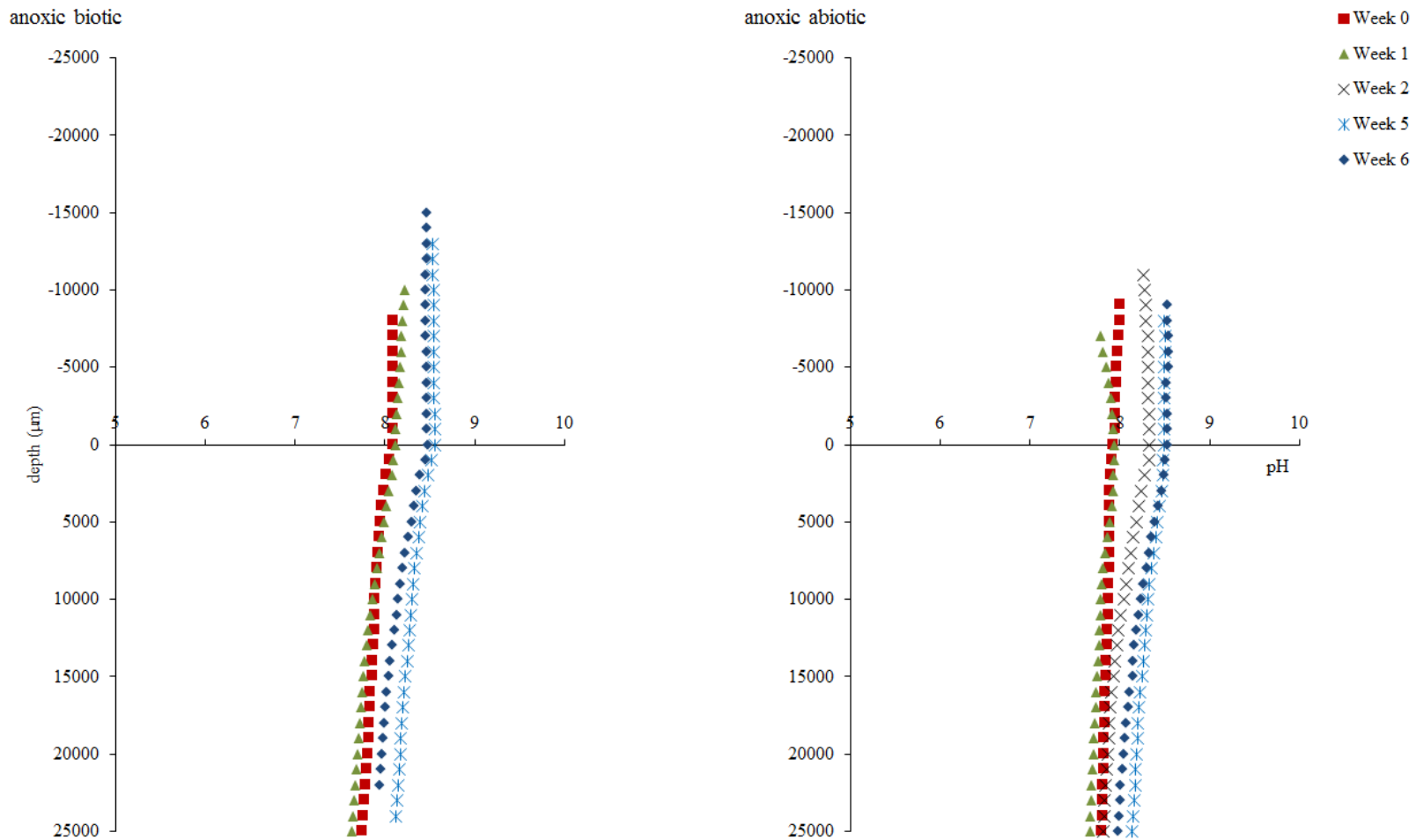
The pH values in the water column for the first 5 mm above the FFT-OSPW interface of the oxic, biotic microcosm (Fig. 2.6) increased moderately over 6 weeks, from ~8.1 to 8.8. The pH in the first 10 mm of FFT below the FFT-OSPW interface was consistently lower than in the overlying water, but also showed a relative increase (from ~7.8 to 8.5) over the 6 weeks. A similar trend was recorded for the oxic, abiotic microcosm system (Fig. 2.6), from ~8.3 to 8.9 in the water column and from 7.8 to 8.7 in the FFT, indicating that changes in pH in these systems were not strongly influenced by biotic activity. In comparison, the less obvious differences in pH were observed across the FFT-OSPW interface in the anoxic microcosms whether or not the microcosm system was biotic or abiotic. The pH in the water column of the anoxic, biotic microcosm (Fig. 2.7) was initially 8.1, increasing to approximately 8.5 after 6 weeks. The pH of the FFT compartment was slightly lower (8.0), increasing only to 8.3 over the 6-week period. The anoxic, abiotic microcosm (Fig. 2.7) had a water column pH value of 8.0 that increased to 8.5 over the 6 weeks, and a FFT pH that increased from 7.9 to 8.4. The relatively similarity of pH values above and below the FFT-OSPW interface suggest that both the water column and FFT compartments are relatively close to reaching steady state in the anoxic microcosms with respect to hydrogen activity during the initial introductory period. Both the oxic and anoxic microcosm pH data suggest little or no biotic influence on pH changes in these systems. However, they seem to point to the fact that there are mechanisms affecting pH, which suggests that oxygen is likely a critical influence for pH changes at the FFT-OSPW interface during this short-term period.



One of many possible reaction pathways leading to increased pH would be a decrease of CO<sub>2</sub> in the system, which suggests methanogenesis is occurring. However, this interpretation is suspect because methanogens generally reside in neutral to acidic conditions (Hatch and Price 2008). The decomposition of organic matter in the system is more likely directly affecting the bicarbonate concentration (Hatch and Price 2008, Stumm and Morgan 1996). This is certainly possible in the context of the anoxic microcosms, since these types of reactions would influence the pH directly, as long as the reduced reaction products remain in a reduced state. The removal of hydrogen ions would occur as the reaction is driven toward creating bicarbonate. The degree of change from the initial pH will depend both on the amount of CO<sub>2</sub> produced and alkalinity, which buffers CO<sub>2</sub> concentration changes (Tucker and D'Abramo 2008).



**Figure 2.6 Comparison of oxic biotic (left) and abiotic (right) pH profiles over the initial (6 week) introductory period. Gradients are observed at the FFT-OSPW interface of the biotic microcosm, and there is an overall increase in pH between week 0 and week 6. There is also a gradient at the FFT-OSPW interface, as well as an overall increase in pH of the abiotic microcosm.**

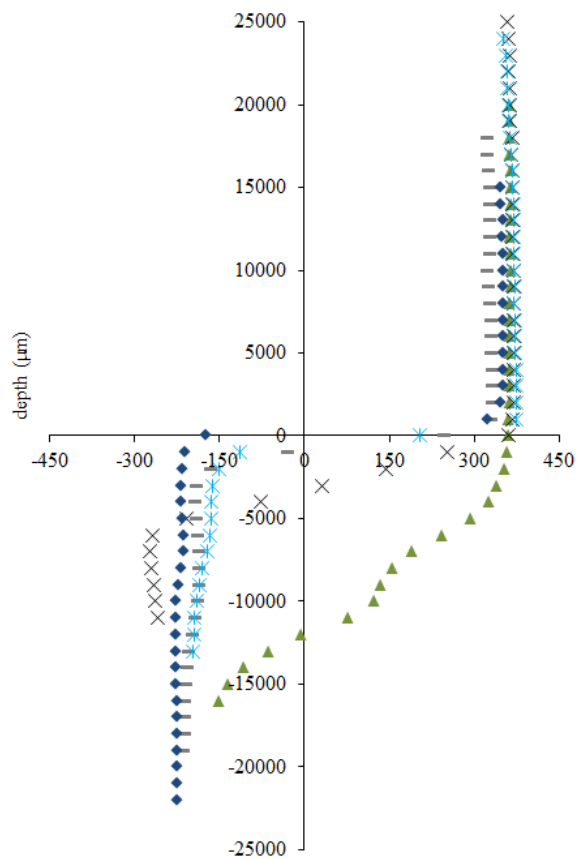


**Figure 2.7 Comparison of anoxic biotic (left) and abiotic (right) pH profiles over the initial (6 week) introductory period. Gradients are much less pronounced, relative to the oxic microcosms, at the FFT-OSPW interface of both the biotic and abiotic microcosms. There is a slight overall increase in pH.**

The Eh in the water column in the oxic, biotic system (Fig. 2.8) was ~335 mV throughout the short-term experimental period, quickly decreasing to reducing values within the FFT. During the first week, Eh gradually decreased with increasing depth within the FFT. However, by week 6, the Eh value within the oxic, biotic microcosm had become constant at approximately -217 mV, regardless of depth (beyond ~1000  $\mu\text{m}$  below the interface). In contrast, the Eh measured in the oxic, abiotic microcosm (Fig. 2.8) water column averaged approximately 354 mV, and became gradually more reducing with increasing depth into the FFT (appearing to approach an estimated maximum reducing Eh value of about -150 mV after reaching below 1000  $\mu\text{m}$  penetration past the FFT-OSPW interface). In comparison, for the anoxic, biotic microcosm (Fig. 2.9), Eh values in the water column decreased rapidly from ~50 mV at week 1 to ~-162 mV by week 5 and 6. During the initial weeks, Eh decreased gradually at the FFT-OSPW interface; notably, the FFT had a similar Eh (-186 mV) to the water column at week 5 and 6. Depth seemed to have little or no effect on Eh at the end of 6 weeks; the decreases of Eh response are immediately evident after contact with the FFT-OSPW interface. The anoxic abiotic microcosm (Fig. 2.9) showed a similar pattern with respect to Eh values across the FFT-OSPW interface; with positive Eh values in the water column, but with an immediate decrease toward reducing values after entering the FFT. These Eh values generally affirm the highly reducing nature of the FFT materials, regardless of whether the water column is exposed to oxygen or not. These observations of a shifting trend towards reducing conditions, suggest that both biotic and abiotic processes are playing significant roles in the distribution of total dissolved salts and ions

in the FFT materials. The systems, however, seem to be more reducing in the presence of biotic activity, which is especially true for the FFT material.

oxic biotic



oxic abiotic

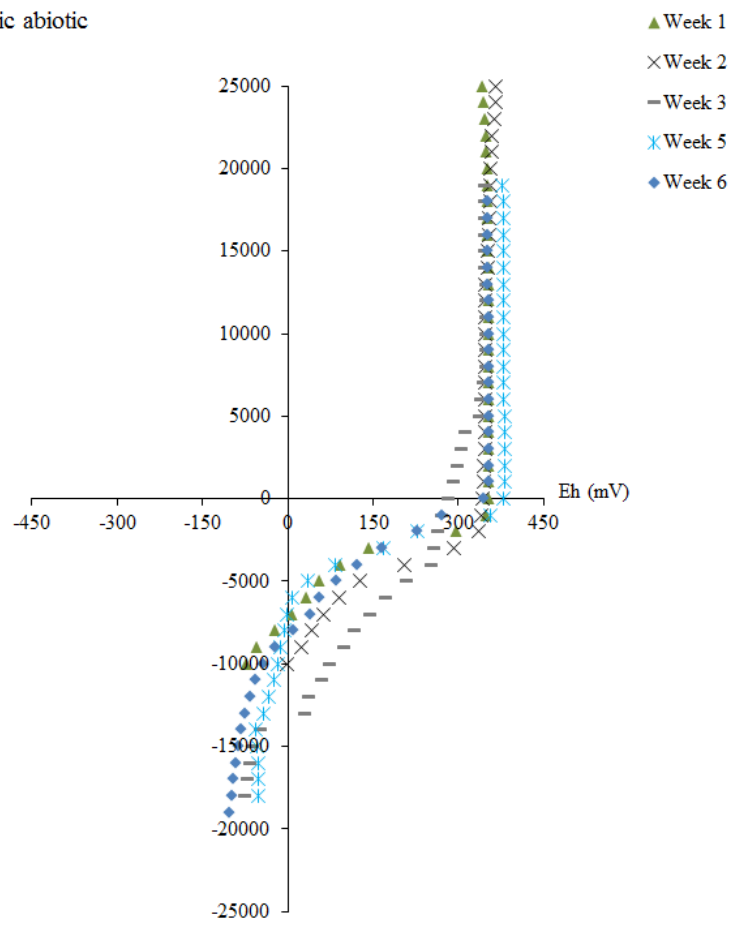
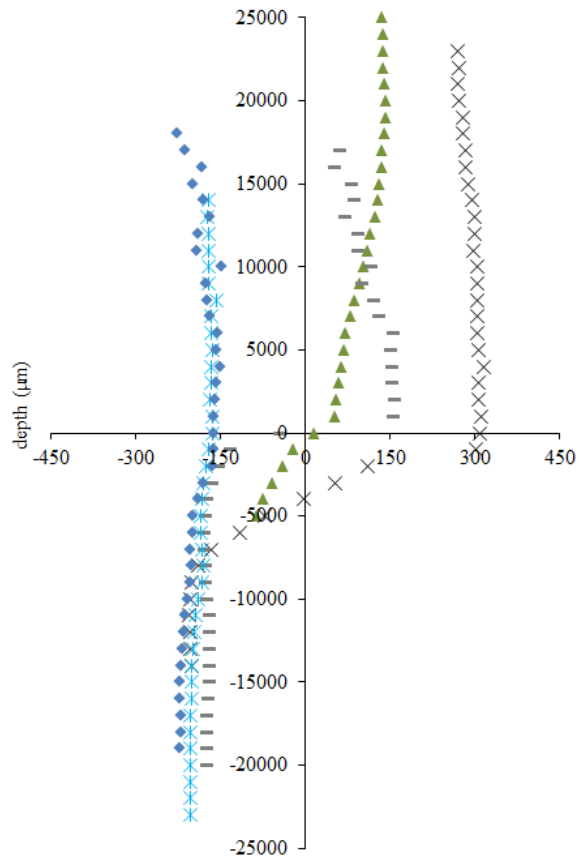


Figure 2.8 Comparison of oxic biotic (left) and abiotic (right) Eh profiles over the initial (6 week) introductory period.

anoxic biotic



anoxic abiotic

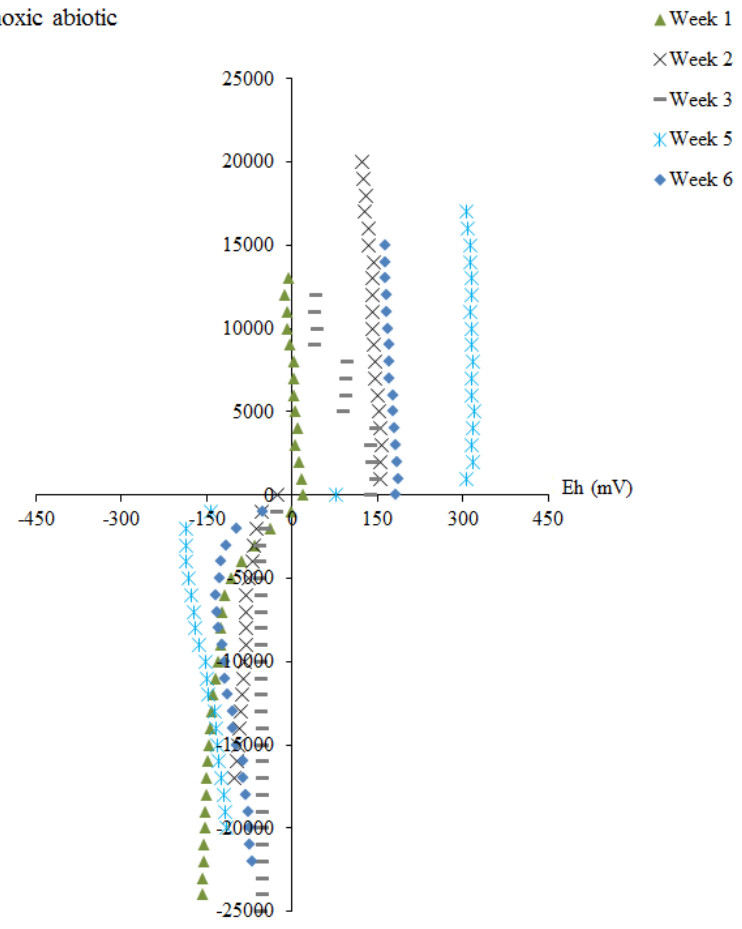


Figure 2.9 Comparison of anoxic biotic (left) and abiotic (right) Eh profiles over the initial (6 week) introductory period.

### 2.3.2 OXYGEN PROFILES

During the initial 6-week period, changes in oxygen concentrations were quantified for the biotic and abiotic microcosms in both oxic and anoxic conditions. Under anoxic conditions (Fig. 2.11), the similarities between the biotic and abiotic systems were immediately apparent. As expected, there was little to no observable difference between the oxygen concentrations measured in the water column and the FFT regardless of whether the FFT was gamma irradiated or not. Both O-ring sealed microcosms contained very low oxygen concentrations (<3 mg/L), which indicates the following: Firstly, it provides evidence that the microcosms remained anoxic over the course of the short-term experimental period. Secondly, the results suggest that no additional processes took place in which oxygen was the driving electron acceptor, as oxygen levels present in the system were negligible (<3 mg/L). Third, there were no processes taking place to introduce oxygen into the system, which is expected of such reducing material. These results are further discussed in Chapter 3. Note that when taking oxygen profiles, the anoxic microcosm had to be temporarily exposed to laboratory atmospheric oxygen to allow for sampling. Despite careful handling of the microcosms, there may still have been trace amounts of oxygen that was given an opportunity to slowly mix into the water column (which can be detected by the sensitivity of the oxygen microsensor). To restrict the amount of oxygen being reintroduced to the systems, the anoxic microcosms were only open to atmospheric conditions when necessary for sampling procedures, and flushed with nitrogen and replaced back in the anoxic gas chamber immediately following the completion of each sampling session.



In the oxic microcosms (Fig 2.10), the concentrations of oxygen in the water column fluctuated slightly from initial concentrations of ~7 mg/L, and decreased to ~3 to 4 mg/L by week 6. Similar to the anoxic microcosms, all oxygen concentrations were below ~1 mg/L within the FFT. Both oxic biotic and abiotic systems illustrated that any residual oxygen (likely left from before the start of the experiment) in the OSPW was immediately consumed by the reducing nature of the FFT. The absence of oxygen at the interface into the FFT is especially important, because it provides a suitable environment for SRB function in both oxic and anoxic microcosms. The reducing nature of the FFT ultimately allows SRB communities to persist and produce sulfide. This will be further explored in the next section and Chapter 3.

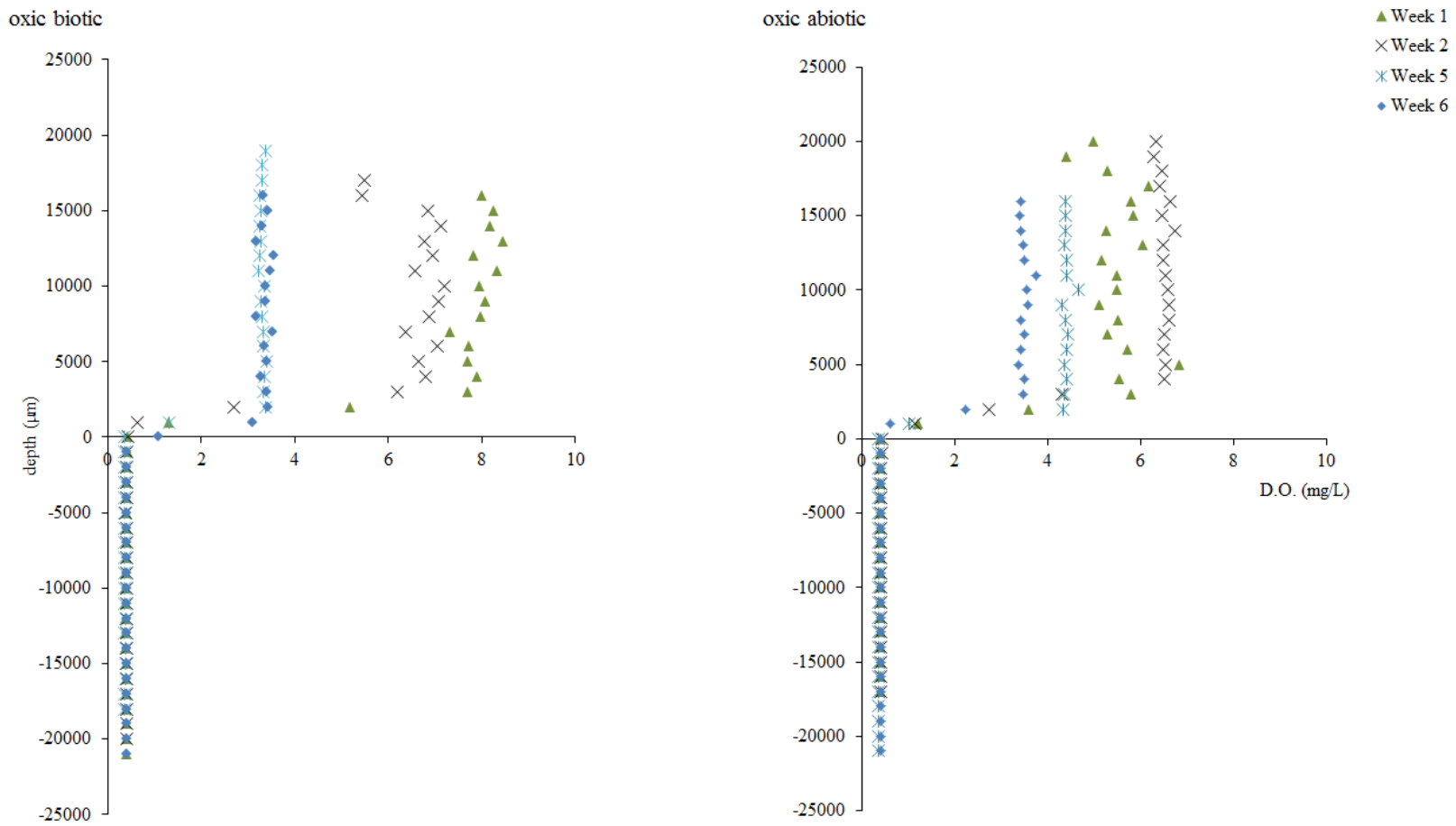
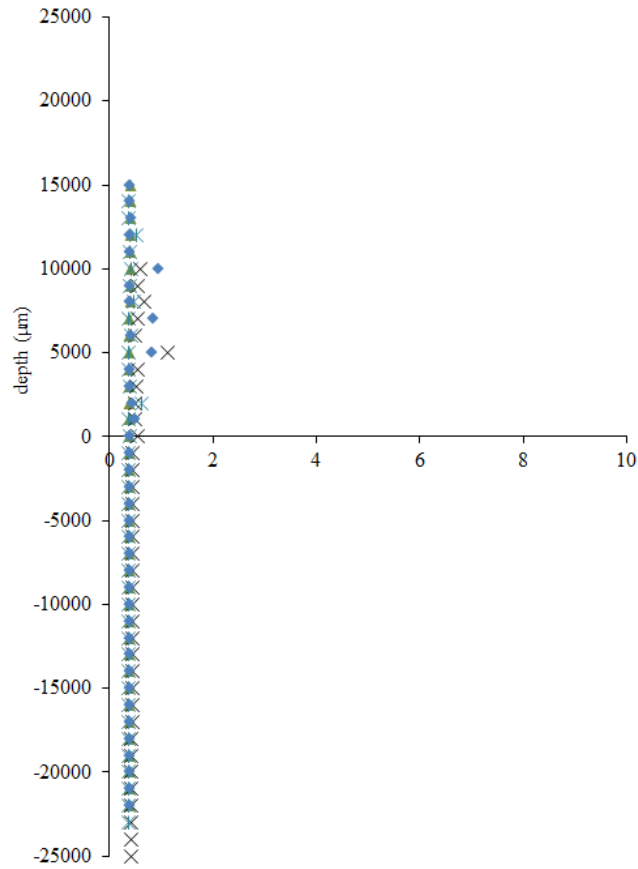


Figure 2.10 Comparison of oxic biotic (left) and abiotic (right) oxygen profiles over the initial (6 week) introductory period.

anoxic biotic



anoxic abiotic

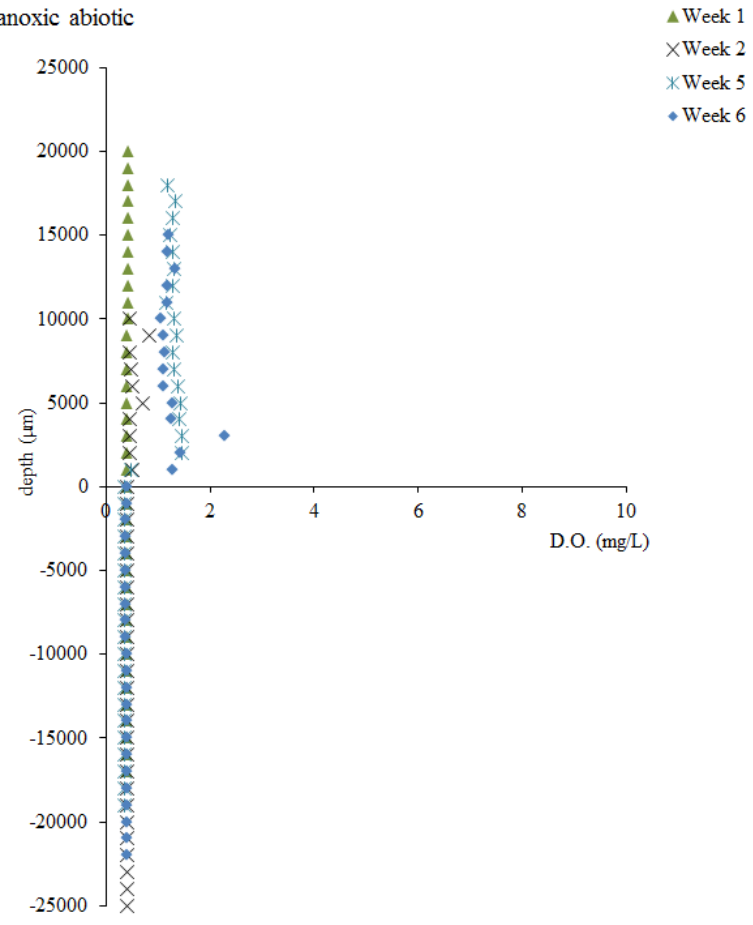


Figure 2.11 Comparison of anoxic biotic (left) and abiotic (right) oxygen profiles over the initial (6 week) introductory period.

### 2.3.3 HYDROGEN SULFIDE FORMATION

The concentrations of  $\text{HS}^-$  measured in both the oxic and anoxic, abiotic microcosms (Fig. 2.12, 2.13) did not change over the initial 6 week short-term period. In both cases,  $\text{HS}^-$  concentrations were negligible throughout the OSPW and FFT microcosm compartments. In contrast,  $\text{HS}^-$  production increased significantly in the oxic and anoxic, biotic microcosms during the short-term study. The evidence is more pronounced in the anoxic treatment, where  $\text{HS}^-$  concentrations increased from  $\sim 40$   $\mu\text{mol/L}$  1 cm below the FFT-OSPW interface after week 1 to a maximum of  $\sim 86$   $\mu\text{mol/L}$  after week 5. The  $\text{HS}^-$  concentrations then started to decrease from 65  $\mu\text{mol/L}$  at week 6, and continued to decrease during the long-term study (Chapter 3). Increases in  $\text{HS}^-$  concentrations in the overlying water column in the anoxic microcosms correlated with peak  $\text{HS}^-$  production after 5 and 6 weeks, reaching maximum concentrations of  $\sim 24$  and 18  $\mu\text{mol/L}$ , respectively. The results are consistent with activity calculations presented in Chapter 3. Oxygen apparently plays a subordinate role in sulfide generation in the FFT materials, likely due to the reducing nature of the material. The oxic biotic microcosm showed relatively low  $\text{HS}^-$  concentrations throughout the entirety of the 6 weeks compared to the anoxic biotic system, but similarly exhibits increasing concentrations with greater depth penetration.

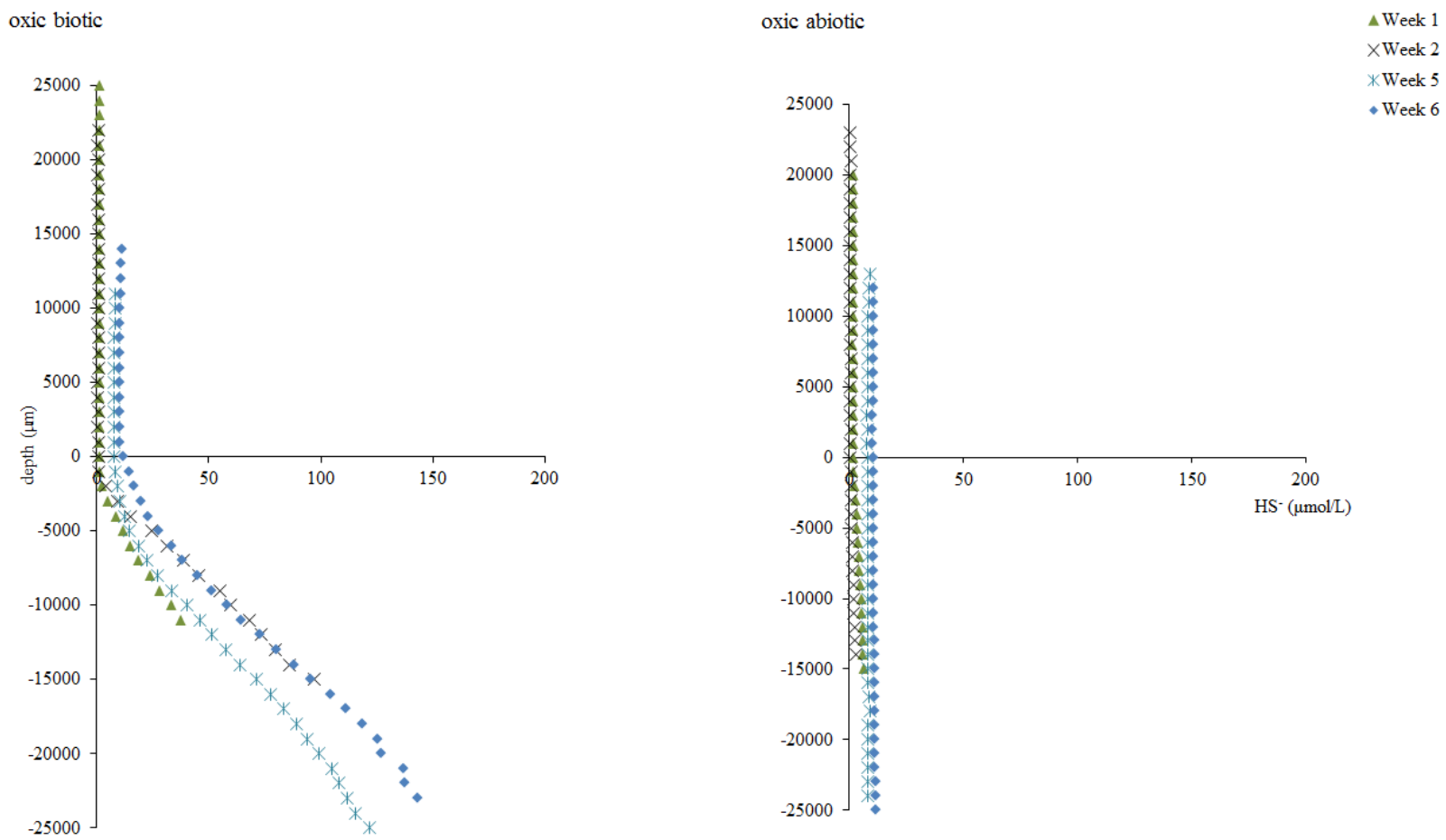


Figure 2.12 Comparison of oxic biotic (left) and abiotic (right) HS<sup>-</sup> profiles over the initial (6 week) introductory period.

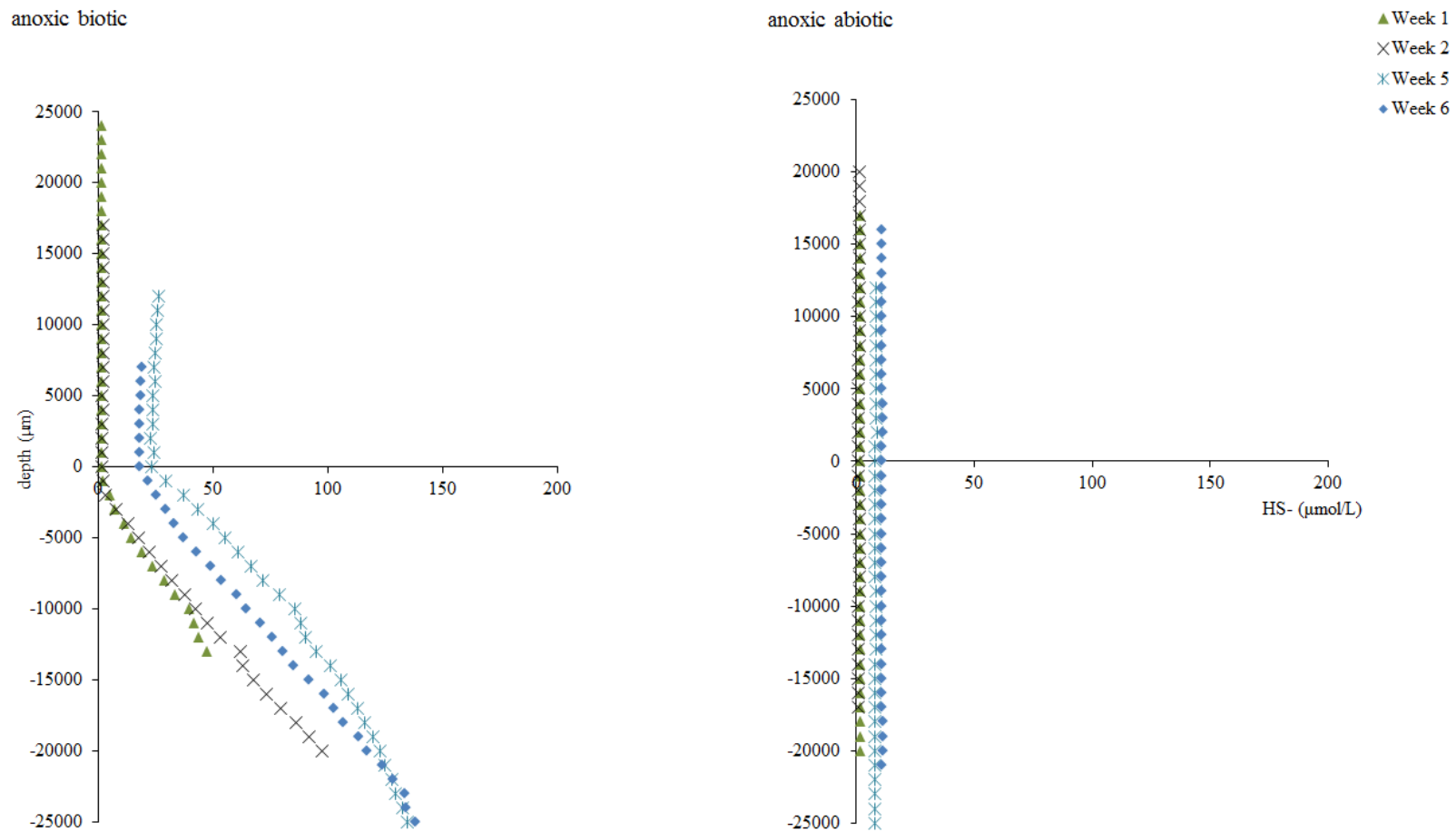


Figure 2.13 Comparison of anoxic biotic (left) and abiotic (right) HS<sup>-</sup> profiles over the initial (6 week) introductory period.

## 2.4 CONCLUSIONS

This study demonstrated early evidence of  $\text{HS}^-$  concentration changes at the FFT-OSPW interface, and shows that strictly biotically-derived  $\text{HS}^-$  production is apparent in the short-term development of FFT. These results are consistent with the original expectation that  $\text{HS}^-$  would be present, inferred from previous studies identifying SRB communities (Sobolewski 1999) and sulfate (i.e., the principal terminal electron acceptor used by SRB for sulfate reduction) availability (Gardner et al. 2011, Appendix B) in similar aquatic systems. In terms of remediation of EPLs and their transformations to other aquatic ecosystems, like reclaimed wetlands, the data shows that the potential for  $\text{HS}^-$  production may be a cause for considerable concern.  $\text{HS}^-$  dispersal into the overlying water column poses a risk to sustaining a viable living environment for higher order organisms (Bagarinao 1992). Predicting when  $\text{HS}^-$  production will cease or how quickly the decrease will occur is not yet possible from these results alone (Chapter 3, Chapter 4).

Generally, the microcosms were found to possess a high SOD. The DO concentrations were much lower below the FFT-OSPW interface, and this can be attributed to both biotic and abiotic factors during the short-term period. The depletion of DO in the FFT, even in the oxic biotic microcosm system, explains why SRB are able to be part of the FFT microbial community when DO is present in the overlying water. Results also suggest that changes in pH are interconnected with oxygen. However, definite conclusions cannot be drawn from this experiment, and only speculations can be made as to why pH increased. Answers may be provided by future studies (Chapter 3, Chapter 4). Eh readings affirmed that the FFT is a reducing environment. The results

indicate that both biotic and abiotic processes play important roles, but the biotic microcosm showed a more reducing FFT environment than the abiotic microcosm. It is additionally important to remember that there are other possible reactions not monitored in this study, which also contribute to the overall Eh.



## CHAPTER 3

### LONG-TERM ASSESSMENT OF THE BIOGEOCHEMICAL DEVELOPMENT OF OXYGEN AND SULFUR IN OIL SANDS FLUID FINE TAILINGS

#### 3.1 INTRODUCTION

In the Athabasca region of north-eastern Alberta, bitumen recovery and commercial production are anticipated to grow, following new advances in extraction technology, coupled with global decline of conventional oil reservoirs (Williams 2003). End-pit lakes (EPLs) are designed using fluid fine tailings (FFT) as part of a strategy to reclaim the landscape following mining activity. The tailings pond water possesses a unique, saline composition consisting mostly of recycled oil sands process water (OSPW) containing elevated  $\text{Na}^+$  concentrations,  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  anions, naphthenic acids, and ammonia (Appendix A). Current evidence suggests natural attenuation mechanisms have the potential for removal or degradation of naphthenic acid compounds and ammonia, as well as a general shift in the water chemistry toward less saline natural water composition as the system ages (Scott et al. 2005). The underlying chemical factors contributing to the diffusive processes operating during maturation of materials in tailing pond basins, however, still require explanation.

This study was designed to investigate sulfide production rates and the behaviour of oxygen in FFT, in order to assess the potential development of EPL settings over a prolonged period of time with the use of microcosms. Continuing from the previous chapter, the observations aimed to determine if FFT development was primarily driven

by biotic or abiotic factors, with respect to oxygen and sulfur, and if the trends observed in the short-term study are consistent with comparatively long-term observations.

The sediment oxygen demand (SOD) function of the FFT directly influences dissolved oxygen (DO) in the water column, and thus, can be affected by a combination of biotic and abiotic processes occurring at the FFT sediment-OSPW interface (Chapter 1, Chapter 2). The principal biological sediment oxygen demand (BSOD) contributors result from the biological respiration associated with bacterial decay processes at the FFT–OSPW interface, together with the release of abiotic chemically-reduced compounds, such as sulfide. The soluble compounds exert a rapid kinetic oxygen demand if the reduced compounds undergo oxidation.

Current data suggest there are various microbial consortia responsible for distinct redox-dependent biogeochemical processes (i.e., development of aqueous molecular and ionic species such as sulfide compounds involving metals like Fe and Mn) that have developed in established FFT settling basins (Penner and Foght 2010, Ramos-Padrón et al. 2011). These complex associations included anoxic heterotrophs, sulfur-reducing bacteria (SRB), and archaea (e.g., methanogens) (Clemente and Fedorak 2005, Siddique et al. 2006, 2007, Penner and Foght 2010, Ramos-Padrón et al. 2011). Substantial evidence also indicated that sulfur cycling is crucial in EPL settings, and a dominant factor for determining the spatial organization of the various microbial communities (Penner and Foght 2010, Ramos-Padrón et al. 2011, Chi Fru et al. 2012) linked to sulfide production and methanogenesis (Holowenko et al. 2000, Salloum et al. 2002).

As mentioned in the previous chapters, oxygen availability contributes to the speed and success of microbial colonization and persistence in EPLs, and especially in laying the foundation for establishing aquatic conditions suitable for development of a food web. Based on findings of the short-term microcosm study, FFT is not an ideal proxy for viable lake sediments. Nevertheless, it is necessary to determine if DO concentrations change (i.e., increase) after the introductory period before making final conclusions.

Rates of oxygen diffusion into the FFT can determine the degradation rates of recalcitrant hydrocarbons, since oxygen is often required for microbial oxygenases to initiate hydrocarbon biodegradation under oxic conditions (Leahy and Colwell 1990). Conversely, specific consortia of microorganisms, like SRB, can degrade hydrocarbons under completely anoxic conditions (Rueter et al. 1994). In particular, naphtha (a residual solvent remaining in the FFT after froth treatment) and naphthenic acids (organic acids present in the original oil sands ore, but concentrated during the extraction process) are likewise biodegraded in the absence and presence of oxygen, respectively (Scott et al. 2005, Siddique et al. 2006, 2007, 2011). Therefore, the development of redox gradients in the FFT, as a function of oxygen availability and demand, is likely to facilitate various biogeochemical processes at different rates.

Little is known, however, about the specific rates of generation and consumption of key oxidants and reductants in EPLs, such as oxygen and sulfur species. The factors

responsible for controlling the biogeochemical dynamics of these redox sensitive species still require attention. This is especially true for HS<sup>-</sup> generation, as this can stress eukaryotic organisms (Williford et al. 2009). The HS<sup>-</sup> production presents potential for critical colonization problems in the overlying water column, where higher trophic level communities are expected to grow following the reclamation of the previously mined areas. In addition, HS<sup>-</sup> plays a key role in regulating trace metal solubility in many aquatic environments, especially iron, which precipitates to form pyritic iron sulfides (Schoonen 2004). Iron plays a fundamental role in microbial growth. It is important both as an essential trace element for microbial growth in the environment (Archer and Johnson 2000, Church et al. 2000), and as a terminal electron acceptor and donor during microbial metabolism (Weber et al. 2006, Crowe et al. 2007, Chi Fru et al. 2012). Thus, HS<sup>-</sup> generation linked to SRB consortia within EPLs may play a fundamental role in both iron cycling and microbial growth.

This study investigated sulfide generation rates and the role of oxygen in FFT, in order to assess their potential behaviour in EPL settings over a long-term time period, using microcosms, which have previously demonstrated the ability to simulate *in situ* tailing ponds and microbial community structures (Chi Fru et al. 2012). Specifically, the experiment posed and answered the question of whether these processes are predominantly regulated by biotic or abiotic driven activity, with respect to sulfur and oxygen.

### *3.1.1 RATIONALE*

Changes in sulfur concentrations at the FFT sediment-water interface will be observed predominantly in the biotic microcosms, due to the presence of the biologically active component (i.e., microbial community). Biological activity is the principal driver of the changes witnessed over time.

## 3.2 MATERIALS AND METHODS

### *3.2.1 SAMPLE COLLECTION AND MAINTENANCE*

FFT and OSPW were obtained from Syncrude Canada Ltd., in the Athabasca oil sands region near Fort McMurray, Alberta (Chapter 2, Fig. 3.1).

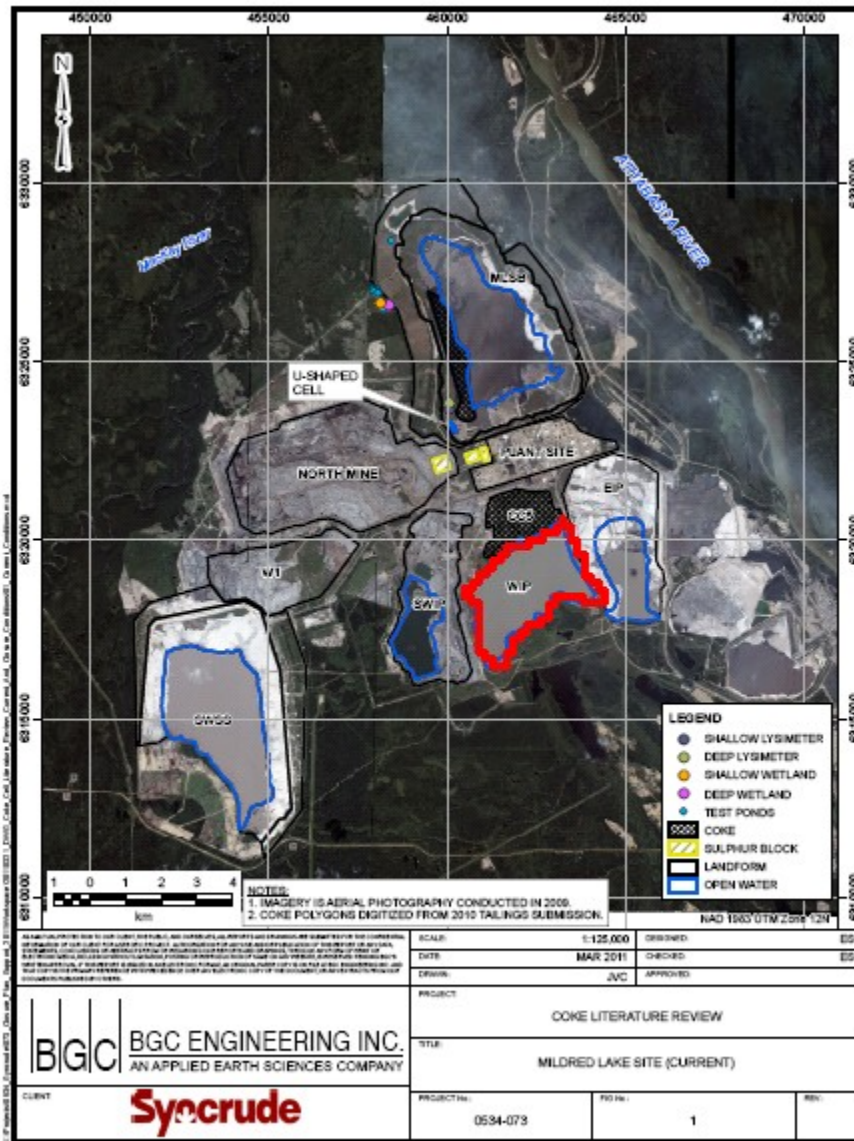


Figure 3.1 Map depicting Syncrude lease site (West In Pit is outlined in red). Aerial photo courtesy of Tara Penner, Syncrude.

### *3.2.2 MICROCOSM SETUP DESCRIPTION AND MAINTENANCE*

A long-term laboratory study was set up over 52 weeks to determine redox gradient changes and chemical flux. This was a continuation of the short-term study, which monitored the initial stages of effects from the tailings treatment (Chapter 2), before leading into monitoring continued changes with aging over a prolonged period for comparison purposes. This component of the study was vital in order to track the changes of oxygen and sulfur in the FFT over time, and to distinguish if microbial communities are responsible for any of the differences found when comparing the short-term and long-term patterns.

### *3.2.3 TAILINGS AND PREPARATION OF MICROCOSM EXPERIMENT*

FFT was collected from Syncrude Canada Ltd and stored at 4°C. Both the FFT and OSPW were sterilized by gamma irradiation at 28 kGy over 24 hrs to eliminate biological activity in half of the samples to serve as abiotic controls. Gamma irradiation was performed at the McMaster Nuclear Reactor using a Gamma Irradiation Hotcell, 10 000 Currie Co-60 Gamma Source in Hamilton, Ontario (Chapter 2). Deoxyribonucleic acid (DNA) quantification of isolates from phenol-chloroform extractions showed that neither gamma irradiated FFT nor OSPW used for abiotic microcosms contained living archaea or bacteria. DNA analysis of materials used in the biotic microcosms (both oxic and anoxic environments), indicated that bacteria were initially prominent, whereas archaea seemed to be completely absent. One possibility accounting for the differences in relative abundances of bacteria and archaea may be that the anoxic cycling of water during the bitumen extraction favoured bacteria over archaea. However, T-RFLP analysis

later confirmed the increasing presence of archaea in the biotic microcosms over time (Chi Fru et al. 2012).

Three sets of microcosms, indicative of the four unique treatment conditions, were arranged for this experiment and were designed to be representative of three major developing long-term (24 weeks, 36 weeks, and 52 weeks) time intervals (each set reproducing the short-term study, as outlined in Chapter 2). These long-term replicates were created and initiated alongside the short-term study microcosms, in order to ensure that each of the three long-term sampling periods were relatively undisturbed until sampling had to occur. Individual microcosms were set up to compare both oxic and anoxic environmental conditions, as well as biologically active and inactive FFT and OSPW. These long-term microcosms comprise twelve individual microcosm units, which were set up in a controlled laboratory environment at the Great Lakes Institute for Environmental Research, University of Windsor in Windsor, Ontario. Each microcosm unit was prepared and initiated at the same time as the short-term microcosm study from Chapter 2, and only individually destructively analyzed at the designated 24, 36, and 52-week periods.

#### *3.2.4 MEASUREMENTS WITH MICROELECTROCHEMISTRY*

For the three long-term study periods of the experiment, all microsensors used the Unisense Microsensor Multimeter, a digital microsensor amplifier, which works with all amperometric and potentiometric sensors without needing manual adjustment of the various polarizations of individual microsensors. Micromanipulator-mounted,



microelectrode sensors purchased from Unisense Science (Denmark), characterized by their linear response to specific media, providing micrometer scale measurements (Damgaard et al. 1995), were utilized to monitor changes in pH, Eh, O<sub>2</sub>, and HS<sup>-</sup> at multiple water and FFT depths within the microcosms to form profiles (Chapter 2). Microsensors were calibrated before each experimental period. The tip diameters used were between 100 and 500 μm. Microsensors for oxygen and HS<sup>-</sup> were constructed and calibrated before every experiment according to Revsbech (1989) and Kuhl et al. (1998), respectively. Eh (Andersen et al. 2001) and pH (Revsbech and Jørgensen 1986) sensors were purchased from Unisense A/S (Denmark), and calibrated according to procedures described in Mohanakrishnan et al. (2009) and Revsbech and Jørgensen (1986).

### *3.2.5 DIFFUSIVITY MEASUREMENTS*

The Unisense diffusivity sensors are a variety of amperometric sensors; there is a micro-transducer surrounded by a tracer gas reservoir with a tip membrane. Hydrogen gas was used as a tracer gas in our experiment, using a 50 μm diameter tip. The diffusivity microsensor used in this experiment was a flow-through type sensor requiring the hydrogen source to be connected to the sensor, and hydrogen flow had to be adjusted so that the outlet flow resulted in a stable signal.

A measurement of apparent gas diffusivity in pore water is based on diffusion from the hydrogen reservoir through the diffusion barrier of the sensor tip, and into the microcosm environment. The signal of the microsensor was calibrated to allow measurement of the apparent diffusivity = porosity · diffusion coefficients, whereas the

diffusivity of stagnant, deionized water and Unisense-provided glass silica beads were known ( $2.22 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  and  $0.57 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ , respectively). A two-point calibration was sufficient for our experiment (Revsbech et al. 1998).

### 3.2.6 INTERPRETATION OF PROFILES

The microsensor assumes that the microcosms are one-dimensional systems, where there is relative homogeneity in one dimension so that a depth profile can be produced from the collected data (Chapter 2).

The concentration value at any given depth in a microcosm can be obtained through the undifferentiated concentration profile. Then utilizing Fick's First Law of Diffusion, the flux of oxygen or sulfide molecules can be obtained. Fick's diffusion law is meant for "homogeneous" case systems. Although it may not be as dependable in heterogeneous environments, Fick's Law has frequently been applied to freshwater and marine aquatic environments. Revsbech (1989), Elberling and Damgaard (2001), Arega et al. (2005), and Sørensen et al. (2009) have shown various applications on environmental samples. Hofman et al. (1991) and Glud (2008) had specifically applied Fick's Law in benthic oxygen availability of marine systems. The FFT and OSPW that result from the oil sands extraction and purification process are relatively homogeneous when compared to native aquatic sediments and water. Therefore, it is reasonable to apply Fick's Law to this particular study.

$$J(x) = -\phi D(x) \frac{dC(x)}{dx} \quad (\text{Eq. 3.1})$$

(Revsbech et al. 1998)

Where:

J is the diffusion flux, which is the amount of substance that will flow through a small area during a small time interval. D is the diffusion coefficient or diffusivity in dimensions of  $\text{length}^2 \cdot \text{time}^{-1}$ ; the diffusivity microsensor measures this as  $\text{cm}^2 \text{ s}^{-1}$ .  $\Phi$  is the concentration in dimensions of  $\text{amount of substance} \cdot \text{length}^{-3}$ . The flux describes the vertical movement of the substance through the specific layer (i.e., between two compartments such as FFT overlaid with OSPW), and can describe the exchange of the substance between the FFT sediment-OSPW interface.

The first derivative of the concentration profile, or the slope, represents the vertical transport of the molecules between specific depths, or the exchange of molecules at the boundary defined by the sediment-water interface. For this study, the slopes of oxygen and sulfide concentration profiles were taken over the first 1000  $\mu\text{m}$  below the FFT surface. This can be interpreted in terms of rates of production and consumption (also referred to as activity) at the FFT-OSPW interface.

The use of the diffusivity microsensor made this a straightforward matter. Simply, after obtaining a depth profile of concentration values (i.e., DO and  $\text{HS}^-$  for this experiment), the slope of the profiles in the FFT and/or the OSPW were calculated, and then multiplied by the diffusivity value measured by the diffusivity microsensor

(approximately  $6.73 \times 10^{-6}$  cm<sup>2</sup>/s at the FFT-OSPW interface) to obtain an activity value (Damgaard et al. 1995).

### 3.2.7 HEADSPACE GAS CHROMATOGRAPHY

Gas samples were analyzed using a Varian 3800 gas chromatograph (GC) equipped with packed sample columns, a vapourization split/splitless sample injector, and a flame ionization and mass spectrometer detector. The GC was calibrated using a calibration standard gas composed of 3.125% CH<sub>4</sub>, 3.125% CO<sub>2</sub>, 3.125% H<sub>2</sub>, 3.125% O<sub>2</sub> (percent volume) and a balance of N<sub>2</sub> gas (provided by Praxair). Both anoxic biotic and abiotic microcosms were sampled separately from the modified push valves, and measured one at a time with the GC. Gas samples (25 µL) were removed from each microcosm for analysis during the last sampling period using specialized airtight gas needle syringes. A Pasteur pipette bulb was fastened to the male-half of the microcosm lid push valves before connecting to the female-half, in order to prevent undesired introduction of external contaminants (e.g. atmosphere, particulates). The needle was then used to penetrate the pipette bulb, and inserted into the headspace gas. The gas needle was opened and the plunger of the syringe was slowly pulled to create negative pressure and draw in gas; and then the gas needle was closed before removing the needle from the microcosm. All gas samples taken from the microcosms were run on the day of sampling, and were stored in the gas needles for no more than 1 hr prior to analysis. Samples taken were run in random order on the GC. Before measuring any gas samples, blanks and atmospheric (air taken from the lab we set up in) samples were also analyzed by the GC as a control. We also measured gas samples taken from the anoxic gas

chamber as an additional control. Concentrations of CO<sub>2</sub>, CH<sub>4</sub>, O<sub>2</sub>, and N<sub>2</sub> were obtained from GC sample elution profiles. The amounts of each gas were calculated from the gas standard used to calibrate the GC.

### *3.2.8 WATER COLUMN AND PORE WATER COLLECTION*

Water was sampled using Rhizon soil moisture samplers purchased from Hoskin Scientific (Fig. 3.2). This method is especially suitable because it allows water to be sampled from both saturated and unsaturated environments in a relatively non-intrusive manner (i.e., without having to physically transfer any material from the microcosms). Each Rhizon sampler possesses a porous membrane (mean pore size of 0.15 µm) making it unnecessary to filter samples before analysis (which typically requires samples to be free of large particulates). Individual samples were taken from each microcosm at the specified sampling period from both the overlying OSPW cap and the FFT pore water using a combination of the Rhizon samplers and vacuum vials to provide suction. Approximately 10 mL of cap water could be obtained over a 10-min period, whereas 8 mL of pore water could be obtained over an 18-hr period.



**Figure 3.2 Rhizon sampler used for obtaining water from microcosms.**

### *3.2.9 TOTAL CARBON ANALYSIS*

Analysis of organic carbon content in the water samples were performed using a TOC/TN analyzer (liquiTOC II, manufactured by Elementar Analysensysteme). The technique is based on high temperature oxidation with the unique capability of estimating additive TIC/TOC concentrations from a single sample injection (1 mL with a 10-fold dilution). This method is suitable for various applications ranging from high purity water to waste water (Elementar, <http://www.elementar.de/>).

### *3.2.10 ION ANALYSIS*

One of the techniques used involves direct analysis of an acidified raw sample (dilution was done using 1% nitric acid), which was filtered to remove suspended solids by the Rhizon samplers. The ionic composition of the solution was then determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, IRIS #701776, Thermo Jarrell Ash Corporation). Liquid samples were introduced into the instrument via a Meinhard concentric glass nebulizer (TK-30-K2, JE Meinhard Associates Inc., California, USA) combined with a cyclonic spray chamber. The plasma high temperature ionizes the sample, which becomes excited and emits light at wavelengths characteristic of the elements present (this chapter will focus on sulfur).

### *3.2.11 SPECTROSCOPIC CHARACTERIZATION*

The compositions of particulate materials in the original starting FFT were compared with samples collected after 6 and 36 weeks from the biotic microcosms, and examined using a FEI Quanta 200F environmental scanning electron microscope (ESEM)

under low vacuum at 15 kV. Both backscattered electron (BSE) and secondary electron (SE) detectors were used. Energy dispersive spectroscopy (EDS) analysis was performed to confirm the elemental composition of minerals. The Genesis Particle Phase cluster analysis software was used for modal mineralogical determinations based on 4000 individual particles. The modal percentages are calculated on the proportion of sulfides to accessory minerals within the matrix material (Weisener and Weber 2010). In addition, X-ray diffraction (XRD) patterns of representative samples of FFT were studied at the National Research Council (NRC) in Ottawa using a RIGAKU D/MAX 2500 rotating-anode powder diffractometer, with monochromatic CuK $\alpha$  radiation at 50kV, 260 mA, a step-scan 0.02°, at a scan rate of 1° per min in 2 $\theta$  from 5 to 70°. Samples for these analyses were collected as cores made from retrofitted cylindrical Costar 25 mL serological pipette tubes. Cores were taken from each microcosm by inserting the stripette into the FFT and creating suction to draw out FFT, while retaining a column of OSPW on top (Fig. 3.3). The bottom of the cores were sealed with sterile rubber stoppers and both ends wrapped in paraffin wax to prevent sample loss, before being set on ice when sampling the remaining microcosms. After the sampling was finished, all cores were immediately frozen at -80°C until the time for DNA extraction.



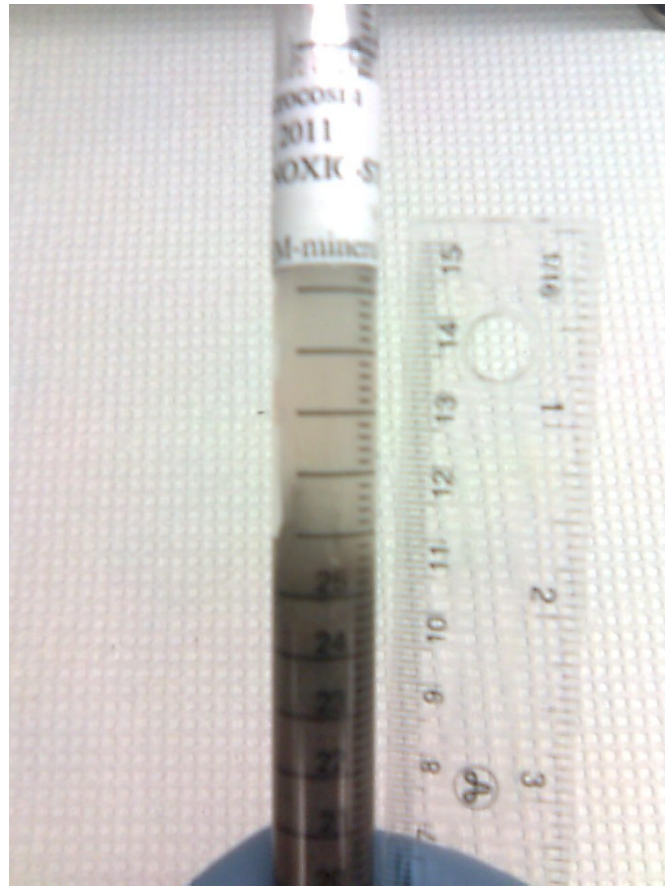
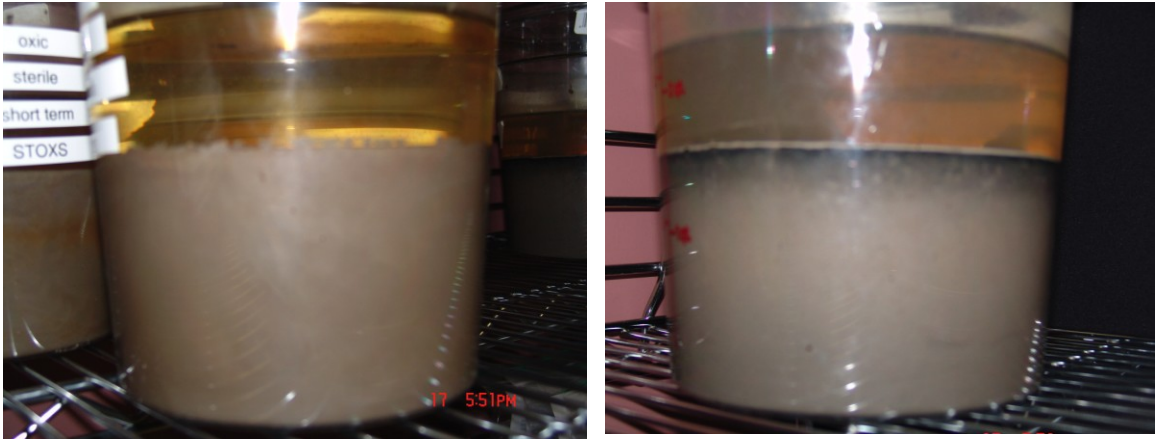


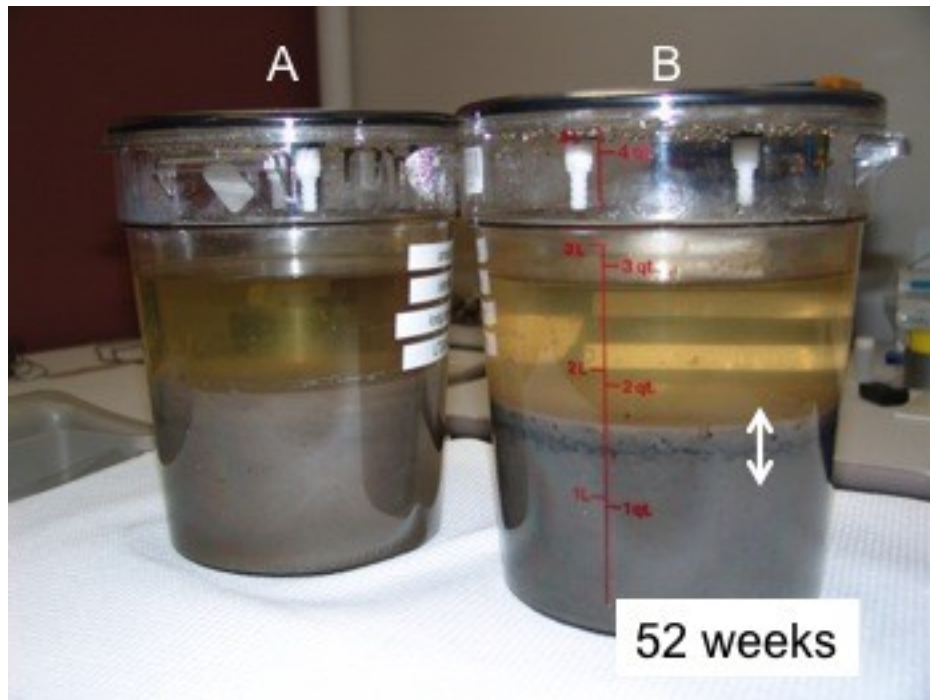
Figure 3.3 Example of a core taken before freezing.

### 3.3 RESULTS AND DISCUSSION

Between the sampling periods, documentation of qualitative changes continued to take place on a weekly basis to maintain a record of microcosm volume changes (e.g. OSPW evaporation, FFT compaction, data cataloguing and archiving) as well as the characteristics of the black, sulfidic banding at the FFT-OSPW interface present in the oxic and anoxic biotic microcosms (Fig 3.4, 3.5). As with the short-term experiment, this banding occurred regardless of whether the microcosms were oxic or anoxic. Such banding was never observed in any of the gamma-irradiated abiotic microcosms, and the recent observation of such banding occurring *in situ* in FFT tailing retention ponds, similarly at the FFT-OSPW interface, at water depths as great as ~15 m (Ramos-Padrón et al. 2011, Chi Fru et al. 2012), continues to support the postulate that the development of sulfide is driven by biotic activity (i.e., SRB communities). The OSPW in the biotic microcosms of both oxic and anoxic treatments became increasingly turbid through the course of the study, relative to the abiotic microcosms, which remained minimally turbid throughout the study. This turbidity, though not yet quantified, has been ascribed to possible microbial growth in these systems (Chi Fru et al. 2012).



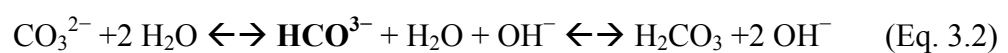
**Figure 3.4 Comparison of oxic biotic (left) and abiotic (right) microcosms after 6 weeks. Note the distinct black sulfidic band at the FFT-OSPW interface.**



**Figure 3.5 Comparison of two sealed anoxic microcosms after 52 weeks. [A] represents the abiotic system; [B] represents the biotic system. The double arrow denotes the black zonation formed by sulfide formation within this system.**

At the end of the 52-week period, pH did not vary in the OSPW above the interface, which was consistent with the short-term observations (Chapter 2). The pH in water column of both the oxic, biotic and abiotic microcosms was approximately 9 (Fig. 3.6), but pH declined below the FFT-OSPW interface. The pH of FFT in the oxic, biotic microcosm gradually decreased as depth increased, approaching a value of ~8.4 at a depth of 25000  $\mu\text{m}$ . The pH of the oxic, abiotic microcosm also decreased with increasing depth, approaching ~8.6. The anoxic, biotic microcosm (Fig. 3.7) had a pH of ~8.5, which gradually approached ~8.3 in the FFT; and the anoxic abiotic microcosm (Fig. 3.7) showed a pH of ~8.6 in both the OSPW and FFT compartments. Clearly, by the end of the experiment, the differences in pH between the FFT-OSPW interface were much smaller in all microcosms than those observed during the initial introductory time period. Also, the results in this study are consistent with the previous chapter's presentiment of approaching relative steady state, with respect to hydrogen activity in the anoxic microcosms, and the postulate that the presence of oxygen affects the pH at the FFT-OSPW interface.

In addition, the presence of relatively basic conditions (pH ~8 to 9) observed in the microcosms, suggests that any carbon dioxide that may be produced in the solid FFT phase would be driven towards becoming bicarbonate by the overlying liquid phase since bicarbonate is the dominant ion at a pH range of 8 to 10 (Stumm and Morgan 1996).





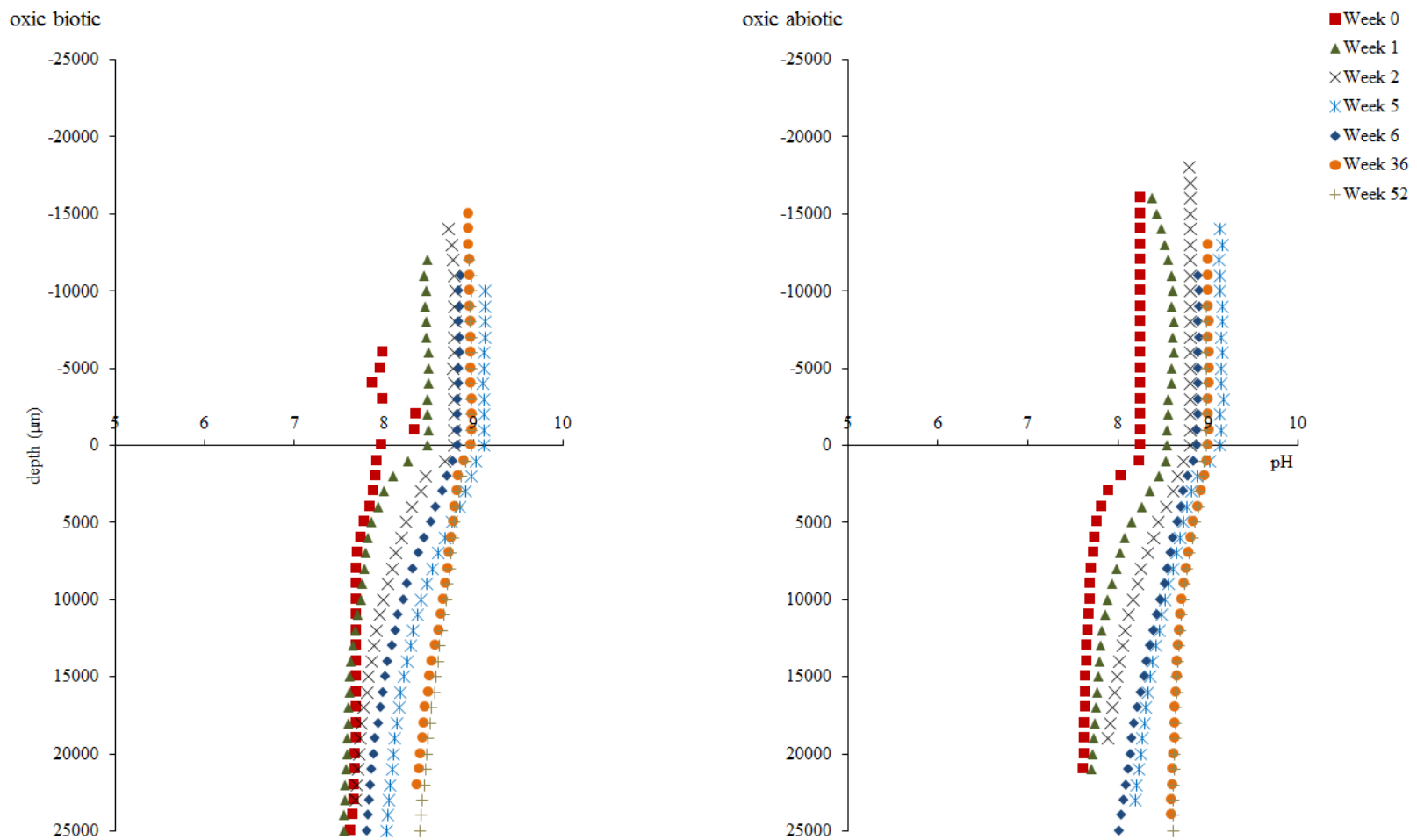


Figure 3.6 Comparison of the oxic biotic (left) and abiotic (right) pH profiles over a 52 week period.

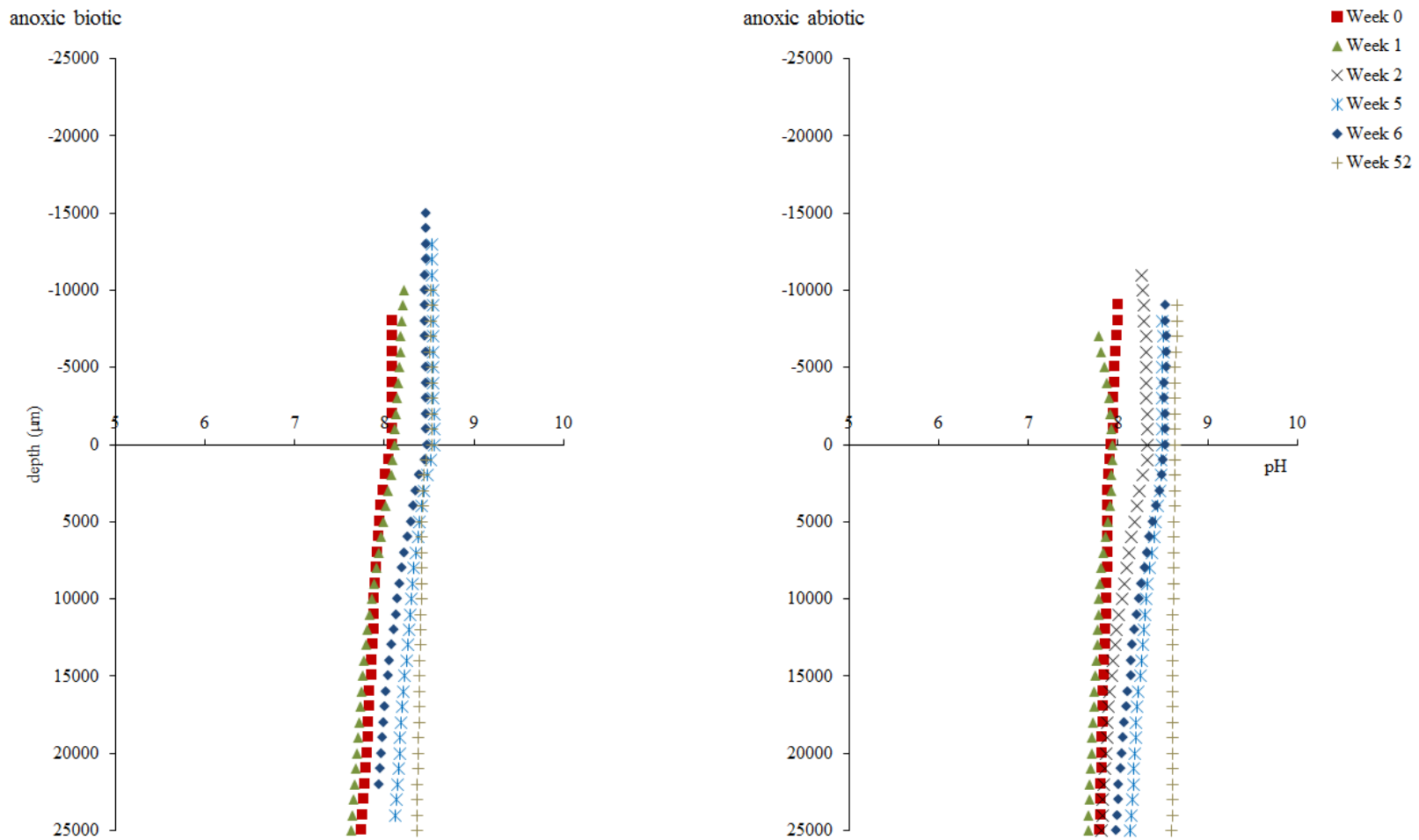
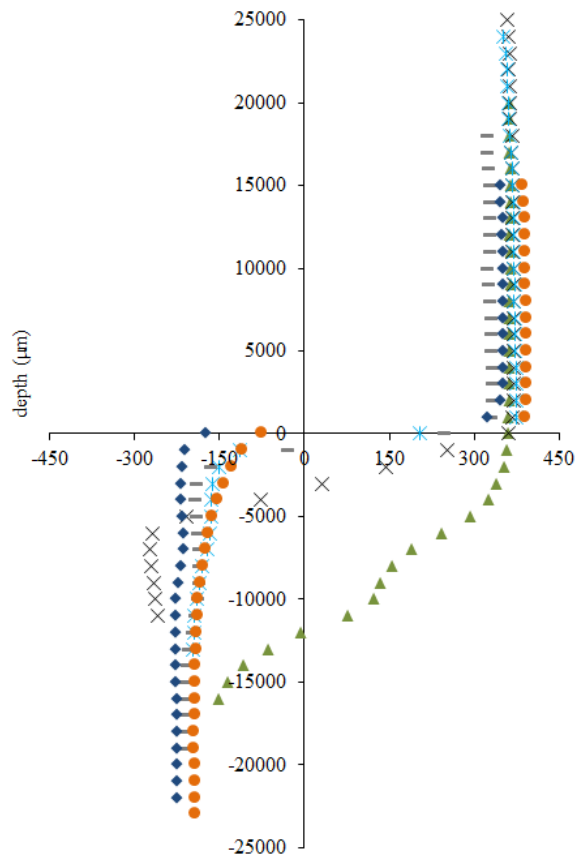


Figure 3.7 Comparison of the anoxic biotic (left) and abiotic (right) pH profiles over a 52 week period.



Generally, Eh profiles (Fig. 3.8, Fig. 3.9) did not change very much through time in either the OSPW or the FFT over the course of the long-term experiment; they were very similar to the values observed at the end of the short-term introductory period observations. The one obvious exception is the oxic abiotic microcosm, which showed the Eh gradient to have moved down to a lower depth (Fig. 3.8). Due to the nature of the sampling, which requires visual observation to detect when the microsensor enters the interface, it is possible that this anomaly may be caused by human procedural error, but because of the consistency of recorded results (both manual transcription of meter readings, the microsensor software recordings detected the anomaly), this observation is apparently valid. Overall, the FFT material remained reducing, whereas the overlying OSPW generally became relatively oxidizing. These Eh values generally affirm the highly reducing nature of the FFT, especially in the case of the microcosms that are biologically active, as they are more reducing than the abiotic microcosms. The Eh in the OSPW also appears to be more reducing in the anoxic treatments, since there is no introduction of oxygen into the closed microcosm systems; this results in a relatively more reducing water column when compared to either oxic microcosms. These observations suggest that both biotic and abiotic processes contribute to the reducing nature of FFT materials, but that an increasingly reducing Eh in FFT sediment appears to be especially emphasized by the presence of biological activity.

oxic biotic



oxic abiotic

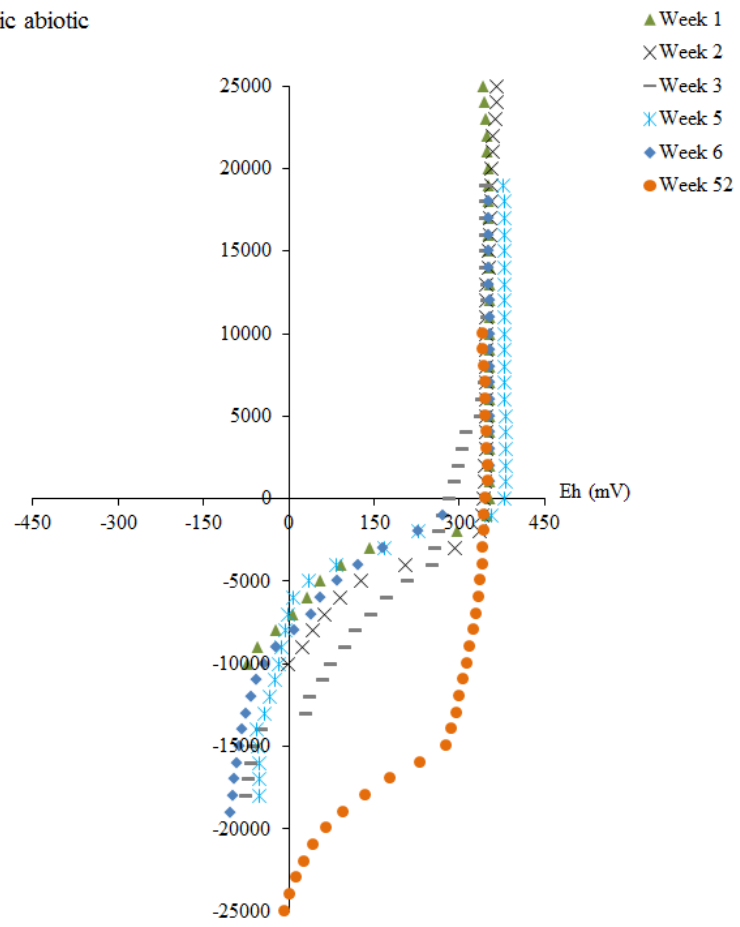
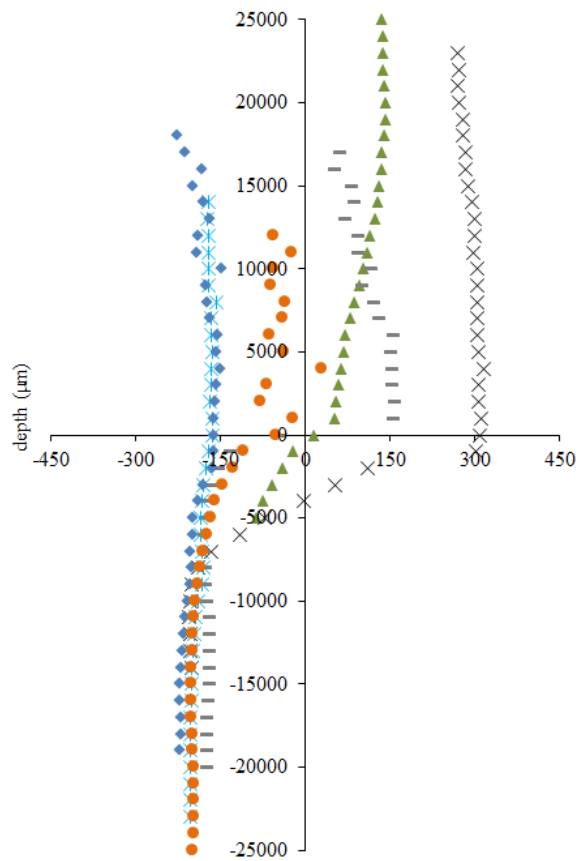


Figure 3.8 Comparison of the oxic biotic (left) and abiotic (right) Eh profiles over a 52 week period.

anoxic biotic



anoxic abiotic

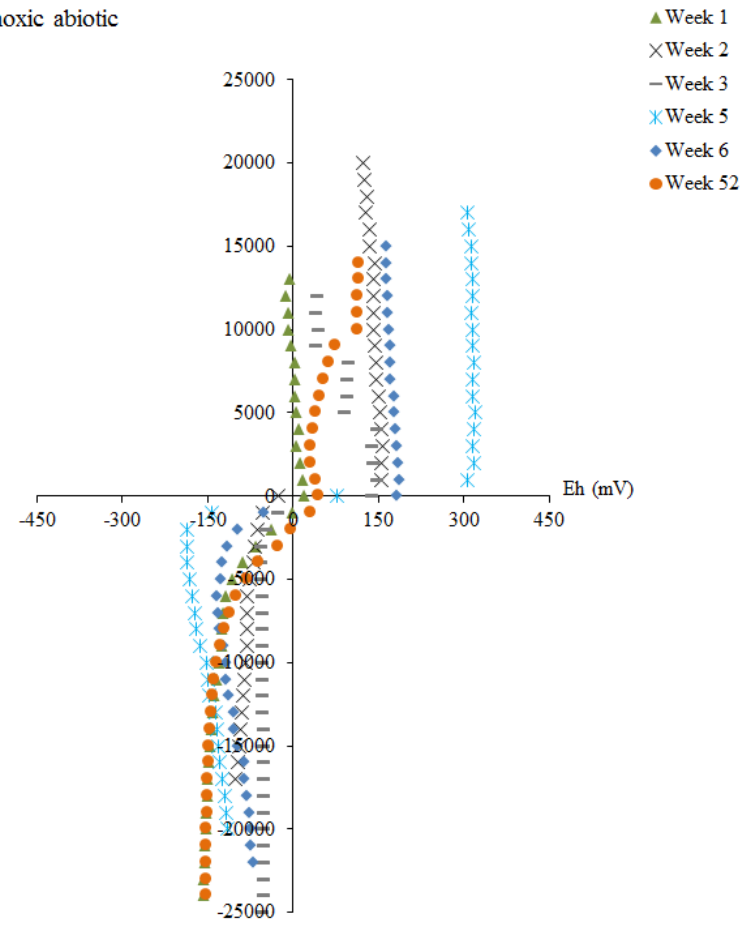
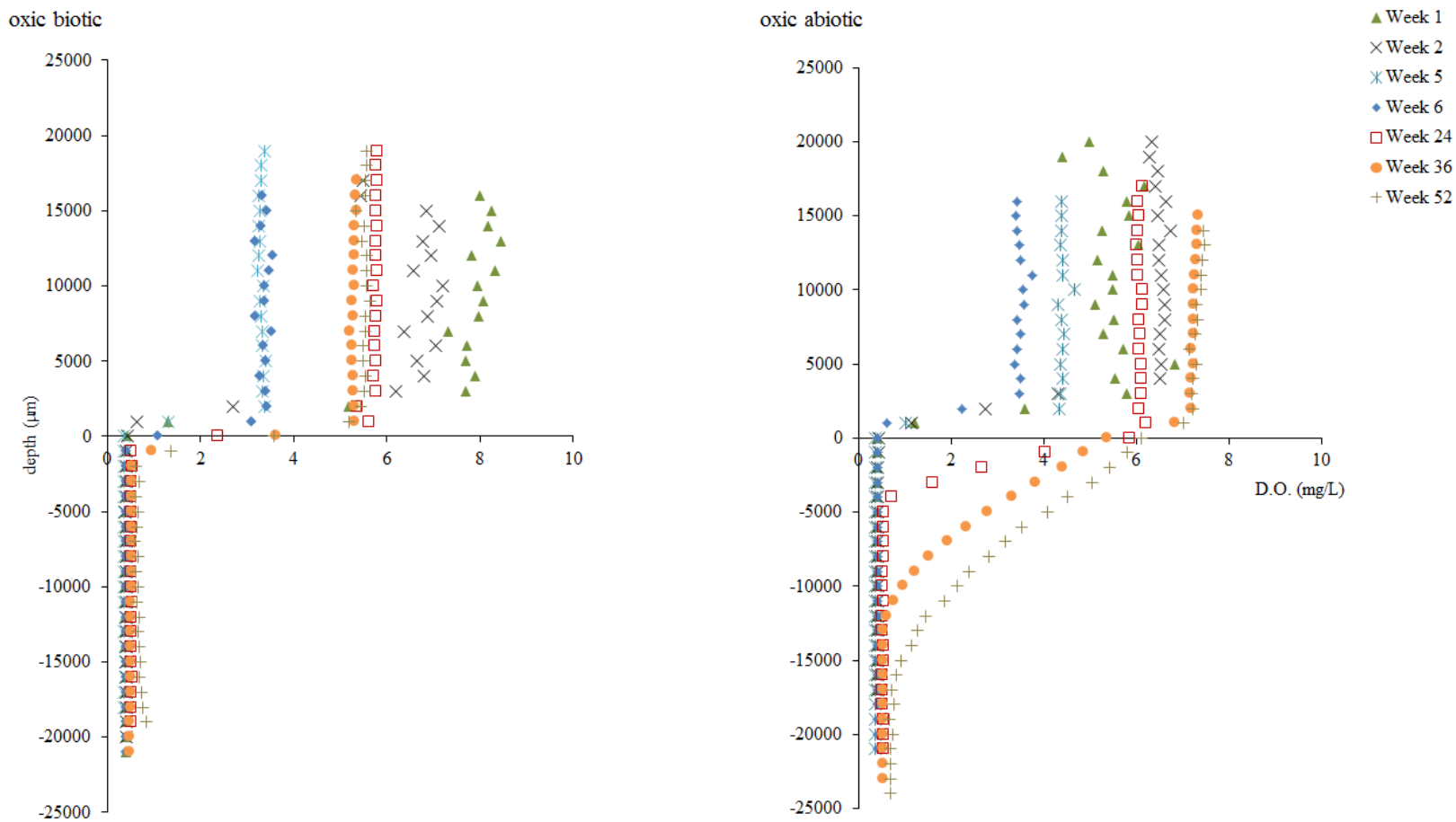


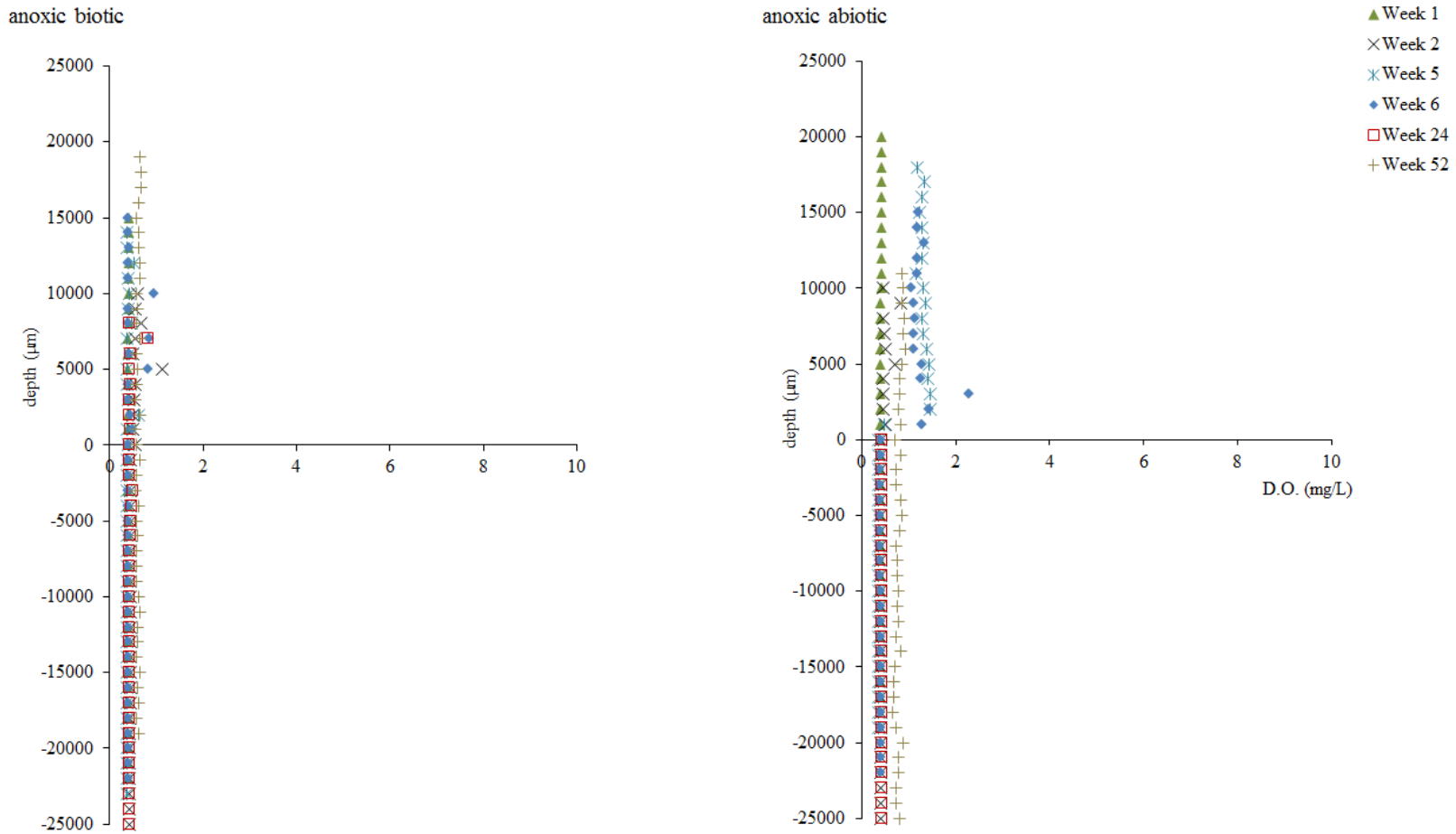
Figure 3.9 Comparison of the anoxic biotic (left) and abiotic (right) Eh profiles over a 52 week period.

### *3.3.1 OXYGEN ACTIVITY*

To verify that: 1) the anoxic microcosms remained oxygen-deficient, and 2) no additional processes took place in which oxygen was the driving electron acceptor after the short-term experiment, the activity values in the biotic microcosms were calculated for oxygen, at the FFT-OSPW interface based on changes in the oxygen concentrations measured during the first six weeks and then compared to the overall net trends observed for the 52-week period (Fig. 3.10, 3.11).



**Figure 3.10 Comparison of the oxic biotic (left) and abiotic (right) oxygen profiles over a 52-week period. Oxygen profiles collected from oxic abiotic microcosms showed significant decrease in oxygen concentrations at the FFT-water interface. Oxygen penetration into the FFT under these abiotic conditions were first observed after 24 weeks, with continual diffusion up to 52 weeks. This contrasts with the behaviour observed in the oxic biotic microcosms, where oxygen concentrations continue to decrease at the FFT-water interface - indicating a strong influence on SOD based on microbial activity present in the FFT over the 52 weeks.**



**Figure 3.11 Comparison of the anoxic biotic (left) and abiotic (right) oxygen profiles over a 52-week period. Note that week 24 experienced some difficulty when taking measurements in the OSPW and was omitted from the graph. In both treatments no observable gradients were observed between the FFT and OSPW, with respect to ability to support higher trophic levels.**

The activities (Table 3.1) shown for the anoxic biotic microcosms indicate that oxygen was initially consumed very quickly, at rates ranging from  $-1.1 \times 10^3 \text{ nmol cm}^{-3} \text{ s}^{-1}$  compared to net activity of  $-6.6 \times 10^2 \text{ nmol cm}^{-3} \text{ s}^{-1}$  for the entire period (52 weeks). Oxygen was rapidly depleted from the overlying water during the initial weeks, and remained depleted in the anoxic microcosms, with negligible ( $<3 \text{ mg/L}$ ) oxygen concentrations observed for the remainder of the experiment, regardless of whether the system was biotic or abiotic in the FFT materials (Fig. 3.11).

Interestingly, a modest amount of DO was present in the water column of the oxic microcosms (Fig. 3.10), even at the end of the experiment; the abiotic OSPW microcosm had an oxygen concentration of  $\sim 7 \text{ mg/L}$ , which is enough to sustain species other than microorganisms, assuming no other toxic effects are present (e.g., salinity) (Kalff 2003). The oxic biotic OSPW had less oxygen, at  $\sim 5$  to  $6 \text{ mg/L}$  at the end of the experiment, but this is also still sufficient to sustain organisms other than microorganisms. Noticeable differences were also observed between the oxic abiotic system and the oxic biotic system. After 36 and 52 weeks, a diffusion gradient developed at the FFT-OSPW interface within the abiotic system, during time which the FFT slowly became more oxygenated under the FFT-OSPW interface, but still more reducing than the OSPW from a chemical context (Fig. 3.10). By comparison, the oxygen demand of the oxic biotic microcosm was relatively more constant over the entire 52-week period, and remained similar to patterns observed during the initial 6-week period (decreasing to below  $1 \text{ mg/L}$  within the FFT (high BSOD at the interface)). Oxygen also appeared to have begun diffusing below the interface by week 52, but this observation requires confirmation by

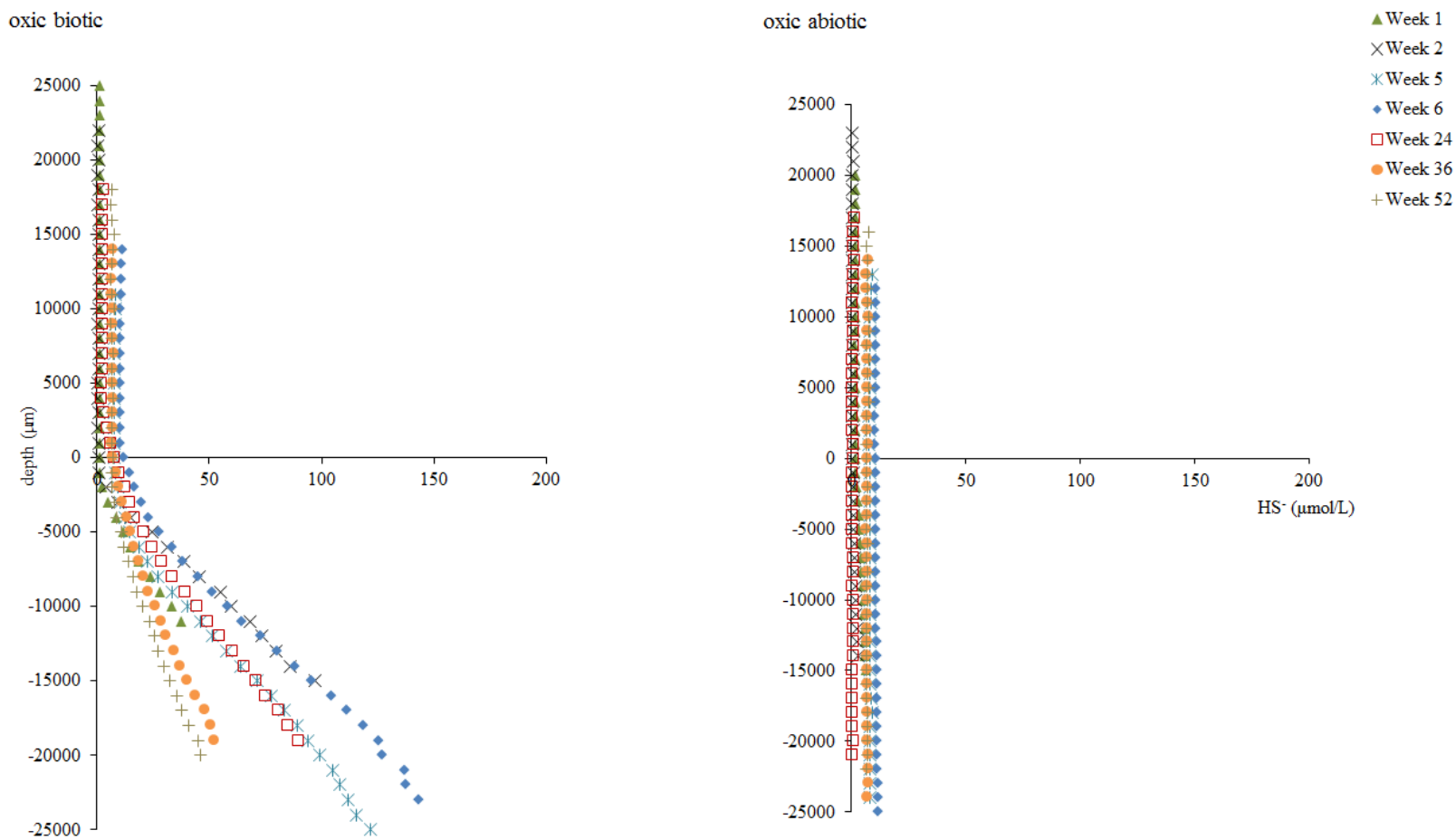
extending the sampling time period. These profiles contrast with the activity values observed for the anoxic biotic microcosms calculated at 52 weeks (Table 3.1), in which no dramatic change was observed over the course of the study (i.e., consumption was constant). This behaviour indicates that there is an overall difference in SOD over time in the microcosms caused by biotic activity. During the initial 6-week monitoring period of the oxic biotic microcosm, a negative oxygen activity of  $-1.0 \times 10^{-1} \text{ nmol cm}^{-3} \text{ s}^{-1}$  was observed at the FFT-OSPW interface, indicating light consumption of oxygen in the system. After week 52, there was a change in the activity for the oxic abiotic system, suggesting there to be indication of oxygen diffusing from the water column into the FFT material, producing a positive activity value of  $1.6 \times 10^1 \text{ nmol cm}^{-3} \text{ s}^{-1}$ . These results support the assertion that microbial activity contributes to oxygen consumption at the FFT-OSPW interface. This activity apparently becomes ever more significant following the depletion of chemical sources of oxygen reduction (or removal of physical barriers) in oil sand aquatic systems. Both biotic and abiotic factors likely play a role in oxygen consumption in FFT material.

### *3.3.2 HYDROGEN SULFIDE ACTIVITY*

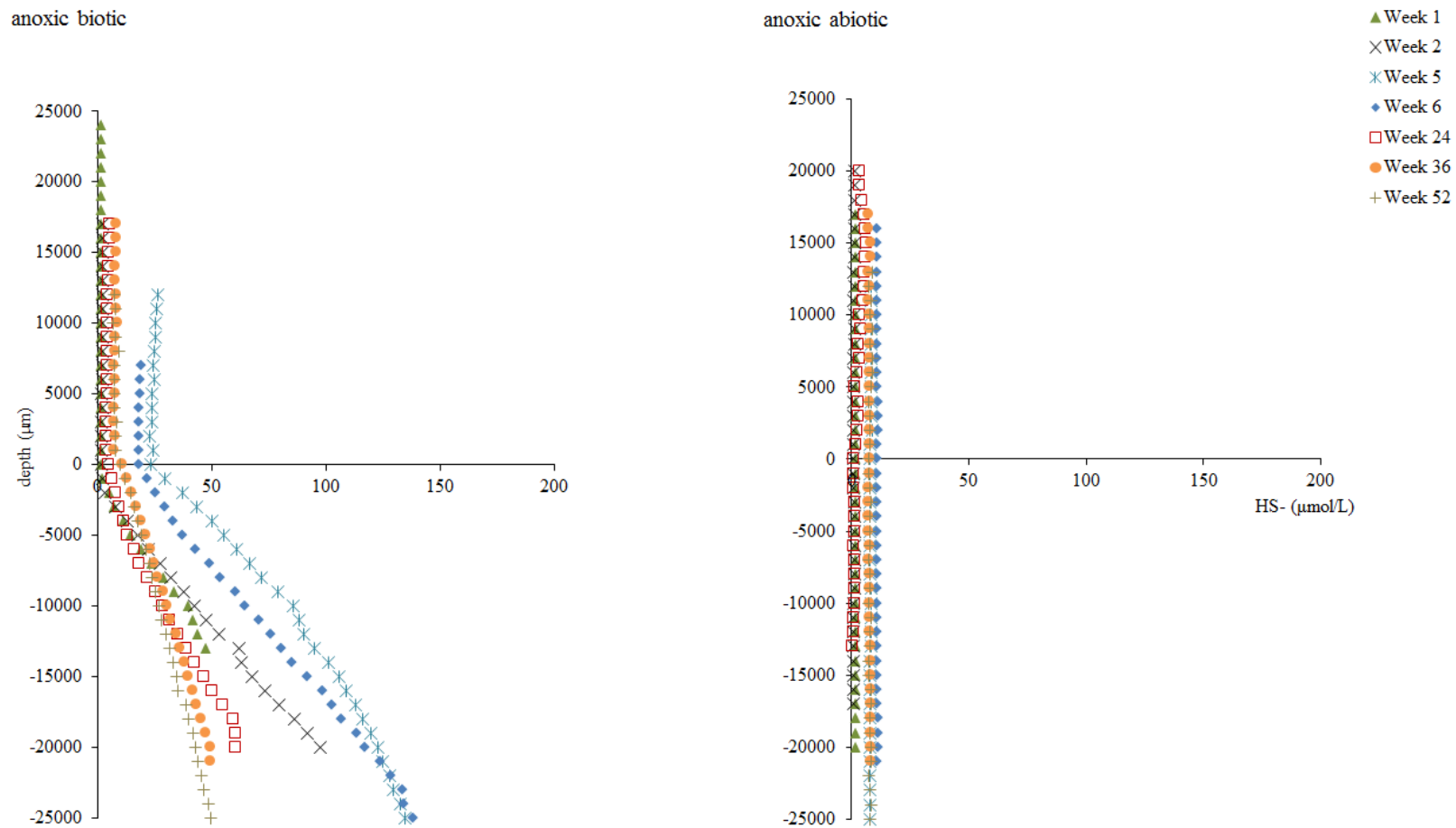
The concentrations of  $\text{HS}^-$  measured in both the oxic and anoxic abiotic microcosms (Fig. 3.12, 3.14) did not change over the 52-week period. In both cases,  $\text{HS}^-$  concentrations were very low ( $<10 \text{ } \mu\text{mol/L}$ ). By comparison,  $\text{HS}^-$  production markedly increased in the oxic and anoxic biotic microcosms over the 52-week study period. The greatest changes occurred in the anoxic biotic treatment, where  $\text{HS}^-$  concentrations increased from  $41 \text{ } \mu\text{mol/L}$   $1000 \text{ } \mu\text{m}$  below the FFT-OSPW interface after one week, to a



maximum of 86  $\mu\text{mol/L}$  at 1000  $\mu\text{m}$  depth after 5 weeks (Chapter 2). Subsequently,  $\text{HS}^-$  concentrations continued to decrease at the 1000  $\mu\text{m}$  depth from 65  $\mu\text{mol/L}$  at 6 weeks, to an asymptotic net concentration of  $\sim 27$   $\mu\text{mol/L}$  for weeks 24, 36 and 52. Increases of  $\text{HS}^-$  concentrations in the overlying water column in the anoxic microcosms correlated with peak  $\text{HS}^-$  production after 5 and 6 weeks, reaching maximum concentrations of  $\sim 24$  and 18  $\mu\text{mol/L}$ , respectively, before decreasing to  $\sim 4$   $\mu\text{mol/L}$  after 24 weeks.



**Figure 3.12 Comparison of the oxic biotic (left) and abiotic (right)  $\text{HS}^-$  profiles over a 52-week period. Hydrogen sulfide profiles in the abiotic treatment show no production during the 52-week period. In contrast, hydrogen sulfide is produced within the biologically active FFT, where the microcosm is exposed to atmospheric conditions - with enhanced production during the initial 6-week period followed by a steady decrease in production over time. During this period, negligible  $\text{HS}^-$  concentrations were observed in the overlying OSPW after 5-6 weeks.**



**Figure 3.13 Comparison of the anoxic biotic (left) and abiotic (right) HS<sup>-</sup> profiles over a 52-week period. Similar to the oxic environmental treatment, hydrogen sulfide activity profiles in the anoxic abiotic microcosms show no production during the 52-week period.**

These HS<sup>-</sup> results are substantiated by the calculated activity values (Table 3.1). The activity for week 6 in the anoxic biotic FFT was calculated to be  $2.1 \times 10^3 \text{ nmol cm}^{-3} \text{ s}^{-1}$ , indicating HS<sup>-</sup> production. For the same time period, the activity in the water column was  $5.8 \times 10^2 \text{ nmol cm}^{-3} \text{ s}^{-1}$ , suggesting elevated HS<sup>-</sup> concentrations in the OSPW. At 52 weeks, the activity in the FFT decreased to  $-1.7 \times 10^2 \text{ nmol cm}^{-3} \text{ s}^{-1}$ . This activity may be viewed as an indication of consumption (or a decrease in production), rather than as a measure of production of HS<sup>-</sup> in the FFT. The profiles presented (Fig. 3.13) clearly indicate that a significant amount of HS<sup>-</sup> was still present in the anoxic biotic FFT at the end of 52 weeks, and that the diffusion of HS<sup>-</sup> into the water column had decreased to  $-9.9 \times 10^{-1} \text{ nmol cm}^{-3} \text{ s}^{-1}$  at the end of the experiment. These results suggest that little or no HS<sup>-</sup> was diffusing out of the FFT after 52 weeks. In the oxic biotic system, similar patterns are evident (Fig. 3.12). Activity suggests there is some HS<sup>-</sup> production in the FFT, starting at  $2.9 \times 10^3 \text{ nmol cm}^{-3} \text{ s}^{-1}$ , and decreases at the end of the 52 weeks, ending at  $-8.2 \times 10^{-1} \text{ nmol cm}^{-3} \text{ s}^{-1}$ .

These data suggest that oxygen plays a minor role in sulfide generation in the FFT materials, likely due to sulfide's reducing nature and to the relative absence of oxygen in biotic FFT sediments. This has implications for designing bioremediation systems and for evaluating what role sulfide production might have in the sustainability of EPLs over a longer time period, especially as the findings being consistent with the fact that SRBs reside in oxygen-deficient environments (Chapter 1, Chapter 2). Microcosm systems appear to rapidly become reducing, with sulfate reduction most pronounced at the onset, but tapering off as the system stabilizes. The occurrence of the active sulfidic zone at the

FFT-OSPW interface is certainly important. Chi Fru et al. (2012) estimated that it takes over a decade for this sulfidic zone to develop *in situ* in tailings ponds. Because sulfide is such a strong reducer, the production of HS<sup>-</sup> in the FFT material likely plays a role in the increase of reducing conditions in the biotic systems; its detection is diagnostic of the presence of anoxic conditions.

The observations presented are pertinent to the understanding of EPLs, as most studies of these systems to date have focused only on methane production in EPLs (Holowenko et al. 2000, Fedorak et al. 2002). Our profiles, however, illustrate that biologically driven HS<sup>-</sup> production, most likely by SRBs, may also present a substantial ecosystem viability concern during the initial development of EPLs. The most noteworthy, and perhaps surprising, finding is that the biotic microcosms used in this study closely duplicate a pattern observed in the field (Ramos Padron et al. 2010). Consequently, the mechanisms deduced in this study may explain this comparable scenario. As shown, the results indicating a peak in microcosm HS<sup>-</sup> production below the FFT-OSPW interface is consistent with *in situ* findings at Syncrude's WIP (Chi Fru et al. 2012), and the presence of a sulfide-rich zone recently reported below the FFT-OSPW interface near the center of Tailing Pond 6 (TP6), an active pond managed by Suncor Energy Inc. (Ramos-Padrón et al. 2011). Ultimately, this microcosm design was only a preliminary attempt at observing the possible types of reaction processes that could occur before scaling up the experiment to a larger field study and apply the knowledge that was gained here. It is important to remember that this study focuses on the processes that occur at the FFT-OSPW interface, which is a boundary that exists in other aquatic

environments too. Furthermore, the microcosms developed microbial profiles whose patterns correlated with the depth and microbial diversity relationships reported *in situ*, in Syncrude's WIP FFT sample core materials (Chi Fru et al. 2012). This suggests that the microcosms and the chemical profiles they generate may serve as important comparative model systems for inferring *in situ* biogeochemical processes and evaluating the results of possible interventions required to manage EPLs.

**Table 3.1 Summary of short and long-term increase (positive values) and decrease (negative values) rate changes for oxygen and hydrogen sulfide concentrations in the biologically active FFT material. Note: only one activity was obtained in the abiotic systems, which was  $1.3 \times 10^1 \text{ nmol cm}^{-3} \text{ s}^{-1}$  for DO in the oxic FFT at 52 weeks.**

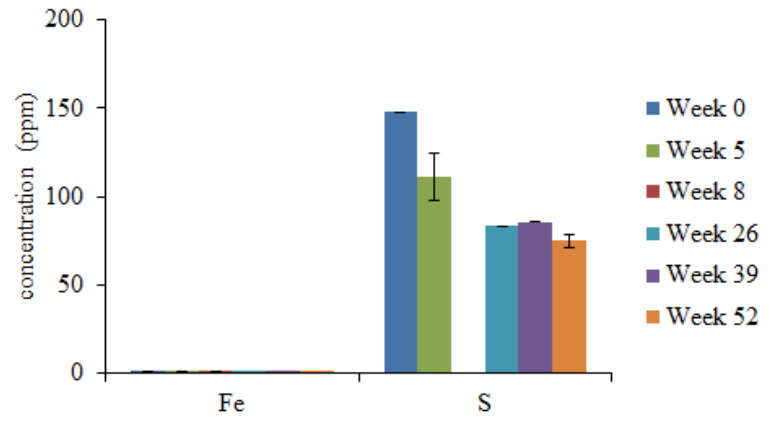
species	time (weeks)	biological FFT activities ( $\text{nmol cm}^{-3} \text{ s}^{-1}$ )	
		oxic	anoxic
dissolved	6	$-1.0 \times 10^{-1}$	$-1.1 \times 10^3$
oxygen (D.O.)	52	$1.6 \times 10^1$	$-6.6 \times 10^2$
hydrogen	6	$2.9 \times 10^3$	$2.1 \times 10^3$
sulfide (HS <sup>-</sup> )	52	$-8.2 \times 10^1$	$-1.7 \times 10^2$

### 3.3.3 TRENDS WITH IONS

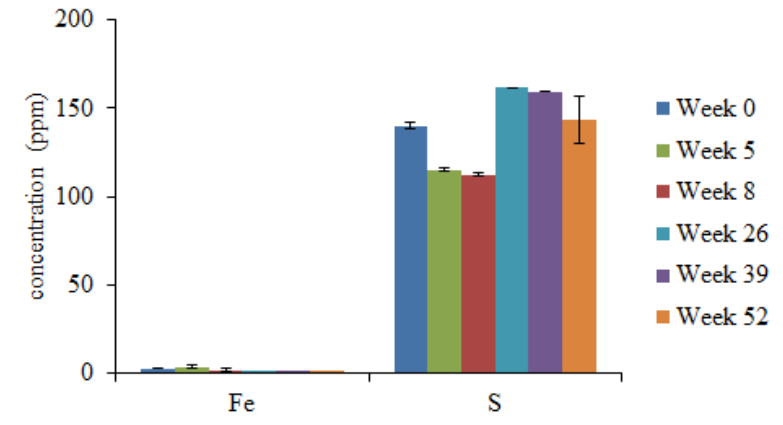
The ICP-OES analysis showed that salinity is generally high (Na concentrations ranging between 700-900 ppm) in all microcosms in both FFT and overlying OSPW cap compartments. This will likely have negative implications for the viability of an EPL design (Chapter 1). In addition, S concentrations in the pore water decreased toward the end of the long-term study (Fig.3.15), consistent with the patterns seen in the microelectrochemistry data and suggesting that the S is in sulfate form. The presence of S is in the water column (also decreasing over time) was also confirmed (Fig. 3.14). There are credible explanation that are both biologically driven: 1) S may precipitate to form  $\text{FeS}_2$  pyrite (aqueous  $\text{HS}^-$  to insoluble black iron sulfide), or 2) sulfate may be biologically converted (sulfate reduction to form  $\text{H}_2\text{S}$  gas emission). In future studies, it would be desirable to be able to track this gas element to account for the loss of S. ICP-OES did not detect Fe, suggesting that the cause may be due to the solid pyrite precipitate being blocked by Rhizon filtration. A possible alternative method to verify this in the future is to use acid digestion to dilute a microcosm-derived sample without filtration before injection to ensure that any  $\text{FeS}_x$  complexes stay in solution. Alternatively, inductively coupled plasma mass spectrometry (ISP-MS) could be used. A preliminary trial of identifying minerals was undertaken by using ESEM and XRD with quantitative particle analysis capabilities, which are described in the following sections.



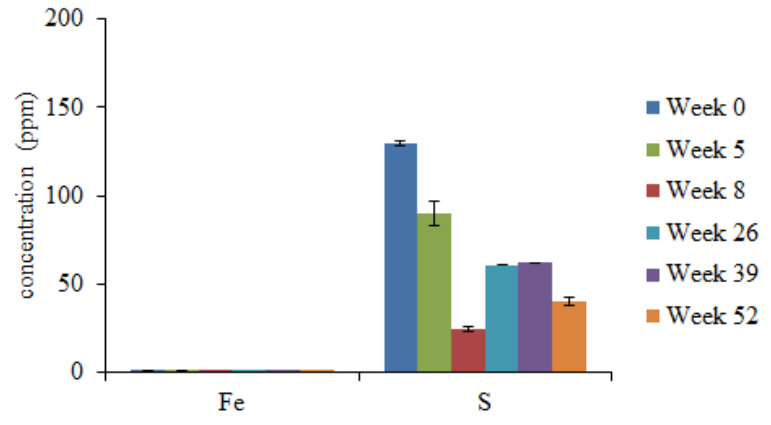
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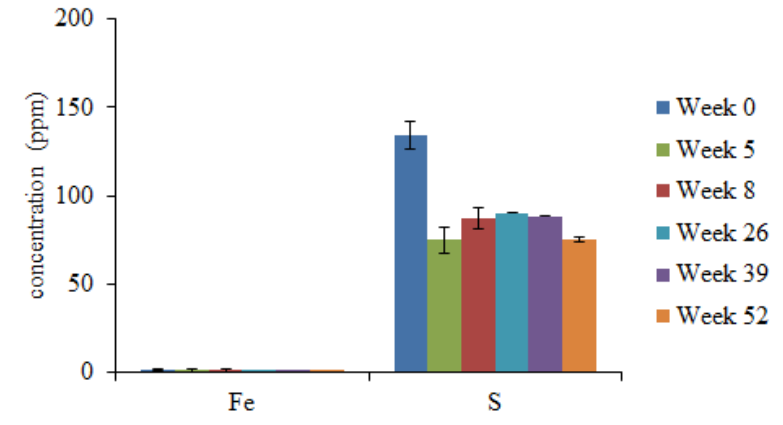
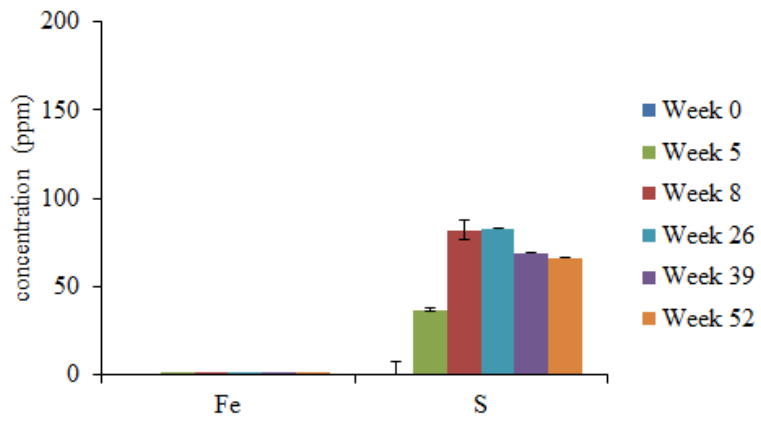
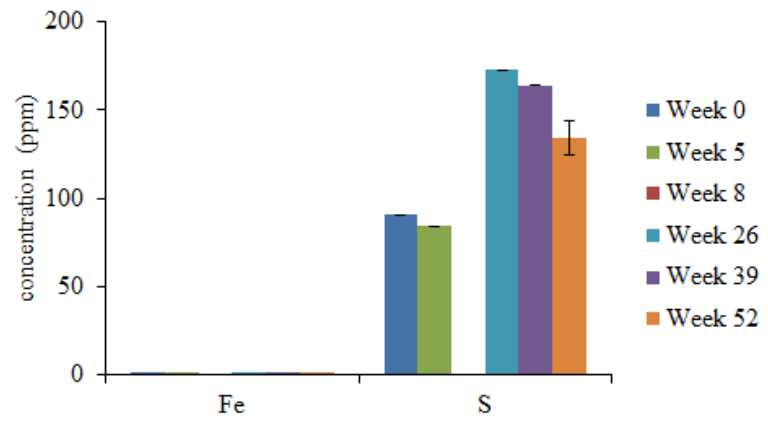


Figure 3.14 Comparison of Fe and S ions in OSPW in overlying water cap.

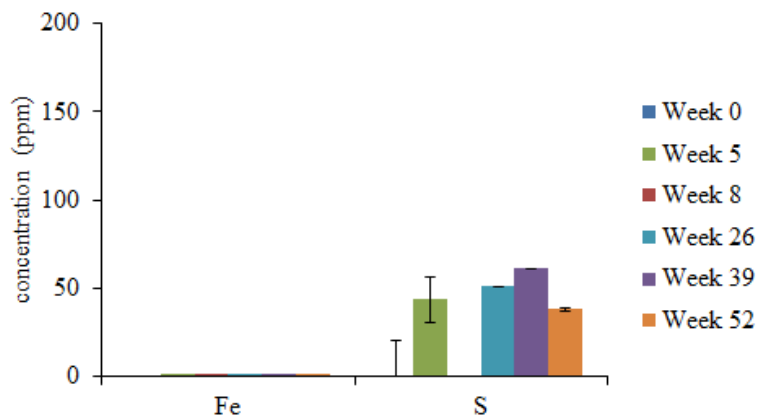
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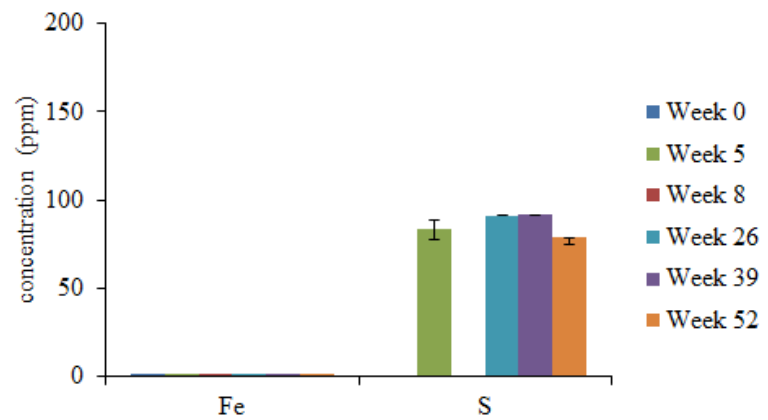


Figure 3.15 Comparison of Fe and S ions in pore water of FFT.

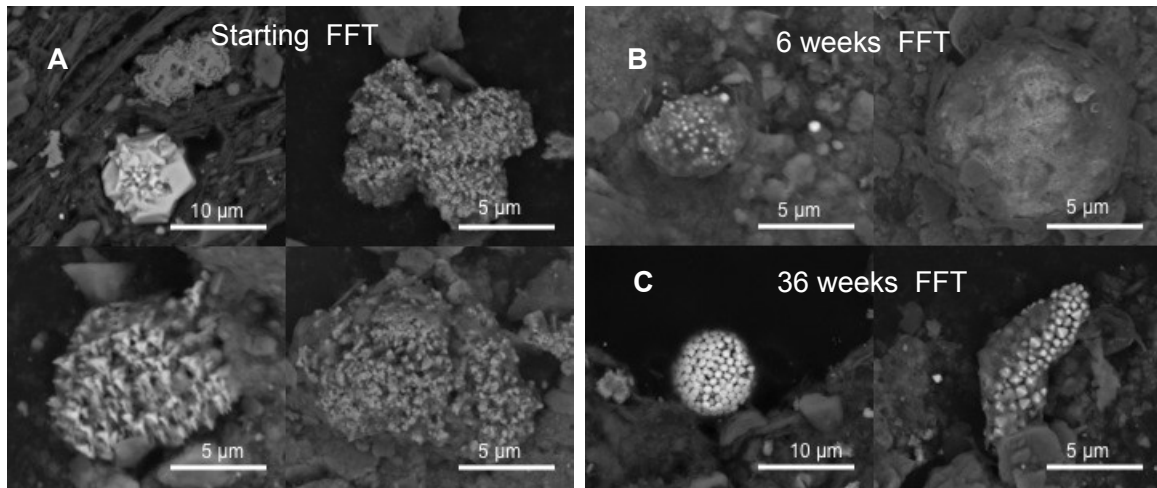
### 3.3.4 *IN SITU MINERALIZATION*

The XRD analysis (archived results) from the 1, 6 and 36-week sample cores identified quartz, mica, feldspar minerals, accessory iron sulfides, and amorphous iron (III) oxides. The bulk mineralogy did not change during the course of the experiment, but an increase in iron sulfide was detected at 36 weeks by XRD, and confirmed by quantitative particle analyses (Table 3.2). Only biotic samples were selected for analyses due to limited resources. There was an iron sulfide increase of almost 100% in the oxic FFT after the 36-week time period, and about 400% in the anoxic FFT.

**Table 3.2 Quantitative particle analysis showing the distribution of iron sulfide particles within the biological FFT treatments over 36 weeks.**

sample	environment	iron sulfides (%)	average number of particles	area sulfide ( $\mu\text{m}^2$ )	area clays ( $\mu\text{m}^2$ )
short-term FFT	oxic	2.2	>4000	2.8	1.7
(bulk)	anoxic	1.8		2.5	1.8
36 week FFT	oxic	4.1		2.8	1.3
	anoxic	7.2		3.1	1.9

To compare background and post formation of *in situ* minerals (e.g., iron sulfides), a combination of ESEM micrographs and particle analyses were performed on samples. ESEM micrographs of the starting material showed both amorphous iron oxides and mature iron sulfide grains (Fig. 3.16 A). The pyrite grains observed in the starting FFT typically consisted of large, single, euhedral grains ranging in size from 5 to 15 $\mu\text{m}$ , in contrast to the aged samples, which illustrated noticeable change in size and shape. The 6 and 36-week FFT samples (Fig. 3.16 B, C) show increasing abundance of framboidal pyrite clusters typified by micromorphological characteristics such as spherical aggregates of discrete equi-regular microcrystallites ( $\sim 0.2\ \mu\text{m}$ ).



**Figure 3.16** Micrographs from FFT collected from the anoxic biotic microcosms collected from a 1000 μm depth below the interface over a 36 week period, (A) Four ESEM micrographs show a variety sulfide and iron hydroxide minerals present from the original starting material after placement in the apparatus; (B) shows an example of newly formed iron sulfide particles collected after 6 weeks coinciding with the onset maximum production of  $\text{HS}^-$  in the system; (C) shows progressive crystallization of iron sulfide framboids within the FFT after 36 weeks.

Analysis of the 6-week FFT samples by EDS showed significant differences in the sulfide mineral stoichiometry (typically 2:1 S:Fe observed in pyrite) from 1:1 to 1.5:1 S:Fe ratio. This suggests the development of a precursor phase resembling a sulfide such as greigite. Analysis of the 36-week FFT samples revealed the presence of mature pyritic framboids with increased diameter ranging from 5 to 10  $\mu\text{m}$ . The morphologies observed at this later stage suggest a maturation effect continues to occur within the sulfide rich band reported in the microcosms following initial precipitation. Particle analysis of the 1 and 36-week FFT samples showed a dramatic increase in the relative proportion of these framboids and iron sulfide particles over the duration of the experiment (Table 3.2). After 36 weeks, an obvious increase was observed from  $\sim 2\%$  in the starting FFT samples (both aerobic and anaerobic treatments) compared to 4.1% in the aerobic and 7.2% in the anoxic treatments. Although the relative dimensional surface areas do not change significantly between the experimental systems, we do observe distinctive morphologies forming, which are distinguishable between later iron sulfides from earlier iron hydroxides. These results suggest that an increase in sulfide production had a direct influence on increasing the pH over time in the FFT (discussed in sections 3.1.1 and 3.1.3), while iron played a role in  $\text{HS}^-$  removal, as has been reported in other natural aquatic systems (Cornwell and Sampou 1995; Chambers et al. 2000. Rickard and Morse 2005). Pyrite formation has been reported at Eh levels as low as -250 mV (Butler and Rickard 2000) in the absence of oxygen, which is similar to conditions reported in the microcosms.

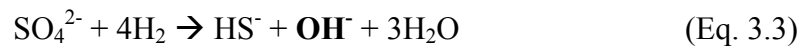
### 3.4 CONCLUSIONS

Bitumen recovery from Alberta oil sands generates waste, retained in tailings ponds, where FFT are formed. The recovered water is recycled for the bitumen extraction process, while the FFT is destined for deposition in EPLs. It is necessary to improve understanding of the mechanisms ensuring environmental viability, as well as enabling biota to develop in EPLs. One concern is the SRB production of hydrogen sulfide, which is highly reducing and toxic to higher life forms. Microcosms have previously simulated the microbial community structure of FFT ponds and are used to predict sulfide generation patterns and the behaviour of oxygen in tailing pond environments. In this part of the study, sulfide generation was positively correlated with increasing depth and with biological activity, with  $\text{HS}^-$  recorded production activities in FFT of over  $2 \times 10^3 \text{ nmol cm}^{-3} \text{ s}^{-1}$ . Oxygen diffusion into the FFT material was controlled by both biotic and abiotic activity. These results provide estimates from which one can quantitatively evaluate the potential impact of  $\text{HS}^-$  production and oxygen availability in the water column of EPLs on the likely survival of biota as well as various biogeochemical cycles linked to functional FFT-containing ecosystems.

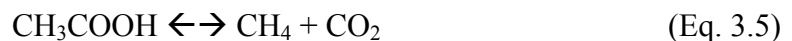
Overall, we found that oxygen is rapidly consumed in all anoxic microcosms, and that the FFT of biotic microcosms had a high BSOD. In the absence of microbial biological activity, FFT slowly becomes oxygenated by diffusion from above the FFT-OSPW interface. The Eh results affirm that the FFT material is reducing in nature, and supports that both biotic and abiotic processes play a role at the FFT-OSPW interface, with biological processes resulting in a relatively more reducing environment. There is



also confirmation from the findings of qPCR analysis that gamma irradiation sterilized the abiotic FFT successfully. Hydrogen sulfate likely serves as the terminal electron acceptor for biological sulfate reducers (i.e., SRB) to produce HS<sup>-</sup>. The HS<sup>-</sup> concentrations also decreased during the long-term study period, after peak production during the short-term period. The decrease may be caused by additional factors affecting production (i.e., stressors, competition by methanogens) (Chapter 1, Chapter 4), or by parallel reactions occurring (iron sulfide complexes forming). In general, pH remained basic, and this observation may also provide answers relating to HS<sup>-</sup> production (Chapter 1).



It is possible that methanogens are being inhibited by sulfate reducers in sulfate-rich sediments (Chi Fru et al. 2012), as is observed in EPLs (Appendix A), where sulfate reduction is the predominant terminal step (Chapter 1) that is competing for common substrates (e.g., hydrogen). Therefore, sulfate reduction may be quantitatively important in the overall oxidation of organic matter at the FFT sediment-water interface.



With the support from Chapter 2 indicating oxygen's interconnection with pH, it has become plain that the basic conditions and decrease in HS<sup>-</sup> concentrations require

more focus on other biologically dependent and independent processes that involve CO<sub>2</sub>, such as the formation of bicarbonate (Tucker and D'Abramo 2008).

## CHAPTER 4

### GENERAL DISCUSSION, RECOMMENDATIONS, AND CONCLUSIONS

The purpose of this final chapter is to summarize parameters that have been measured in this study, and to consider them from an integrated perspective while applying this understanding to the biogeochemistry of oil sands-affected end-pit lakes (EPLs) and improve the predictive capacity of the existing EPL model. This chapter also proposes future research that should be undertaken to assess fluid fine tailing (FFT) mixtures.

The oil sands are a unique feature found in few locations on Earth. With an estimated 2.5 trillion barrels recoverable bitumen, Canada has one of the largest known deposits of accessible and/or mineable oil sands ore (Penner and Foght 2010). Extraction byproducts, however, reside in tailings ponds comprising 840 million m<sup>3</sup> oil sands process material (OSPM), occupying more than 170 km<sup>2</sup> of Alberta's surface area (Siddique et al. 2011). The environmental consequences of extracting bitumen from the oil sands require urgent attention, and strategies assessing the fate of stored OSPM must be developed immediately. Recently, there has been widespread awareness of the oil sands industry in the media, along with studies illustrating the impacts on habitats and water quality. The data obtained that relate to biogeochemical changes can contribute to the effort for understanding degradation processes, ultimately leading to improved, reliable environmental reclamation strategies.

Because discharge of process water and tailings material into the natural environment is prohibited (FTFC 1995), FFT must be returned to previously mined areas, which will eventually become EPLs. The EPLs represent a reclamation strategy to create viable and sustainable lake systems able to support fish and waterfowl.

The industry proposes to use gravity settling and subsequent storage of the OSPM in large basins. The gravimetrically-separated oil sands process water (OSPW) can then be used for landscape reconstruction, or may be recycled for use in further oil sand extraction and purification processes. The densified FFT material will become a sediment layer of EPLs.

The proposed use of FFT as the sediment layer in EPLs raises the question of suitability in contributing toward the establishment of a sustainable, long lasting viable aquatic system. The industry is presently interested in processes influencing the initial stages of reclamation success: tailings consolidation, lake deepening, pore water release, gas production, sediment resuspension, erosion, and salinity (CEMA Oil Sands Pit Lake Model Draft Report, May 2011). Lake water must have appropriate pH and oxygen levels, and can not contain toxic substances, such as hydrogen sulfide and high salinity.

Many biotic and abiotic factors interact in lake systems. Monitoring the biogeochemical development of FFT over time is an important component of the reclamation in the Athabasca oil sands lands that have undergone mining activities. Data are needed to relate the biotic and abiotic processes contributing to the development of

key elemental cycles in reclaimed areas. Results from studies, such as the investigation of methane ( $\text{CH}_4$ ) and ammonia ( $\text{NH}_3$ ) oxidation, and their contribution to sediment oxygen demand (SOD) (DiToro et al. 1990), relating sulfate reservoirs to dissolved iron (Canfield 2004), or linking trace metals with sulfidic water (Öztürk 1995), can be used in strategies such as the EPL conceptual model. This will ultimately aid in predicting the success of proposed reclamation efforts.

The goal of this study was to characterize and quantify oxygen and sulfur flux within FFT sediments across the sediment-water interface, in constructed microcosms. The microelectrochemistry employed for this study improves on existing methods for determining spatial and chemical changes that occur at small scales across the FFT-OSPW interface. Such techniques provide a spatial perspective of the sediment-water interface from a fine scale resolution that was previously impossible, and could contribute to development of a standardized protocol for continual assessment of reclamation efforts in EPLs.

Cabirol et al. (1998), Holowinko et al. (2000), and Matthews et al. (2005) have documented that methane production is an important consideration in oil sands tailings. This study, however, shows that  $\text{HS}^-$  production is also a significant characteristic of the short-term, biophysicochemical processes that affect FFT development. In terms of reclamation options, such as EPLs, and other aquatic ecosystems, such as constructed wetlands (i.e., fens, marshes), this  $\text{HS}^-$  production may be of significant concern if it disperses into the overlying water column, which would be detrimental to the biota that

comprise for higher trophic levels of the lake community. Elevated sulfide concentration in the water column and its potential to affect aquatic biota should be assessed in future studies.

#### 4.1 OXYGEN AND SULFIDE DEVELOPMENT OVERVIEW

The dissolved oxygen (DO) analysis indicated that negligible amounts of oxygen were present in both the water column and FFT compartments of the anoxic microcosm treatments, regardless of the presence or absence of biological activity. This was expected because of their isolation from atmospheric conditions (i.e., oxygen). The water column of oxic treatments, on the other hand, showed relatively higher levels of oxygen due to exposure to the atmosphere. The oxic, abiotic microcosms showed increasing oxygen concentrations in the FFT over time. An oxygen diffusion gradient at the interface had developed 6 weeks, and had continued to develop by the end of the 52-week study, indicating a slowly oxygenating FFT compartment. The oxic, biotic treatment, however, did not show nearly as prominent a gradient change, and there was only a small indication of initial diffusion during the long-term sampling period. This indicates the presence of high biological sediment oxygen demand (BSOD) at the interface, characterized by a rapid consumption of the oxygen by microorganisms throughout the entirety of the FFT during the 52-week experiment. Oxygen consumption at the sediment-water interface is mainly attributed to the microbes, an inference that is further supported by the Eh measurements. In the biotic treatments, Eh measurements show the interface to be relatively strongly reducing, while the interface in the abiotic microcosms is only moderately reducing in comparison.

Hydrogen sulfide analysis showed that no  $\text{HS}^-$  was formed in the abiotic microcosms, regardless of the presence or absence of oxic conditions. In contrast, increasing concentrations of  $\text{HS}^-$  were observed during the initial 6 weeks in the biotic microcosms, after which a gradual decline in concentrations was detected. The trends suggest that microorganisms use sulfate as a principal terminal electron acceptor to create sulfide in the oxygen-deprived FFT compartment. Sulfates occur naturally in numerous minerals, including barite ( $\text{BaSO}_4$ ), epsomite ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), and gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ). These dissolved minerals are components of many different types of sediment (Greenwood and Earnshaw 1984), and although sediment analysis was not analyzed as part of this study, preliminary analysis of OSPM composition suggested that these compounds are present (Appendix A). The gradual continual increases of pH over time are consistent with these findings, because  $\text{OH}^-$  groups are released during biological  $\text{HS}^-$  production (Chapter 1). However, pH increased in all treatments, including the abiotic microcosms. It therefore remains unclear if the changes in pH in the biotic treatments are solely attributable to the sulfate reduction conversion to sulfide, though it may contribute to the total reaction. Other possible explanations for the consistent increase in pH are microbial methanogenesis (Chi Fru et al. 2012), accomplished by oxidizing (i.e., removing from the OSPM) readily available  $\text{CO}_2$ , and creating bicarbonates (Hatch and Price 2008, Tucker and D'Abrano 2008, Gardner Costa et al. 2010). The presence of methanogens is very possible, as indicated by Chi Fru et al. (2012) detecting newly emerging archaea during the long-term study using terminal restriction fragment length polymorphism (T-RFLP); the process of methanogenesis is exhibited only by microbes

identified as archaea, which are distinct from bacteria. Details of the microbiological genetic work were not included as a part of this thesis. Methanogenesis, however, is a purely microbiologically driven process, so future research should focus on changes in carbonate species (i.e., alkalinity) to help account for the pH increase.

The biologically active treatments were further investigated by determining fluxes for the biotic activity; the slopes of the concentrations of the oxygen and sulfide were used to calculate the rates of change using Fick's Law of Diffusion (Chapter 3). In the biotic microcosm treatments, oxygen is consumed most rapidly at the beginning of the study. Evidence of the initiation of diffusion into the FFT compartment was observed near the end of the 52-weeks. The production of  $\text{HS}^-$  was very high at the beginning of the study, peaking after 6 weeks, and subsequently decreased over time. A possible explanation for the long-term decrease in  $\text{HS}^-$  is that the sulfide was used in a simultaneously occurring formation of iron sulfide complexes (first suspected when dark banding in the microcosms became evident at the sediment-water interface during week 3). Intermediate comparisons of the biotic FFT were done using environmental scanning electron microscopy (ESEM) and applying quantitative particle analysis. Preliminary results showed that iron sulfides had increased by a factor of 2 after 36 weeks in the oxic biotic treatment, and by a factor of about 4 in the anoxic biotic treatment. The ESEM micrographs shown in Chapter 3 also illustrate the presence of iron hydroxides, which could serve as a potential reservoir for iron in the starting material. Fe (III) could also plausibly serve as a possible terminal electron acceptor during bacterial metabolism in this system, resulting in the formation of Fe(II) (Weisener et al. 2008, 2010); this would



in turn complex with sulfide to form  $\text{FeS}_x$ . Additional research to identify the exact iron sulfide species would be advantageous (e.g.,  $\text{FeS}_2$  would remove  $\text{HS}^-$  from the FFT much faster than  $\text{FeS}$ ). This would help to explain the long-term decrease in  $\text{HS}^-$ . Future investigations could also monitor and quantify iron binding agents (i.e., siderophore) production, in general, via specific bacterial and/or archaeal species that are capable of using iron as a metabolic pathway.

This thesis shows that the oxygen demand at the sediment-water interface is primarily driven by biotic rather than abiotic processes, as is evidenced by the development of a defined oxygen diffusion gradient in the abiotic system. The  $\text{HS}^-$  production at the interface is also biologically influenced, but may be self-limiting, in that gradually decreasing  $\text{HS}^-$  concentrations were detected after the initial 6-week increase. Therefore, this study supports the proposed hypothesis that the biotic component is predominantly responsible for SOD and  $\text{HS}^-$  changes over time at the sediment-water interface.

The changes in concentration of oxygen and sulfur within the FFT material at the sediment-water interface are more pronounced in the biotic microcosms (which had not undergone the gamma irradiation treatment). This reflects the important influence of the biotic component present in the material. The microbial community is largely responsible for the reducing nature of the FFT, and causes oxygen concentrations to decrease. Additionally there is an indication of increasing sulfide production.

The results in this thesis suggest that this HS<sup>-</sup> production may be a self-limiting process, which will begin to decrease after a suitable period of time. The variables and rates measured for oxygen and HS<sup>-</sup> consumption and production will be useful for calibrating current and proposed models designed to understand EPL behaviour. One can not predict specifically when HS<sup>-</sup> production will cease or how quickly a decrease will occur from these results, because other variables (e.g., hydrogen sulfide gas, nutrient loading, methanogenesis, bicarbonate production) also influence these systems and must be taken into account.

#### 4.2 IMPLICATIONS, LIMITATIONS, AND UNCERTAINTIES

Supplementary studies on concentration and composition of organic carbon, anions, and end-point gas analysis of the headspace in the anaerobic treatments are required to understand the nutrient sources that support the biological microbial community in FFT. Constraints on the design of the study (i.e., speed of obtaining depth profiles, microsensor shelf life), time restrictions, and the necessity to minimize microcosm disturbance precluded the measurement of several important covariates in this experiment. In addition to restrictions in sample size, limited data led to ambiguous or inconclusive results in some cases. For example, planned analyses of carbon, anions, and gas were not completed. Furthermore, logistical constraints in terms of coordination and access to some of the experimental equipment, as well as instrumental limitations (i.e., inappropriate columns) also affected aspects of the study execution. Future studies should be conducted with more frequent sampling and should be designed with sample quantities large enough to support organic carbon, anionic, and end-point gas analyses.

The elevated salinity of FFT-affected aquatic systems is expected to reduce freshwater productivity at many trophic levels (Gardner Costa et al. 2011). This may also be true for other organisms, like the microbial community, but more work must be done to evaluate these effects (Baldwin et al 2006, Gardner Costa et al. 2011).

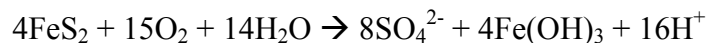
Oxygen, sulfur, and additional nutrients (i.e. organic matter, metals) may also be newly introduced into EPL systems on occasion (Chapter 1). Deep boreal lakes become stratified into three defined layers: the epilimnion (i.e. upper layer of circulating warm water, where dissolved oxygen concentrations are relatively high), the thermocline (i.e., transitional layer with rapid temperature and oxygen decrease with depth), and the hypolimnion (i.e. lower non-circulating layer with cold, deep water, where SOD can cause oxygen concentrations to become low or absent). Most stratified freshwater lakes become completely mixed, replenishing oxygen when the water becomes isothermal in spring and autumn. The thermocline in these EPLs may or may not be disrupted over time, by temperature changes during spring and autumn, depending on lake morphometry and whether the hypolimnion becomes isolated by a dense halocline. This is also a consideration that must be taken into account when extrapolating a microcosm study to full scale.

#### 4.3 FUTURE RESEARCH DIRECTIONS

The high pH observed in the microcosms in the previous chapters, suggests that any carbon dioxide that may have been present (inconclusive gas results) in the OSPM

would occur in the form of bicarbonate, which is the dominant ion at a pH range of 8 to 10 (Stumm and Morgan 1996). In the future, it would be appropriate to analyse the alkalinity of water samples from the water column and FFT (pore water). Headspace gas analysis for hydrogen sulfide and methane will also need to be refined (e.g., septum installation).

Parallel studies (not part of this thesis) detected sulfide in the experimental starting FFT material using stable isotope analysis techniques to identify acid volatile sulfide (AVS) (Appendix B). Stable isotopes are a useful tool to aid in identifying AVS, which are specific zones in sediments that release H<sub>2</sub>S when acidified (Rickard and Morse 2005). AVS does not accurately measure iron sulfides in aquatic sediments because it is a component that does not represent any single or simple group of sediment components. However, AVS measurement may still be a useful indirect method by which to infer the presence of H<sub>2</sub>S in future studies. This is especially important for the experiment presented by this thesis; the microcosm systems possess a relatively alkaline setting, and oxidation of iron and sulfur (see equations below) would allow prediction of potential H<sub>2</sub>S gas release.



(Nicholson et al. 1988, Elberling and Damgaard 2001)

At the same time, the parallel study measured nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) in addition to HS<sup>-</sup>. This raised the question as to why NO<sub>2</sub><sup>-</sup> seems to accumulate in a system

where no biotic processes were taking place (the absence of microorganisms in the abiotic treatments was confirmed using qPCR). This may relate to the chemical gradient (i.e., oxygen) observations described in Chapter 2, for which further investigation is warranted. Future work must also consider the kinetic constraints on  $\text{HS}^-$  and  $\text{H}_2\text{S}$  production versus the role that iron and other metals will have on metal sulfide production and the ultimate mass balance within the system.

A thorough understanding of the role of biological activity in nutrient cycling (i.e., key elements as discussed in the previous chapters), in relation to aquatic environments in oil sand regions is important for predicting the characteristics of basal community establishment that can allow for the possible development and sustainability of other components of aquatic food webs. The biological and chemical drivers responsible for changes in SOD and sulfur concentrations must be tracked to assist in making guidelines for management of these systems in areas of oil sands mining.

In the oxic, biotic microcosms, oxygen introduced into the OSPW from the atmosphere is consumed primarily by the microbial community present at the interface of the FFT. In the oxic, abiotic microcosms, the depth of the redox boundary gradually became deeper, as was indicated by the oxygen measurements taken over the 52-week experimental period. Ammonia present in the FFT (Table I) can possibly be chemically oxidized as oxygen diffuses in, but in the absence of biotic processes (i.e., nitrifying bacteria) the detectable layer of  $\text{NO}_2^-$  is more likely a residual concentration from pre-

gamma sterilization. Nonetheless, this presents an opportunity for further investigation of the role of N to further explain complex biotic and abiotic reactions.

Understanding of EPL dynamics can also be increased by examining other nutrients that may be mobilized (i.e. C sources, metals such as Fe and Mg, etc.). Tracking nitrogen and other recurring compounds present will also help to confirm their role in chemical oxygen consumption.

While small scale analyses, such as this microcosm study, give insight into the principal processes taking place, other factors must also be considered if these results are to be extrapolated to a field scale. Large scale field studies will need to consider effects such as spatial heterogeneity. Fick's Law can be applied for the present small scale experiments (Chapter 3). However, the assumption of spatial homogeneity must be validated before lab data can be applied to field scale investigations (depending on the properties of the media). Furthermore, possible effects of seasonality, stratification, and haloclines need to be investigated, as well as addressing the question of the influence of mixing fresh and old material for the input and outputs of the EPL.

Refinement of the methods used in this study will enable improved and properly-designed reclamation strategies to be made in the future aside from EPLs. The present study investigated the changes in the FFT material over one year, but the estimated settling time for FFT is on the order of 125-150 years (Eckert et al. 1996), during which pore water is released while tailings become consolidated. However, methanogenesis

might accelerate the settling rate considerably (Fedorak et al. 2003, Foght et al. 2010). *In situ* fieldwork is in progress to assess the differences in SOD and  $\text{HS}^-$  production between young (7 years) and old undisturbed FFT (35 years), in order to evaluate conditions in the field and help identify the most informative types of data that can be obtained from biogeochemical studies to provide more reliable indicators of EPL conditions. Longer-term experimentals are needed to track the development of the oxic zone of which the present study was only able to show the initiation. Extensive long-term studies would also enable one to assess other reactions, such as ammonia oxidation. The methods used in the laboratory microcosm study could be eventually employed under field conditions to better understand likely field-based processes in EPLs.

Another important consideration of FFT and OSPW is the release of gases (mainly  $\text{CH}_4$  and  $\text{CO}_2$ ). Methane is of concern, which is relatively insoluble in water compared to  $\text{CO}_2$ , and would potentially rise to the surface and diffuse into the atmosphere (Blumenberg et al. 2007). The appearance of archaea communities during analysis of FFT from the long-term microcosms of parallel studies (Chi Fru et al. 2012), suggests the potential for methane to be formed (DeLong 1992). Although previous studies have analysed gas releases from tailing ponds (Matthews et al. 2005), more research will be needed to understand the mechanisms of gas emission related microbial community development in the EPL.

The research in this thesis contributes importantly to our understanding of oxygen and sulfide development in FFT sediments. FFT-associated biotic oxygen consumption

and hydrogen sulfide production have been quantified. Oxygen is an essential requirement for organisms at higher trophic levels in the food web, whereas sulfide is toxic to most biota and therefore undesirable within an EPL environment. The bench test scale of this study does not simply survey the flow of oxygen and sulfide from microbiological attributors, but it quantifies the changes over time. Because the microbial activity was found to be a dominant component regulating fluxes in the microcosms, future research will undoubtedly lead to characterization of the microbial functional groups beyond simply grouping into bacteria and archaea (Chi Fru et al. 2012). Further resolution of the causes changing overall pH will also tie into this work. This thesis lays the foundation for future studies on estimating oxygen and sulfide concentrations and fluxes, and whether or not EPLs will eventually be able to resemble native lake systems.



## APPENDICES

### APPENDIX A: Additional information on sample material

**Table I. Summary analysis of FFT and OSPW by Syncrude comparing before (A) and after (B, highlighted) gamma irradiation. Average particle size = 3 µm. Relatively no major change found in major/trace chemistry, alkalinity.**

	FFT		OSPW	
	FFT "A"	FFT "B"	OSPW "A"	OSPW "B"
SCL ID number	E77768	E77769	E77770	E77771
pH	7.82	8.19	7.96	8.31
Cond (uS/cm)	3550	3200	3320	3300
Naphtha (% by wt)	0.05	0.05	not analyzed	not analyzed
Naphtenic acids (ppm)	67	34	64	4
<b>Anions (ppm)</b>				
CO3=	123	137	15	12.3
HCO3-	1250	1190	574	639
F-	BDL	BDL	BDL	BDL
Cl-	570	580	510	510
NO2	BDL	BDL	BDL	BDL
NO3	BDL	BDL	68	BDL
PO4	BDL	BDL	BDL	BDL
SO4	3.37	12.2	518	528
Br	BDL	BDL	BDL	BDL
<b>Cations (ppm)</b>				
Na+	863	866	796	761
K+	16.6	17.9	16.6	16.2
Mg++	12.3	11.7	13.4	13.0
Ca++	21.7	20.2	24.2	23.2
NH4+	10.4	13.4	BDL	4.0
<b>Elemental Analysis</b>				
<b>Trace metals (ppm)</b>				
SCL ID number				
Aluminum (Al)	0.092	0.660	0.178	1.240
Antimony (Sb)	BDL	BDL	BDL	BDL
Barium (Ba)	0.529	0.484	0.218	0.192
Boron (B)	4.19	3.47	2.54	2.45
Cadmium (Cd)	BDL	BDL	BDL	BDL
Calcium (Ca)	21.7	20.2	24.2	23.2
Chromium (Cr)	BDL	BDL	BDL	BDL
Cobalt (Co)	BDL	BDL	BDL	BDL
Copper (Cu)	BDL	BDL	BDL	BDL
Iron (Fe)	BDL	0.172	0.020	0.396
Lead (Pb)	BDL	BDL	BDL	BDL
Lithium (Li)	BDL	BDL	BDL	BDL
Magnesium (Mg)	12.3	11.7	13.4	13.0
Manganese (Mn)	0.073	0.049	0.058	0.083
Molybanum (Mo)	BDL	BDL	0.15	0.15
Nickel (Ni)	BDL	BDL	BDL	BDL
Phosphorus (P)	BDL	BDL	BDL	BDL
Potassium (K)	16.6	17.9	16.6	16.2
Selenium (Se)	BDL	BDL	BDL	BDL
Silicon (Si)	6.25	5.79	2.45	3.99
Sodium (Na)	863	866	796	761
Strontium (Sr)	0.794	0.745	0.825	0.800
Sulfur (S)	5.12	7.00	185.00	177.00
Titanium (Ti)	0.027	0.037	0.028	0.055
Vanadium (V)	0.031	0.036	0.035	0.035
Zinc (Zn)	BDL	BDL	BDL	BDL
Zirconium (Zr)	BDL	BDL	BDL	BDL

APPENDIX B: Companion study

**Table II. Preliminary isotope analysis observations of preserved starting FFT sample material done by Sean Crowe in 2011.**

sample	$\text{NO}_2^-$ ( $\mu\text{mol L}^{-1}$ )	$\text{NO}_3^-$ ( $\mu\text{mol L}^{-1}$ )	$\text{SO}_4^{2-}$ ( $\mu\text{mol L}^{-1}$ )
oxic biotic	ND	1.6	1198
oxic abiotic	158.5	3.6	1874

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