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**Bio-Physicochemical Effects of Gamma Irradiation Treatment for Naphthenic
Acids in Oil Sands Fluid Fine Tailings**

By

Ryan Boudens

A Thesis
Submitted to the Faculty of Graduate Studies
Through the Great Lakes Institute for Environmental Research
In partial fulfillment of the requirements for
The Degree of Master of Science
At the University of Windsor

Windsor, Ontario, Canada

2015

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**Bio-Physicochemical Effects of Gamma Irradiation Treatment for Naphthenic
Acids in Oil Sands Fluid Fine Tailings**

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I. Declaration of Previous Publication

This thesis includes 1 original paper that will be submitted for publication in peer reviewed journals, as follows:

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ABSTRACT

Naphthenic acids (NA) are components of most petroleum, including those found in the Athabasca oil sands and in some systems are considered to be persistent compounds. Their presence in the environment can pose significant problems since freshly released NAs are toxic to aquatic organisms. In this investigation gamma irradiation (GI) was used to reduce the toxicity and concentration of NAs in oil sand tailings and assess the impact on biogeochemical processes. GI reduced NA concentration by up to 97% in Oil Sands Process Water (OSPW) and 85% in fluid fine tailings (FFT). Dissolved oxygen flux was stimulated directly in the GI-treated FFT in both fresh and aged sources, while increases in hydrogen sulfide flux was restricted to the fresh FFT source material. Acute toxicity to *Vibrio fischeri* was immediately reduced following GI treatment of fresh OSPW. GI treatment followed by 28-day incubation reduced toxicity of aged OSPW to *Vibrio fischeri*.

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CHAPTER 1: INTRODUCTION

Introduction to Oil Sands

The Athabasca oil sands deposits in northern Alberta, Canada, with an estimated 170.4 billion barrels remaining of recoverable bitumen, make up the third-largest oil reserve in the world (Energy Resource Conservation Board of Alberta (ERCB), 2012). Oil sands are naturally occurring bitumen deposits that generally consist of 80-87% inorganic materials, 6-16% bitumen and 1-8% water (Liu et al. 2005). Approximately 20% by area of the Athabasca deposit is close enough to the surface to permit open pit mining. Bitumen extraction from the mined sands typically involves the Clark caustic hot water extraction process (Chalaturnyk et al., 2002). Extraction of bitumen to produce oil consumes on average 3 barrels of Athabasca river water for every barrel of oil produced (Allen, 2008). This extraction process has created large volumes of waste materials termed fluid fine tailings (FFT), which consists of a mixture of water, dissolved salts, sand, clay, residual bitumen and naphtha products (Allen, 2008). The oil sands industry operates under a zero discharge policy. As a result, these materials are stored on-site in large tailings ponds, which promotes the separation of the oil sands process-affected water (OSPW) from FFT (Figure 1.1) (Quagraine et al. 2005). Approximately one billion m³ of FFT and OSPW are presently impounded in oil sands lease areas covering over 176 km² within mine lease areas (Simieritsch et al. 2009; Gosselin et al. 2010; ERCB, 2012). The OSPW recovered from the surface of tailings ponds is recycled into the bitumen extraction process to reduce freshwater demand; however

various organic (e.g., Naphthenic Acids, benzene, toluene, and polycyclic aromatic hydrocarbons) and inorganic (e.g., sodium, chloride and sulfate ions) constituents become concentrated, further increasing their toxicity (Hwang et al. 2013; Clemente and Fedorak, 2005).

Figure 1.1 Tailings pond construction (Alberta Government, 2012)

The Alberta Environmental Protection and Enhancement Act prohibits the release of toxic by-products created during bitumen extraction outside of oil sands lease areas, and requires the companies to remediate disturbed areas to a pre-mining state (Madill et al., 2001). The “wet landscape” option involves transferring FFT into previously mined-out pits and capping it with clean water to create a series of interconnected wetlands, which drain into end-pit lakes (FTFC, 1995). A major concern with tailings management is the extremely slow settling rate of the fine tailings particles releasing pore water into the capping layer. As FFT compresses and becomes denser (a process that can continue over decades), pore water and its dissolved constituents are released into the overlying surface water, increasing contaminant concentrations. Many constituents contribute to the overall toxicity of OSPW and FFT. However toxicity to aquatic organisms has mainly been attributed to a persistent mixture of naturally occurring acid extractable organics known

collectively as naphthenic acids (NAs). Adding to the toxicity of OSPW is the osmotic stress due to the high salinity from chemicals added during extraction. Sodium, calcium, chloride, sulfate and bicarbonate are the major ions contributing to the salinity of OSPW (Gosselin et al., 2010).

Naphthenic Acids

Naphthenic Acids refer to a group of acid extractable organics mainly comprised of alkyl substituted, acyclic and cyclo-aliphatic saturated carboxylic acids with/without S and/or N atoms as impurities (Clemente and Fedorak, 2005; Grewer et al. 2010; Kannel and Gan, 2012). The general empirical formula $C_nH_{2n+z}O_2$; where n represents the number of carbon atoms in the molecule and z specifies hydrogen deficiency due to the bonding of cyclic structures, has been used to describe NA mixtures (Scott et al., 2008) (Figure 1.2). NAs are a natural component found in most petroleum sources, including bitumen from the Athabasca oil sands, and are thought to originate from aerobic microbial degradation of petroleum hydrocarbons (Holowenko et al., 2002). During the process of extraction with water, NAs are released from bitumen, particularly under the alkaline pH conditions associated with oil sands processing for which NAs are more water-soluble and exist as naphthenate salts (Quagraine et al., 2005). Resulting NA concentrations can range from 20 to 120 mg L⁻¹ in OSPW (Holowenko et al., 2002). Natural biodegradation reduces NA concentrations over time. However, concentrations seldom decline below 20 mg L⁻¹ because of the presence of persistent non-biodegradable fractions contributing to the overall NA mixture (Quagraine et al. 2005).

Figure 1.2 Examples of Naphthenic Acid structures where *R* represents an alkyl side chain, *Z* represents the hydrogen deficiency due to the bonding of cyclic structures and *m* represents the number of CH_2 units (from Clemente and Fedorak, 2005)

NAs are toxic to a wide range of aquatic organisms and may have endocrine disrupting properties (Kavanagh et al. 2011; van den Heuval et al. 2012). The toxicity of NAs is often associated with their surfactant properties, leading to narcosis, or through reactive effects at a biological receptor site (Frank et al. 2008). NA extracts were found to be weak estrogen receptor agonists and androgen receptor antagonist in yeast (Thomas et al., 2009). Kavanagh et al., (2011) determined that NA concentrations greater than 25 mg L^{-1} and salinities typical of OSPW would not be conducive for successful fish reproduction due to altered sex steroid levels in fish. Bioassays on yellow perch determined an LC_{100} as low as 3.6 mg L^{-1} , due to gill and liver disorders (Nero et al., 2006). Toxicity assays using *Vibrio fischeri* (Frank et al., 2008; Martin et al., 2010) and *Photobacterium phosphoreum* (Holowenko et al., 2002) determined that NAs are acutely toxic to microorganisms. Frank et al. (2008) suggested that low molecular weight NAs is more toxic than

higher molecular weight NAs. However, the low molecular weight NAs are considered more readily accessible for microbial metabolism (Frank et al., 2008).

Various treatment options have been explored to promote or accelerate the degradation process and reduce in situ effects of NAs on environmental systems. Some plausible treatments to further biodegrade NAs in OSPW include bioaugmentation with bacteria selected to degrade persistent NAs (Quagraine et al., 2005), biodegradation by microorganisms from rhizosphere soils (Biryukova et al., 2007), and biostimulation by indigenous microbial communities with nutrient supplementation (Toor et al., 2012). Biodegradation studies have determined that NAs with greater alkyl side chain branching are more persistent than those with short chain linear compounds. The findings suggest that the more persistent NAs found in OSPW contain larger alkyl branched side chains that are less bioavailable due to intramolecular hydrogen bonding (Johnson et al., 2011). However, due to the long half-lives associated with biodegradation of NAs and the difficulty in degrading larger NA fractions several chemical treatments have been explored to further enhance the breakdown of NA congeners.

There are several treatments that rely on the production of hydroxyl radicals to oxidize NAs. These include ultraviolet photolysis (McMartin et al., 2004), photocatalysis with TiO_2 (Mishra et al., 2010) and ozonation (Scott et al., 2008; Martin et al., 2010). Photo-catalysis using UV fluorescent lamps in the presence of TiO_2 was able to degrade OSPW NAs with half-life values between 1.55-4.80 h, leading to a

reduction in the acute toxicity (up to 5 min. IC_{50} v/v > 90%) based on MicroTox assays (Mishra et al., 2010). Ozonation treatment for 50 min reduced NA concentrations by ~70%, resulting in an overall decrease in the proportion of high molecular weight NAs (Scott et al., 2008). McMartin et al., 2010 determined that mild ozonation alone did not significantly affect OSPW toxicity. However toxicity reduction was significantly accelerated following ozonation and incubation with indigenous microbes. These findings suggest that chemical oxidation of NAs may degrade some of the most bio-persistent NA fractions, creating a more labile carbon source for microbial metabolism, potentially accelerating further bioremediation of OSPW. The structure-reactivity hypothesis put forward by Martin et al., 2010 for the improved biodegradability of NAs observed following ozonation is that the hydroxyl radicals produced through the decomposition of ozone in water can abstract hydrogen more easily from tertiary carbon (larger, more alkyl branched NA congeners) than primary or secondary carbon atoms. This hypothesis has yet to be proven because of the analytical limitations in distinguishing between highly branched isomers and more linear ones.

Although these treatments hold promise, their use is limited in that they can only treat OSPW released from FFT, which typically occurs only after long-term settling. Thus, there is still a need for an alternative treatment method that can not only degrade NAs in OSPW, but also catabolize NAs within the FFT slurry. Also, both photocatalysis and ozonation technologies may have limited ability to treat the large volumes of OSPW encountered in the oil sands industry. Ultraviolet radiation cannot

penetrate turbid fluids, requiring OSPW samples to be filtered thoroughly before treatment. Ozone must bubble through the OSPW for reactions to take place.

Gamma Irradiation Treatment

A less explored treatment option to degrade NAs in OSPW is gamma irradiation (GI), which is a technology well developed for the treatment of complex persistent organic compounds in industrial wastewaters, contaminated groundwater and sewage sludge (Wang and Wang, 2007). The effect of ionizing radiation is dominated by its reaction with water. The radiolysis of water exposed to ionizing radiation can be described by the following equation:

Wang and Wang, 2007 (1)

The transfer of energy produces an H_2O molecule in an excited state that undergoes reactions to produce oxidizing hydroxyl radicals ($\text{OH}^\bullet$), reducing hydrated electrons (e^-) and hydrogen radicals ($\text{H}^\bullet$) which are the predominant products responsible for degrading the molecular structure of wastewater pollutants and a reduction of pathogens (Wang and Wang, 2007). The radical refers to an atom that contains an unpaired valence electron; in the case of $\text{OH}^\bullet$ this is the neutral form of the hydroxide ion OH^- . In aqueous radiation chemistry the absorbed dose is defined as the energy absorbed divided by the mass of material irradiated. The SI unit used to describe the absorbed dose is Gy, which is equivalent to 1 J/kg (Wang and Wang, 2007). Previous investigations have shown that GI effectively

degrades persistent organic compounds such as phenols (Chitose et al. 2003), tetrachloroethane and trichloroethane (Gerhinger and Matschiner, 1998), polychlorinated biphenyls (Arbon and Mincher, 1996) and trinitrotoluene (Lee and Lee, 2005). Unlike photolytic or ozonation technologies, GI can penetrate turbid fluids and sediments, providing the potential to treat large volumes of oil sands process material (OSPM) as a means to speed the degradation process once effluents have been released. Ideally, GI could be used to treat FFT, and eliminate NAs prior to its return into the environment for settling and eventual reclamation of tailing ponds.

The energy of ionizing radiation is great enough to break molecular bonds and ionize atoms. However, the energy is not high enough to impart radioactivity to the irradiated chemical species because there is not enough energy to disrupt the structure of the atomic nucleus (Wang and Wang, 2007). Facilities that treat wastewater with radiation technology using ^{60}Co and ^{137}Cs radiation sources have been in use since the 1960s. A treatment plant in South Korea currently disinfects and breaks down the persistent organic contaminations in 10,000 m³ of industrial wastewater per day (Han et al., 2008). In Albuquerque, NM, USA a sewage sludge treatment plant with a capacity for treating 8 tonnes of dried sludge per day at a dose of 10 kGy was built and commissioned in 1979 (Wang and Wang, 2007).

Biogeochemistry of Tailings Pond

Oil sand tailings ponds are chemically and physically stratified, harboring a diverse microbial community (Penner and Foght, 2010; Chi-Fru et al., 2013) involved in the aerobic and anaerobic degradation of organic compounds (Siddique et al., 2011) and elemental reduction/oxidation (redox) cycling within the ponds (Chen et al. 2013; Stasik et al., 2014). Current evidence suggests that natural attenuation mechanisms are occurring in the FFT and surface water, characterized by the removal or degradation of NA compounds and a general reduction in salinity due to leaching (MacKinnon et al., 2005). However, the underlying chemical factors contributing to the diffusive processes at work during maturation of the basin still require elucidation in order to predict the long-term behavior of the ponds.

Another important facet for the sustainable management of reclaimed tailings pond environments under the wet-landscape approach is determination of the sediment oxygen demand (SOD) exerted by the sediment itself, which is comprised of biological oxygen demand (BOD) and chemical oxygen demand (COD) (Chen et al., 2013). In the presence of dissolved oxygen (DO), aerobic bacteria will utilize O_2 as a terminal electron acceptor because it is the most energetically favorable electron acceptor. This will in part determine the degradation rates of persistent hydrocarbons, as hydrocarbons are more rapidly degraded under oxic conditions (Leahy and Colwell, 1990). Maintaining sufficient DO concentrations to prevent epibenthic anoxia in the overlying pond water will also be crucial to establishing conditions suitable for development of a typical boreal limnological food web.

Periods of extended hypoxia or anoxia can cause direct toxicity to macrobenthic communities and indirectly through the accumulation of reduced compounds in sediments and the water column (Middleburg and Levin, 2009).

When oxygen is depleted in a system the progression of terminal electron acceptors is determined by the thermodynamic energetic favorability a microbe can receive from that reaction (Middelburg and Levin, 2009) (Figure 1.3). Currently, there is limited knowledge of the variability and distribution of toxic compounds and the potential for their biotransformation within each of the redox-critical zones under sub-oxic conditions (with nitrate, sulfate, Mn(IV) and Fe(III) as electron acceptors) that is relevant for many potentially affected freshwater habitats. As FFT consolidates, pore water containing dissolved constituents (NAs, HS⁻, Fe, nitrates, and NH₄⁺) will be released into the overlying water. Many of these chemically reduced compounds produced via microbiological respiration within the sediment become rapidly oxidized at the sediment-water interface potentially leading to anoxia of the overlying waters. The diffusion-controlled flux of these constituents and the overall oxygen demand exerted by the bulk tailings will determine the FFT's influence on the quality of the overlying water comprising the pond, wetland (Chen et al., 2013; Matzinger et al., 2010). Understanding which redox-sensitive species dominate in a given system or stratum can provide insight into sediment chemistry dynamics and overlying water chemistry, an essential component for predicting the long-term behavior of settling basins and their eventual development into reclaimed

landscapes that sustain the ecological processes and food webs expected in a boreal ecosystem.

Figure 1.3 Microbial respiration processes under aerobic and anaerobic conditions

Sulfur Geochemistry

Sulfur is a key component of global biogeochemical cycling because it is highly reactive, redox-sensitive and microbial active. Sulfur can exist in various valence states, ranging from -2 (H_2S), 0 (S^0), +2 ($S_2O_3^{2-}$) to +6 (SO_4^{2-}). Thus, it is reactive under both oxidized and reduced conditions in both biotic and abiotic settings (Sturman et al., 2008). Sulfur can behave as an electron donor or electron acceptor, and it reacts with most metals to form metal sulfides. Microbial catalyzed processes play a key role in determining the fate of S in aquatic environments. Dissolved sulfide from influent water or sulfide diffusion from anoxic sediments, may be subject to oxidation via abiotic processes in more aerobic zones or through reactions catalyzed by sulfur oxidizing bacteria (SOB) (Figure 1.4). Under anoxic

conditions sulfide can precipitate with heavy metals, forming insoluble metal sulfides such as iron sulfide (FeS). The most important biotic reaction involving S in aquatic sediments is often illustrated and in most cases catalyzed by dissimilatory sulfate-reducing bacteria (SRB), which generate energy through the transfer of electrons from organic carbon sources to sulfate, producing sulfide (Sturman et al., 2008). The reduction of sulfate can account for more than half of organic carbon mineralization in anaerobic environments such as marine and wetland sediments (Sturman et al., 2008). Depending on the pH of the system and the presence of oxygen, H₂S may potentially outgas. Under acidic conditions, sulfide will be present as H₂S, due to its lower solubility than the deprotonated form. However, under alkaline conditions such as in oil sands FFT, HS⁻ will predominate.

Figure 1.4 Sulfur cycling and precipitation within aerobic and anaerobic sediment layers

In many environments S dynamics are tightly coupled to Fe cycling and release. Elevated Fe^{2+} concentrations can buffer the release of H_2S gas and prevent dissolved H_2S concentrations from becoming toxic, through the precipitation of Fe^{2+} sulfides (Middelburg and Levin, 2009). The minerals formed usually start as amorphous FeS precipitates and transition to more thermodynamically stable precipitates such as pyrite (FeS_2) (Bura-Nakic et al., 2009). In tailings ponds, the concentration of acid soluble metal sulfide precipitates can be up to 10-fold higher than dissolved sulfide due to the presence of reactive metals (Ramos-Padrón et al., 2011). Although most sulfide produced in a system will form FeS precipitates, up to 70% of the sulfide may be subject to continual cycling within the system, and the remainder will be sequestered permanently (Sell and Morse, 2006).

Production of HS^- in oil sands FFT has been observed in both field (Ramos-Padron et al. 2011) and microcosm studies (Chen et al. 2013). Hydrogen sulfide is toxic and subjects aquatic organisms to oxidative stress (Joyner-Matos et al. 2010), which may limit microbial and metazoan colonization in the pond water following reclamation. Several studies indicate that S cycling is a critical determinant of the spatial organization of various microbial communities linked to sulfide production and methanogenesis within FFT (Chi Fru et al. 2013; Holowenko et al. 2000; Ramos-Padron et al. 2011). Holowenko et al. (2000) found that sulfate addition inhibited methanogenesis in FFT by stimulating SRB, which outcompete methanogens for more energetically favorable electron donors. Methanogenesis in tailings ponds speeds the densification of FFT material. However it poses another threat to

successful wet-landscape reclamation as the flux of methane from the FFT into the surface water provides a route for the release of toxic NAs and other contaminants that are trapped in the FFT. Holowenko et al. (2000) examined the flux of methane across the sediment-water interface of a large tailings pond. They discovered that 60-80% of the gas flux was methane, and that the FFT samples released 0.1-0.25 mL of CH₄ mL⁻¹ of FFT under laboratory conditions. Methane is a potent greenhouse gas; therefore, the suppression of methanogens by SRBs may be a favorable option for the management of tailings ponds.

Research Scope

If GI-treatment alters the chemical composition of NAs, then a clear understanding of its impacts on the development and composition of the indigenous microbial community is warranted. Also, biogeochemical processes and the generation of metabolites related to the cycling of organic and inorganic materials must be assessed, especially in the context of potential oxygen and sulfur consumption and release of potentially harmful greenhouse gases. The rates at which these processes occur may depend on the quality and bioavailability of carbon being utilized by the microbes. Gamma irradiation of OSPM and FFT sterilizes the treated material by disrupting the DNA and other cellular structures of biota, without affecting the materials physical geochemistry (Bank et al., 2008; Chen et al., 2013). Therefore, inoculation of FFT and OSPW re-establishes the native microbial community, allowing for assessment of the bio-physicochemical responses to an altered organic carbon source in a relatively unchanged geophysical environment. Previous work has shown that laboratory microcosms can effectively simulate *in situ*

geochemical and microbial community structure development within oil sands tailings (Chi Fru et al., 2013; Chen et al., 2013). The tailings in the laboratory microcosm simulate and are a proxy for the most recently deposited layer of material in a basin environment (Chen et al., 2013). This layer will likely exert the greatest short-term influence on the quality of the water cap (Matzinger et al., 2010).

Hypothesis

In this thesis I hypothesize that the ionizing effects of GI-treatment will degrade NA congeners within OSPW and FFT, creating a more bioavailable carbon source for microbial respiration processes, thus stimulating the chemical response gradient which in turn is stimulated by the biological activity of indigenous microorganisms inoculated into GI-treated process materials. I also postulate that the reduction in NA concentration will result in reduced toxicity as determined through standard (MicroTox) assays.

Research Objectives

The remainder of this thesis consists of 2 chapters that investigate the influence of GI treatment on microbial processes and responses, and chemical fluxes, as well as a final chapter that integrates and interprets the results, and makes recommendations for further research. The primary research objectives for chapter 2 of this thesis are to assess the biological and physicochemical responses of microbes to GI-treated OSPW and FFT collected from tailings ponds composed of fresh and aged materials that are inoculated with native microbes from each ponds

respective untreated source material following treatment. The aged FFT harbors a relatively high proportion of refractory NA isomers (because it has undergone natural biodegradation), and supports a microbial community that has undergone succession as a consequence of aging. In contrast, material from a tailings pond containing fresh FFT contains the broader suite of NA congeners characteristic of freshly processed FFT and an early-successional microbial community. It is predicted that the microbial community of GI-treated material will exhibit greater respiration rates than the community of untreated materials due to an increase in bioavailable carbon, leading to altered (increased) fluxes and quantities of microbial respiratory end products (carbon dioxide, nitrogen, hydrogen sulfide and methane).

The research objectives for Chapter 3 are to determine the efficacy of GI-treatment on the breakdown of NAs in fresh and aged OSPW and FFT, and to assess toxicity reduction in OSPW. It is predicted that the ionizing effects of GI-treatment will result in reduced NA concentrations for each pond material. The fresh and aged process materials are expected to contain differing NA composition. In part this is due to breakdown of the original NAs over the ~40 year period giving rise to a refractory mixture of NA compounds. It is predicted that GI-treated OSPW will be markedly less toxic to *Vibrio fischeri* than will untreated OSPW.

In chapter 4 the implications and feasibility of GI treatment for tailings pond management and reclamation will be discussed. The major findings from chapters 2 and 3 are reviewed, as are the unknowns that must be addressed before GI can be

confidently implemented for the treatment of OSPM. The results of this research will contribute to an improved understanding of the value of GI as a potential waste treatment for oil sands operators in northern Alberta. This will provide a better understanding of the short and long-term processes influencing the biogeochemistry in detoxifying OSPM and document the byproducts of interest produced.

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CHAPTER 2: BIO-PHYSICOCHEMICAL EFFECTS OF GAMMA IRRADIATION TREATMENT FOR NAPHTHENIC ACIDS IN OIL SANDS FLUID FINE TAILINGS

Introduction

The goal of this chapter was to determine the bio-physicochemical effects of GI-treatment in oil sand FFT and OSPW. It is expected that the ionizing effects of GI would alter the organic carbon characteristics, leading to a breakdown of persistent NAs and other hydrocarbons into forms that are more bioavailable for microbial metabolism. This could potentially stimulate microbial respiration, leading to increased concentration of their byproducts such as hydrogen sulfide and methane. Alternatively, the rates of microbial respiration may be limited by the availability of other substrates necessary for metabolism, leading to similar biogeochemical evolution between GI-treated and untreated materials. In order to predict how GI-treatment will alter the bio-physicochemical properties of materials placed in tailings ponds and the suitability of this treatment for promoting enhanced detoxification of FFT and OSPW to allow for its incorporation into reclaimed wetland and end-pit lakes landscapes; there is a need to determine the factors controlling the rates of microbial respiration, the resulting chemical composition of the FFT and OSPW, and the diffusional flux of important chemical species within FFT and across the diffusive boundary layer (DBL) between sediments and the overlying water.

The DBL is the epibenthic layer of water overlying the sediment surface of aqueous environments, through which molecular diffusion is the dominant

transport mechanisms of dissolved species due to the low water velocity within this layer (Elberling et al., 2001). The flux of dissolved constituents across the DBL and into or from the sediment is a measure of the rate of mineralization of organic matter within the sediment and the dissolution or precipitation of minerals (Glud et al., 2007). Due to the highly reducing nature of FFT, managing dissolved oxygen (DO) concentrations within the surface waters of reclaimed ecosystems and the sediment oxygen demand (SOD) at the sediment-water interface will be a key factor in determining the sustainability of those ecosystems (Chen et al., 2013). In this thesis I define sustainability as the capacity of ecosystems to maintain their essential functions and processes over the long-term. Sulfate reduction is a dominant terminal electron accepting process in many high organic carbon-rich environments, accounting for more than half of organic carbon mineralization (Sturman et al., 2008). Determination of high-resolution spatiotemporal trends in HS⁻ flux provides a proxy for the rate of anaerobic organic matter mineralization in the sediment (Jørgensen et al., 2001).

Methods

Source of the materials

Two sets of microcosms were created. One which contained fresh (6-9 old) materials (FFT and OSPW) from South Tailings Pond (STP) (Lat: 56.868783° Long: -111.33164°) and one of which contained aged (~40 years) materials from Pond 1A (P1A) (Lat: 56.985213° Long: -111.47803°) operated by Suncor Energy Inc., located in the Athabasca region, Alberta (Figure 2.1). The creation of P1A dates back to

1968, making it the oldest tailing basin operated by Suncor (Fedorak et al., 2003). P1A does not receive fresh tailings but is used for the recycling of OSPW on the surface. Recycling of the process water concentrates certain chemicals but no additional chemicals are added with the exception of sodium hydroxide, which was used in the past to disperse fine particles. From a NA treatment perspective, P1A represents a worst-case scenario, as only the refractory NA fractions that have not biodegraded over a period of over 4 decades remain in the FFT and water. Suncor STP, constructed in 2006 was 9 years old at the time of sampling and still received freshly processed FFT. This material was expected to contain the full suite of both labile and persistent NA congeners in its OSPW and FFT, and a microbial community at a relatively young successional stage.

Figure 2.1 Location of P1A (top left) and STP (bottom right) tailings ponds on the Suncor lease site ~30 km north of Fort McMurray, Alberta (.maps.google.com)

Microcosm Design

The study design consisted of 2 treatments - non-GI (100% untreated) vs. GI-exposed (90% gamma-irradiated material mixed with 10% untreated inoculum from its parent pond source) x 2 sources of material x 2 atmospheric maintenance conditions, x 6 time points, with n=2 replicates per treatment. GI-treatment sterilizes OSPW and FFT without affecting its physical properties or geochemistry (Bank et al., 2008; Chen et al., 2013). The inoculum of untreated material re-introduced the native microbial community from the source pond to the GI-treated, sterile source. Representative microcosms were maintained under either oxic or anoxic conditions, resulting in 8 different treatments (Table 2.1). The anoxic microcosms were maintained in an anaerobic chamber. Oxic microcosms were incubated under ambient atmospheric conditions on laboratory bench tops. The different environmental conditions provide several important functions: 1) allowing assessment of the role of oxygen availability on degradation pathways of NA congeners and the breakdown products produced following GI-treatment, 2) determining the rate of oxygen consumption at the sediment-water interface within oxic microcosms, 3) determining the fate of microbiological respiratory end-products in the presence/absence of oxygen. The oxic microcosms represent biogeochemical functions expected to occur near the sediment-water interface where DO is present. The anoxic microcosms represent the biogeochemical functions expected below the sediment-water interface where DO has already been consumed by biotic and abiotic processes and potentially at the sediment-water interface of anoxic surface water where the DO has already been consumed before

reaching the sediment interface. All microcosms were covered with opaque foil to exclude light and incubated at an average temperature of 23°C.

Table 2.1 *Experimental design summarizing source of materials, irradiation treatment, and storage conditions. Two small microcosms of each treatment were created and harvested 2, 4, 8, 20, 32 and 52 weeks after creation.*

STP pond material		P1A pond material	
oxic	anoxic	oxic	anoxic
<i>STP_{Gl}</i>	<i>STP_{Gl}</i>	<i>P1A_{Gl}</i>	<i>P1A_{Gl}</i>
<i>STP_{non-Gl}</i>	<i>STP_{non-Gl}</i>	<i>P1A_{non-Gl}</i>	<i>P1A_{non-Gl}</i>

Approximately 100 L of FFT and 100 L of OSPW were collected separately from each pond source in August 2012 and shipped in 20 L plastic pails to the University of Windsor. Upon arrival, samples were stored at 4°C at the University of Windsor – Great Lakes Institute for Environmental Research walk in freezer. A subset of FFT samples and OSPW were gamma irradiated on October 2012 at 28 kGy over 24 h, at the McMaster Institute of Applied Radiation Services (McIARS), Hamilton, Ontario, Canada.

The small microcosms were destructively analyzed after incubation periods of 2, 4, 8, 20, 32 and 52 weeks. Microcosms were incubated in the dark under ambient temperature (~22°C). Small microcosms used for destructive analysis were comprised of clear plastic vessels with a diameter of 10.16 cm and height of 20 cm with a flat base and a white PVC cap, designed by the University of Windsor technical support center (Figure 2.2). Approximately

800±1 g of FFT and 400±1 g of OSPW were added to each small vessel (n=2 per treatment).

In addition, one large microcosm per treatment was prepared and used for repeated, non-destructive sampling. These microcosms were prepared with a 2000±1 g aliquot of FFT overlain by 1000±1 g of OSPW (n=1 per treatment) in an ethanol sterilized 3.8 L Camwear® container (Cambro Manufacturing Company, Huntington Beach, CA) (Figure 2.3). These containers had a diameter of 21 cm and a height of 22 cm. Large microcosms were created and maintained for the purpose of collecting measurements on which to base flux calculations. Repeated measurement of the same microcosms produced greater consistency in the determination of concentration profiles, used to calculate flux over the 52-week study period.

Figure 2.2 Example of small microcosms (diameter of 10.16 cm and height of 20 cm) used for destructive sampling

Figure 2.3 Large microcosms (diameter of 21 cm and height of 22 cm) (used for non-destructive sampling over the 52 weeks

Chemical Analyses

In situ electrochemical and diffusivity measurements were made in each microcosm 2, 4, 8, 20, 32 and 52 weeks after microcosms were filled with materials (see below). Each microcosm identified for destructive sampling on a date was placed in an anoxic glove box. Subsequently, the OSPW surface water was carefully siphoned from above the FFT into a 500-mL Nalgene container. The remaining FFT was then emptied into a separate 1000-mL Nalgene container. The OSPW and FFT samples were flushed with nitrogen and stored frozen at -20°C. Chemical and physical characteristics of these materials were subsequently determined using standard operating procedures for OSPW and extracted FFT pore water (mean $\pm$ SE (n=2)) (Syncrude Canada Ltd., 1995).

In Situ Electrochemical Analysis

Automated micromanipulator-mounted, micro-electrode sensors were purchased from Unisense A/S (Aarhus, Denmark) to monitor changes in pH, O₂, HS⁻ and diffusivity across the OSPW-FFT interface within the microcosms. Sensors were purchased with tip diameters ranging between 100 and 500 μ m. The response to chemical gradients is characterized by their linear response to specific media, allowing for high-resolution detection of chemical gradients with low detection limits (Elberling et al., 2011). Prior to use micro-sensors were polarized using a Unisense Multimeter model PA2000 and calibrated according to the manufacturer's protocol. The sensors were mounted to a Unisense LS18 lab stand and controlled using a Unisense model MC-232 motorized microprofiling system, which is operated

through the SensorTrace Pro software system (Unisense A/S, 2015) (Figure 2.4). Measurements were taken at 1000- μm intervals to give high-resolution concentration profile gradients from 1 cm above the OSPW-FFT interface to >5 cm into the FFT below the OSPW-FFT interface.

Figure 2.4 Unisense LS18 lab stand and microprofiling system controlled through the Sensortrace software program

Apparent diffusivity measurements within FFT were performed using procedures as described in Chen et al. (2013). The diffusivity sensor uses an amperometric sensor measuring concentrations of a tracer gas in an internal H_2 gas reservoir within the sensor tip to provide a measure of the diffusion coefficient ($\text{m}^2 \text{ day}^{-1}$) (Revsbech et al., 1998). A mathematical model that describes the diffusivity is based on a two-point calibration using two different media with known porosity and

diffusion coefficient (stagnant water and 40-75 µm unsorted glass beads in water) (Revsbech et al., 1998). The collection and interpretation of diffusivity coefficients assumes that diffusion properties in the microcosms behave as one-dimensional horizontal systems so that the undifferentiated vertical concentration profile from HS- and O₂ sensors can be applied for calculating fluxes using Fick's first law of diffusion as follows:

$$J(x) = -\phi \frac{D(x) \left(\frac{dC(x)}{dx} \right)}{dx}$$

where J is the diffusion flux (concentration per unit area per unit time), ϕ the porosity, D the diffusion coefficient and $\frac{dC(x)}{dx}$ the concentration gradient for which the flux is calculated. Fluxes were calculated for dissolved oxygen (DO) concentrations across the diffusive boundary layer obtained from just above (within 5 mm of) the OSPW-FFT interface to just below (within 10 mm of) the OSPW-FFT interface, where the steepest change in concentration gradient with depth occurs as outlined by Elberling and Damgaard, 2001 (Figure 2.5). However, in this study the concentration gradient across the DBL could not be distinguished from the concentration gradient across the FFT. Therefore, a single gradient was assumed to exist, extending from the top of, and through the DBL, and into the FFT below the OSPW-FFT interface. Similarly, hydrogen sulfide flux was calculated using the largest gradient change in concentration recorded within the FFT.

Figure 2.5 Schematic depiction of the DO profile within saturated cap water and the consumption across the DBL and into the FFT, where h_{dbl} represents the height of the DBL, d_p is the DO penetration depth within the FFT, C_o is the DO concentration at the FFT-OSPW interface, C_{sat} is the DO concentration in atmospherically saturated cap water, $dC_w/dx(0)$ is the DO concentration gradient in the DBL and $dC_s/dx(0)$ is the concentration gradient in the FFT (Figure from Elberling and Damgaard, 2001)

Headspace Gas Analysis

On each sampling date, two 5-mL samples of headspace gas were collected from the large microcosms incubated in the anaerobic chamber using a sterile needle and syringe. Each headspace sample was transferred into a separate glass vial, previously filled with helium-flushed water and sealed with a crimp-top rubber septum for preservation. To maintain atmospheric pressure within the vial, a separate needle was injected through the septum to collect water displaced by the gas sample that was added to the inverted vial. A 0.2-mL subsample of the headspace gas was used for analyses of H₂S and CH₄ by gas chromatography. A Variant model CP3800 equipped with a Thermal Conductivity Detector (TCD) and an HP column (part # 19095p-Q04) was used for analysis. An oven temperature of 90°C, column temperature of 95°C and a flow rate of 1.3 mL min⁻¹ was used for all samples.

Results and Discussion

Impact of GI-treatment on FFT materials

The effect of GI treatment was tested on OSPM materials from P1A (old pond) and STP (newer pond), respectively to determine the efficacy of GI treatment on the two NA sources. The older FFT from P1A is assumed to be more dominated by persistent NA isomers (and to contain a microbial community that is more mature) because it has been subject to biodegradation for over 30 years. In contrast, STP is more likely to be constituted of materials containing the full suite of NA congeners

because this pond continuously receives freshly processed FFT, which would be metabolized by a relatively early-succession microbial community.

Microcosms were visually inspected daily to document changes that might be occurring in the OSPW surface water and FFT. The OSPW in the P1A_{non-GI} and STP_{non-GI} microcosms was transparent and pale yellow. The clarity and color remained unchanged in both treatments throughout the study. In the GI-treated P1A_{GI} and STP_{GI} microcosms, OSPW was transparent with a much darker orange coloring that persisted throughout the entire study, possibly caused by the production of humic substances, which constitute the main component causing color in effluent containing organic materials (Jung et al., 2002). Other NA treatments were found to reduce the intensity of coloration in OSPW (Scott et al., 2008). Jung et al. (2002) observed that the humic substances in effluent containing organic contaminants from sewage sludge were effectively decomposed by gamma rays, leading to color removal. However, depending on the size and structure of the parent compounds (NA isomers) and radiation dosage, an increase in humic substances may have occurred due to incomplete decomposition of the parent NA compounds.

At the end of two weeks, black discolorations were observed on the inner walls of the microcosms in the FFT, extending downward from below the OSPW-FFT interface in both the anoxic P1A_{non-GI} (Figure 2.6) and P1A_{GI} (Figure 2.7) treatments. The patches continued to develop, covering the entire portion of the FFT visible through the walls of the microcosm by week 8. Chen et al.

(2013) observed similar behavior, which reflects the precipitation of Fe sulfides created through the activity of SRBs in the material. In contrast, the STP FFT in anoxic microcosms did not become discolored until the end of week 8. Unlike P1A, the sulfide front in STP FFT developed at the bottom of the microcosm and expanded upwards towards the sediment-water interface (Figures 2.8 and 2.9). This may be related to difference in the sulfur metabolizers (e.g. obligate anaerobes) present in the STP material. Also, P1A material contains an older microbial community than does STP. Presumably, the HS⁻ front was faster to develop in P1A because the microbial assemblage is more adapted to the FFT conditions and may contain facultative anaerobes that can survive in the presence of oxygen. The sulfidic zone continued to develop at a slower rate in STP than in P1A microcosms, discoloring sediments over the bottom 50% of the microcosm by the end of week 20 and the entire outer surface of the FFT microcosms outer surface at week 52. These observations suggest that the rate of sulfide precipitation was similar between the anoxic GI and non-GI treatments of each pond material.

In the oxic P1A FFT microcosm, a distinct ochre coloration was observed at the end of week 4 suggestive of the formation of iron oxides proximal to the FFT interface. Interestingly, an isolated, black sulfidic zone was observed within the bulk of the FFT as had been noted in oxic P1A_{non-GI} (Figure 2.10) and P1A_{GI} (Figure 2.11) microcosms. This suggests significant heterogeneity with respect to redox behavior for this particular FFT material under oxic conditions. In

contrast, the oxic STP microcosms initially showed no ochre discoloration, suggesting that iron oxide formation was minimal until the end of week 16 in both the STP_{non-GI} (Figure 2.12) and STP_{GI} (Figure 2.13). As well, no localized sulfidic zones were visible in the oxic STP FFT. The reason for this could be related to differences microbial community composition and diversity between the two ponds since the P1A microbial community is older than that of STP material.

In general, pH values ranged from 8.4-8.7 within the FFT and 8.3-8.9 within the OSPW over the 52-week period regardless of treatment and pond material examined. Overall, the temporal trends in the concentrations of dominant ions (alkalinity, SO_4^{2-} , NH_4^+ , Ca^{2+} , Mg^{2+} and Na^+) in the bulk aqueous OSPW and the pore water extracted from FFT was similar between GI and non-GI treatments for each respective pond source (Tables 2.2-2.5). Some potential microbial respiration pathways occurring in the FFT of P1A and STP will be discussed based on the OSPW and extracted pore water FFT chemical expressions.

The observed reduction in alkalinity may be related to alkalinity consuming reactions via nitrification processes occurring within the treatments (Li and Irvin, 2007). The nitrification process is a two step reaction: in the first step ammonia is oxidized to nitrite followed by the oxidation of nitrite to nitrate:

Li and Irvin, 2007 (2)

The loss in buffering capacity suggests an excess production of H^+ protons due to possible microbial nitrogen cycling (e.g. nitrification), responsible for consuming 7.14 g of alkalinity for every gram of N oxidized under laboratory settings (Li and Irvin, 2007). Sulfate reduction coupled with organic matter oxidation is an alkalinity generating reaction in oil sands materials (Fedorak et al., 2003):

Fedorak et al., 2003 (3)

However, in order to contribute alkalinity, the products of sulfate reduction must be of a form that prevents reoxidation, an acid-generating process that causes a loss of alkalinity (Giblin et al., 1990). For example, pyrite (FeS_2) and iron monosulfide (FeS) can be oxidized in the presence of O_2 , typically at the sediment surface or within the anoxic sediment if NO_3^- , Fe (III) oxides or MnO_2 are available as potential electron acceptors (Schippers and Jørgensen, 2002):

Schippers and Jørgensen, 2002 (4)

Although most sulfide in sediments will form FeS precipitates, Sell and Morse (2006) estimated that up to 70% of the sulfide will be subject to continual cycling within the system. Fedorak et al. (2003) found that bicarbonate

concentrations increased as sulfate concentrations decreased in sulfate amended FFT. Siddique et al., (2014), who studied microbial metabolism in FFT amended with a labile organic substrate, observed lowered pH, which resulted in a release of divalent cations (Ca^{2+} , Mg^{2+}) and increased HCO_3^- in pore waters of amended FFT. These findings contrast with our results. We found that the concentration of Ca^{2+} , Mg^{2+} and HCO_3^- decreased over time regardless of the altered carbon source in GI-treated FFT pore water; and pH remained at 8.3 for these FFT pond materials (Tables 2.2-2.5) which suggests bicarbonate salts of calcium and magnesium are being produced:

Siddique et al., 2014 (5)

Headspace Gas Evolution

To understand the impact of GI on biogenic gas production, the concentration of CH_4 was measured in the headspace gas of both anoxic P1A and STP microcosms. At the end of 2 weeks, methane was detectable in the headspace of the anoxic P1A microcosms. At week 2 a trace amount was detected in the P1A_{non-GI} (0.18% CH_4 in N_2) treatment (Figure 2.14). However methane was undetectable for the remainder of the study up to week 52. In contrast, in the P1A_{GI} treatment a CH_4 concentration of ~3% CH_4 in N_2 was detected at the end of weeks 2 and 4, after which concentrations gradually declined until they were below the limits of detection at week 52. The increase in methane concentrations in the P1A_{GI} headspace over the first 8 weeks is

believed to be from the release of residual methane entrapped in the FFT that has not been consumed from Anaerobic Oxidation of Methane (AOM) processes coupled to sulfate reduction:

Jørgensen et al., 2001 (6)

Methanogenesis is a well documented process in aged FFT (Holowenko et al., 2000) and there is an apparent drawdown of methane in the P1A_{non-GI} headspace because of AOM being coupled to sulfate reduction. However, the P1A_{GI} FFT started with only 10% of the microbial biomass as the P1A_{non-GI} treatment at the beginning of the experiment and was therefore not able to consume methane at a similar rate as the P1A_{GI} FFT. By week 36 the methane concentration dropped down to below 0.5% CH₄ in N₂ (Figure 2.14) because it is being consumed by AOM processes as the microbial community is becoming established and we also observe a decrease in sulfate concentrations (Table 2.3).

Interestingly, an increase in CH₄ evolution has often been correlated to higher ratios of labile:persistent carbon pools (Updegraff et al., 1995; Siddique et al., 2014). Siddique et al., (2014) observed increased CH₄ emissions in FFT that had been amended with canola hydrolysate but only minor gas production from unamended FFT microcosms. Holowenko et al. (2000) found that sulfate addition inhibited methanogenesis in FFT by stimulating SRB, which can outcompete methanogens for more energetically favorable electron donors which might explain why there appears to be no further production of CH₄ in the headspace of both P1A and STP. In

contrast to P1A, no CH₄ was detected in the headspace of STP microcosms over the duration of the 52 week study period. Since STP FFT contains fresh tailings, complex microbial development may be limited due to this dynamic nature. Also, the continual influx of more energetically favorable electron acceptors (such as nitrate, sulfate, Mn(IV) and Fe(III)) used by respiring microorganisms may result in these reducers suppressing methanogens until those oxidants are consumed (Middelburg and Levin, 2009). No H₂S gas was observed in the headspace or overlying OSPW regardless of treatment in P1A and STP.

Figure 2.6 Development of sulfide precipitate formation within anoxic $P1A_{non-GI}$ microcosms at weeks 2 (left), week 8 (middle) and week 52 (right).

Figure 2.7 Development of sulfide precipitate formation within anoxic $P1A_{GI}$ microcosms at weeks 2 (left), week 8 (middle) and week 52 (right).

Figure 2.8 Development of sulfide precipitate formation within anoxic STP_{non-GI} microcosms at weeks 2 (left), week 20 (middle) and week 52 (right).

Figure 2.9 Development of sulfide precipitate formation within anoxic STP_{GI} microcosms at weeks 2 (left), week 20 (middle) and week 52 (right).

Figure 2.10 Development of iron oxide formation within oxic $P1A_{non-GI}$ microcosms at weeks 2 (left), week 20 (middle) and week 52 (right).

Figure 2.11 Development of iron oxide formation within oxic $P1A_{GI}$ microcosms at weeks 2 (left), week 20 (middle) and week 52 (right). Sulfide precipitates were also visible at week 20 (middle) but were only slightly visible at week 52 (right).

Figure 2.12 Development of iron oxide formation within oxic STP_{non-GI} microcosms at weeks 2 (left), week 20 (middle) and week 52 (right).

Figure 2.13 Development of iron oxide formation within oxic STP_{GI} microcosms at weeks 2 (left), week 20 (middle) and week 52 (right).

Figure 2.14 Mean ($\pm$ SD, $n=2$) concentration of methane detected in headspace gas of anoxic $P1A_{non-GI}$ (filled circles, solid line) and $P1A_{GI}$ (open squares, dashed line) microcosms. No methane was detected in the headspace of any STP microcosms

Table 2.2 P1A oxia OSPW and FFT water chemistry (mean $\pm$ SE (n=2))

Treatment	Time	pH	Total Alkalinity mg/L CaCO ₃ eq.	HCO ₃ mg/L	SO ₄ mg/L	Cl mg/L	NO ₃ mg/L	Na mg/L	K mg/L	Mg mg/L	Ca mg/L	NH ₄ mg/L	Fe mg/L
P1A Oxia OSPW													
Non-GI	Week 2	8.72 $\pm$ 0.02	814 $\pm$ 2	946 $\pm$ 5	198 $\pm$ 3	280 $\pm$ 0	BDL	620 $\pm$ 9	19.5 $\pm$ 1	12.6 $\pm$ 1	8.45 $\pm$ 1	22.8 $\pm$ 1	0.15 $\pm$ 0.1
Non-GI	Week 4	8.81 $\pm$ 0.05	884 $\pm$ 65	1014 $\pm$ 66	202 $\pm$ 16	270 $\pm$ 10	BDL	629 $\pm$ 48	19.6 $\pm$ 2	11.9 $\pm$ 1	6.26 $\pm$ 1	23.7 $\pm$ 1	0.11 $\pm$ 0.1
Non-GI	Week 8	8.81 $\pm$ 0	795 $\pm$ 1	913 $\pm$ 1	179 $\pm$ 5	246 $\pm$ 3	0.65 $\pm$ 0.5	564 $\pm$ 6	18.3 $\pm$ 0.7	9.53 $\pm$ 0.1	7.28 $\pm$ 1	18.0 $\pm$ 1	1.18 $\pm$ 0.5
Non-GI	Week 20	8.78 $\pm$ 0	729 $\pm$ 0	841 $\pm$ 0	165 $\pm$ 0	242 $\pm$ 0	BDL	528 $\pm$ 0	16.2 $\pm$ 0	8.18 $\pm$ 0	5.5 $\pm$ 0	17.7 $\pm$ 0	1 $\pm$ 0
Non-GI	Week 34	8.90 $\pm$ 0.1	590 $\pm$ 86	669 $\pm$ 95	185 $\pm$ 34	249 $\pm$ 5	0.38 $\pm$ 0.2	509 $\pm$ 7	14.5 $\pm$ 0.1	7.05 $\pm$ 0.2	7.08 $\pm$ 1	13.8 $\pm$ 0	0.94 $\pm$ 0.2
Non-GI	Week 52	8.69 $\pm$ 0.1	640 $\pm$ 9	746 $\pm$ 13	141 $\pm$ 6	248 $\pm$ 5	0.20 $\pm$ 0.1	515 $\pm$ 7	14.1 $\pm$ 0.1	6.68 $\pm$ 0	6.08 $\pm$ 1	13.2 $\pm$ 1	1.38 $\pm$ 0.1
GI-Treated	Week 2	8.77 $\pm$ 0.1	811 $\pm$ 11	937 $\pm$ 16	196 $\pm$ 2	280 $\pm$ 0	BDL	591 $\pm$ 5	19.1 $\pm$ 1	11.9 $\pm$ 1	7.84 $\pm$ 1	22.6 $\pm$ 1	1.09 $\pm$ 0.1
GI-Treated	Week 4	8.74 $\pm$ 0	822 $\pm$ 0	954 $\pm$ 1	188 $\pm$ 1	265 $\pm$ 5	0.21 $\pm$ 0	591 $\pm$ 2	18.8 $\pm$ 1	11.5 $\pm$ 0.2	8.89 $\pm$ 0.2	22.7 $\pm$ 0.3	1.35 $\pm$ 0.6
GI-Treated	Week 8	8.80 $\pm$ 0.1	822 $\pm$ 0	945 $\pm$ 6	171 $\pm$ 1	244 $\pm$ 2	BDL	553 $\pm$ 2	19.1 $\pm$ 1	9.91 $\pm$ 0.1	8.75 $\pm$ 1	20.7 $\pm$ 0.1	0.56 $\pm$ 0.2
GI-Treated	Week 20	8.85 $\pm$ 0.1	744 $\pm$ 6	851 $\pm$ 1	167 $\pm$ 1	240 $\pm$ 1	0.94 $\pm$ 1	512 $\pm$ 15	16.6 $\pm$ 1	8.41 $\pm$ 0.3	5.92 $\pm$ 1.2	18.3 $\pm$ 0	1.26 $\pm$ 0.1
GI-Treated	Week 34	8.88 $\pm$ 0.1	646 $\pm$ 21	734 $\pm$ 21	184 $\pm$ 3	242 $\pm$ 5	BDL	531 $\pm$ 9	14.8 $\pm$ 0.1	7.65 $\pm$ 0.1	5.79 $\pm$ 0.8	13.8 $\pm$ 0.6	0.99 $\pm$ 0.2
GI-Treated	Week 52	8.86 $\pm$ 0	614 $\pm$ 6	701 $\pm$ 7	204 $\pm$ 10	250 $\pm$ 13	0.67 $\pm$ 0.1	531 $\pm$ 14	14.8 $\pm$ 0.4	7.33 $\pm$ 0.3	5.99 $\pm$ 0.3	13.2 $\pm$ 2	1.69 $\pm$ 0.3
P1A Oxia FFT													
Non-GI	Week 2	8.54 $\pm$ 0.1	789 $\pm$ 7	932 $\pm$ 10	197 $\pm$ 22	196 $\pm$ 22	BDL	520 $\pm$ 23	17.0 $\pm$ 0.3	10.6 $\pm$ 0.7	18.4 $\pm$ 0.1	16.5 $\pm$ 0.1	0.19 $\pm$ 0.1
Non-GI	Week 4	8.46 $\pm$ 0.1	762 $\pm$ 20	904 $\pm$ 24	227 $\pm$ 22	215 $\pm$ 15	1.14 $\pm$ 0.6	523 $\pm$ 11	17.5 $\pm$ 0.3	11.0 $\pm$ 0.3	19.4 $\pm$ 1	16.8 $\pm$ 0.6	0.25 $\pm$ 0.1
Non-GI	Week 8	8.39 $\pm$ 0	787 $\pm$ 3	938 $\pm$ 3	210 $\pm$ 9	239 $\pm$ 7	0.21 $\pm$ 1	547 $\pm$ 9	17.9 $\pm$ 0.2	10.4 $\pm$ 0.2	17.5 $\pm$ 0.2	17.9 $\pm$ 0.3	0.13 $\pm$ 0.1
Non-GI	Week 34	8.47 $\pm$ 0.1	545 $\pm$ 39	646 $\pm$ 44	181 $\pm$ 32	248 $\pm$ 1	0.58 $\pm$ 0.3	477 $\pm$ 2	14.4 $\pm$ 0.4	5.44 $\pm$ 0.3	7.59 $\pm$ 0.5	9.25 $\pm$ 2	1.10 $\pm$ 0.7
Non-GI	Week 52	8.47 $\pm$ 0.1	607 $\pm$ 11	720 $\pm$ 17	174 $\pm$ 7	287 $\pm$ 8	1.24 $\pm$ 0.5	525 $\pm$ 4	13.5 $\pm$ 0.2	5.83 $\pm$ 0.1	8.33 $\pm$ 0.1	11.6 $\pm$ 0.3	0.15 $\pm$ 0.1
GI-Treated	Week 2	8.49 $\pm$ 0.1	783 $\pm$ 20	928 $\pm$ 25	188 $\pm$ 20	190 $\pm$ 21	BDL	501 $\pm$ 22	16.8 $\pm$ 0.4	10.0 $\pm$ 0.6	17.4 $\pm$ 1	16.2 $\pm$ 0.4	0.33 $\pm$ 0.1
GI-Treated	Week 4	8.46 $\pm$ 0.1	791 $\pm$ 31	940 $\pm$ 38	266 $\pm$ 46	218 $\pm$ 16	0.28 $\pm$ 0.2	555 $\pm$ 15	19.9 $\pm$ 0.7	13.0 $\pm$ 0.6	22.7 $\pm$ 1	17.6 $\pm$ 0.7	1.00 $\pm$ 0.4
GI-Treated	Week 8	8.41 $\pm$ 0.1	795 $\pm$ 8	947 $\pm$ 11	220 $\pm$ 7	236 $\pm$ 1	BDL	559 $\pm$ 3	18.5 $\pm$ 0.7	11.0 $\pm$ 0.1	18.6 $\pm$ 0.2	18.6 $\pm$ 0.2	0.18 $\pm$ 0.1
GI-Treated	Week 20	8.34 $\pm$ 0.1	753 $\pm$ 27	900 $\pm$ 34	277 $\pm$ 0	234.5 $\pm$ 0.5	BDL	583 $\pm$ 20	21.2 $\pm$ 2	12.9 $\pm$ 2	22.0 $\pm$ 5	19.4 $\pm$ 1.1	0.18 $\pm$ 0.1
GI-Treated	Week 34	8.51 $\pm$ 0	566 $\pm$ 2	670 $\pm$ 3	191 $\pm$ 6	242 $\pm$ 9	0.74 $\pm$ 0.5	482 $\pm$ 10	14.0 $\pm$ 0.1	5.75 $\pm$ 0.1	7.32 $\pm$ 0.2	11.6 $\pm$ 0.3	0.79 $\pm$ 0.1
GI-Treated	Week 52	8.41 $\pm$ 0.1	593 $\pm$ 4	705 $\pm$ 5	261 $\pm$ 7	294 $\pm$ 13	0.76 $\pm$ 0.5	558 $\pm$ 6	15.5 $\pm$ 0.5	6.91 $\pm$ 0.1	9.93 $\pm$ 0.1	12.2 $\pm$ 0.8	0.43 $\pm$ 0.1

Table 2.3 P1A anoxic OSPW and FFT water chemistry (mean $\pm$ SE (n=2))

Treatment	Time	pH	Total Alkalinity mg/L CaCO ₃ eq.	HCO ₃ mg/L	SO ₄ mg/L	Cl mg/L	NO ₃ mg/L	Na mg/L	K mg/L	Mg mg/L	Ca mg/L	NH ₄ mg/L	Fe mg/L
P1A Anoxic OSPW													
Non-GI	Week 2	8.71 $\pm$ 0.1	848 $\pm$ 0.5	986 $\pm$ 2	188 $\pm$ 4	265 $\pm$ 5	BDL	594 $\pm$ 1	18.1 $\pm$ 0.3	12.7 $\pm$ 0.1	11.0 $\pm$ 1	23.7 $\pm$ 0.2	0.08 $\pm$ 0.1
Non-GI	Week 4	8.73 $\pm$ 0.1	828 $\pm$ 12	961 $\pm$ 14	174 $\pm$ 2	255 $\pm$ 5	BDL	571 $\pm$ 4	18.0 $\pm$ 0.1	11.2 $\pm$ 0.4	8.35 $\pm$ 0.2	21.6 $\pm$ 1	0.17 $\pm$ 0.1
Non-GI	Week 8	8.69 $\pm$ 0.1	815 $\pm$ 27	949 $\pm$ 38	154 $\pm$ 0.5	235 $\pm$ 0.5	3.59 $\pm$ 0.6	537 $\pm$ 12	18.0 $\pm$ 0.8	9.42 $\pm$ 1	7.76 $\pm$ 0.5	21.9 $\pm$ 1	0.35 $\pm$ 0
Non-GI	Week 20	8.58 $\pm$ 0.1	852 $\pm$ 6	1005 $\pm$ 6	114 $\pm$ 1	228 $\pm$ 0.5	3.51 $\pm$ 2	527 $\pm$ 3	16.8 $\pm$ 0.1	10.1 $\pm$ 0.2	7.87 $\pm$ 0.1	21.9 $\pm$ 0.1	0.22 $\pm$ 0.1
Non-GI	Week 34	8.59 $\pm$ 0.1	740 $\pm$ 23	870 $\pm$ 27	48 $\pm$ 1	216 $\pm$ 1.5	0.68 $\pm$ 0.5	466 $\pm$ 15	15.2 $\pm$ 0.1	6.34 $\pm$ 0.5	4.82 $\pm$ 0.4	18.6 $\pm$ 0.5	BDL
Non-GI	Week 52	8.50 $\pm$ 0.1	759 $\pm$ 6	899 $\pm$ 2	47 $\pm$ 27	243 $\pm$ 12	0.48 $\pm$ 0.1	492 $\pm$ 15	20.4 $\pm$ 0	6.30 $\pm$ 0.8	5.03 $\pm$ 0.4	17.2 $\pm$ 0.9	0.65 $\pm$ 0
GI-Treated	Week 2	8.68 $\pm$ 0.1	841 $\pm$ 23	980 $\pm$ 30	193 $\pm$ 0	285 $\pm$ 5	BDL	602 $\pm$ 3	18.9 $\pm$ 0.2	12.7 $\pm$ 0.6	11.8 $\pm$ 0.1	23.9 $\pm$ 1	0.87 $\pm$ 0.6
GI-Treated	Week 4	8.73 $\pm$ 0.1	803 $\pm$ 103	934 $\pm$ 117	171 $\pm$ 25	255 $\pm$ 15	BDL	564 $\pm$ 62	18.1 $\pm$ 1	11.2 $\pm$ 2	6.78 $\pm$ 0.1	23.5 $\pm$ 2	0.30 $\pm$ 0.1
GI-Treated	Week 8	8.72 $\pm$ 0.1	829 $\pm$ 12	963 $\pm$ 17	156 $\pm$ 0.5	240 $\pm$ 3	0.85 $\pm$ 0.7	548 $\pm$ 8	19.5 $\pm$ 1	10.5 $\pm$ 0.1	8.65 $\pm$ 0.8	23.1 $\pm$ 0.5	1.89 $\pm$ 1
GI-Treated	Week 20	8.55 $\pm$ 0.1	863 $\pm$ 2	1015 $\pm$ 5	127 $\pm$ 2	229 $\pm$ 1	0.24 $\pm$ 0	525 $\pm$ 2	18.0 $\pm$ 0.9	10.8 $\pm$ 0.4	7.58 $\pm$ 0.2	22.7 $\pm$ 0.2	1.42 $\pm$ 0.8
GI-Treated	Week 34	8.68 $\pm$ 0.1	771 $\pm$ 11	899 $\pm$ 16	100 $\pm$ 6	228 $\pm$ 3.4	0.63 $\pm$ 0.3	509 $\pm$ 11	15.7 $\pm$ 1.2	7.62 $\pm$ 0.4	6.70 $\pm$ 0.8	15.0 $\pm$ 4.6	0.62 $\pm$ 0.5
GI-Treated	Week 52	8.70 $\pm$ 0.1	716 $\pm$ 67	834 $\pm$ 76	50.3 $\pm$ 28	226 $\pm$ 2.1	0.44 $\pm$ 0.1	474 $\pm$ 15	14.0 $\pm$ 0.6	5.71 $\pm$ 0.4	4.31 $\pm$ 0.3	17.2 $\pm$ 0.9	0.16 $\pm$ 0.1
P1A Anoxic FFT													
Non-GI	Week 2	8.38 $\pm$ 0.3	783 $\pm$ 36	927 $\pm$ 46	170 $\pm$ 10	180 $\pm$ 17	BDL	494 $\pm$ 21	16.8 $\pm$ 1	10.4 $\pm$ 1	18.5 $\pm$ 2	16.6 $\pm$ 1	0.19 $\pm$ 0.1
Non-GI	Week 4	8.60 $\pm$ 0.1	802 $\pm$ 10	941 $\pm$ 8	204 $\pm$ 12	205 $\pm$ 15	BDL	525 $\pm$ 11	18.9 $\pm$ 0.3	11.3 $\pm$ 0.7	19.4 $\pm$ 2	17.3 $\pm$ 1	1.13 $\pm$ 0.6
Non-GI	Week 20	8.39 $\pm$ 0.1	796 $\pm$ 2	950 $\pm$ 2	207 $\pm$ 1	233 $\pm$ 3	0.37 $\pm$ 0.1	575 $\pm$ 16	19.1 $\pm$ 0.2	11.8 $\pm$ 0.3	20.6 $\pm$ 0.4	18.7 $\pm$ 0.4	0.07 $\pm$ 0
Non-GI	Week 34	8.55 $\pm$ 0.1	657 $\pm$ 8	775 $\pm$ 9	30.4 $\pm$ 2	237 $\pm$ 2	1.05 $\pm$ 0.9	442 $\pm$ 0	13.3 $\pm$ 0.2	5.07 $\pm$ 0.1	6.33 $\pm$ 0.3	11.4 $\pm$ 0.2	2.39 $\pm$ 0.5
Non-GI	Week 52	8.50 $\pm$ 0.1	621 $\pm$ 15	735 $\pm$ 17	25 $\pm$ 2	234 $\pm$ 4	BDL	432 $\pm$ 4	13.9 $\pm$ 0.1	5.03 $\pm$ 0.1	5.16 $\pm$ 0.4	11.6 $\pm$ 0.2	3.86 $\pm$ 0.4
GI-Treated	Week 2	8.49 $\pm$ 0.1	828 $\pm$ 40	981 $\pm$ 49	150 $\pm$ 21	210 $\pm$ 24	BDL	513 $\pm$ 6	16.8 $\pm$ 0.8	10.1 $\pm$ 0.8	17.4 $\pm$ 2	16.9 $\pm$ 0.8	0.27 $\pm$ 0.1
GI-Treated	Week 4	8.56 $\pm$ 0.1	797 $\pm$ 42	939 $\pm$ 53	149 $\pm$ 22	218 $\pm$ 13	0.45 $\pm$ 0.2	520 $\pm$ 7	17.1 $\pm$ 0.6	10.0 $\pm$ 0.8	16.6 $\pm$ 2	15.8 $\pm$ 0.9	0.92 $\pm$ 0.2
GI-Treated	Week 8	8.37 $\pm$ 0.1	970 $\pm$ 22	1155 $\pm$ 25	180 $\pm$ 71	261 $\pm$ 2	0.74 $\pm$ 0.6	636 $\pm$ 16	20.9 $\pm$ 2	14.7 $\pm$ 2	25.9 $\pm$ 3	18.2 $\pm$ 0.8	0.14 $\pm$ 0
GI-Treated	Week 20	8.37 $\pm$ 0	757 $\pm$ 31	903 $\pm$ 37	206 $\pm$ 3	217 $\pm$ 18	0.82 $\pm$ 0.7	523 $\pm$ 27	18.1 $\pm$ 0.5	10.4 $\pm$ 0.5	18.3 $\pm$ 0.9	18.6 $\pm$ 0.8	0.11 $\pm$ 0.1
GI-Treated	Week 34	8.49 $\pm$ 0.1	632 $\pm$ 6	749 $\pm$ 7	96.5 $\pm$ 3	247 $\pm$ 4	0.65 $\pm$ 0.5	459 $\pm$ 11	13.6 $\pm$ 0.7	5.51 $\pm$ 0.3	6.17 $\pm$ 0.3	11.4 $\pm$ 0.3	2.47 $\pm$ 0.4
GI-Treated	Week 52	8.48 $\pm$ 0.1	600 $\pm$ 21	711 $\pm$ 24	79.5 $\pm$ 5	241 $\pm$ 5	BDL	451 $\pm$ 2	14.5 $\pm$ 1	5.52 $\pm$ 0.4	5.89 $\pm$ 0.5	12.1 $\pm$ 0.5	3.82 $\pm$ 2

Table 2.4 STP oxalic OSPW and FFT water chemistry (mean $\pm$ SE (n=2))

Treatment	Time	pH	Total Alkalinity mg/L CaCO ₃ eq.	HCO ₃ mg/L	SO ₄ mg/L	Cl mg/L	NO ₃ mg/L	Na mg/L	K mg/L	Mg mg/L	Ca mg/L	NH ₄ mg/L	Fe mg/L
STP Oxidic OSPW													
Non-GI	Week 2	8.87 $\pm$ 0.1	745 $\pm$ 9	849 $\pm$ 16	254 $\pm$ 4	650 $\pm$ 0	0.77 $\pm$ 0.5	888 $\pm$ 6	14.0 $\pm$ 0	6.70 $\pm$ 0.2	4.44 $\pm$ 0.4	4.52 $\pm$ 0.2	0.60 $\pm$ 0.2
Non-GI	Week 4	8.85 $\pm$ 0.1	729 $\pm$ 16	833 $\pm$ 22	249 $\pm$ 0.5	640 $\pm$ 10	0.51 $\pm$ 0.3	886 $\pm$ 9	13.0 $\pm$ 0.1	6.43 $\pm$ 0.2	4.09 $\pm$ 0.3	3.89 $\pm$ 1	0.16 $\pm$ 0.1
Non-GI	Week 8	8.83 $\pm$ 0.1	614 $\pm$ 106	705 $\pm$ 125	213 $\pm$ 39	580 $\pm$ 80	7.85 $\pm$ 2	776 $\pm$ 115	11.2 $\pm$ 1	5.71 $\pm$ 1	4.95 $\pm$ 0.9	BDL	0.24 $\pm$ 0.1
Non-GI	Week 20	8.90 $\pm$ 0.1	703 $\pm$ 0.5	797 $\pm$ 3	267 $\pm$ 0.5	693 $\pm$ 0.5	7.14 $\pm$ 0.1	851 $\pm$ 6	13.0 $\pm$ 0.2	6.04 $\pm$ 0.1	6.35 $\pm$ 0.4	BDL	0.14 $\pm$ 0
Non-GI	Week 34	8.89 $\pm$ 0.1	695 $\pm$ 11	788 $\pm$ 5	279 $\pm$ 8	720 $\pm$ 13	1.77 $\pm$ 1	896 $\pm$ 27	12.5 $\pm$ 0	6.17 $\pm$ 0.1	8.11 $\pm$ 0.4	BDL	0.10 $\pm$ 0.1
Non-GI	Week 52	8.92 $\pm$ 0.1	553 $\pm$ 103	623 $\pm$ 111	217 $\pm$ 40	579 $\pm$ 84	0.42 $\pm$ 0.2	766 $\pm$ 141	11.0 $\pm$ 2	4.52 $\pm$ 1	5.76 $\pm$ 2	BDL	BDL
GI-Treated	Week 2	8.81 $\pm$ 0	731 $\pm$ 8	841 $\pm$ 10	249 $\pm$ 3	635 $\pm$ 5	0.47 $\pm$ 0.3	834 $\pm$ 33	14.0 $\pm$ 1	7.26 $\pm$ 0.1	5.91 $\pm$ 0.2	4.90 $\pm$ 0.2	2.38 $\pm$ 0.1
GI-Treated	Week 4	8.82 $\pm$ 0.1	713 $\pm$ 22	818 $\pm$ 18	250 $\pm$ 3	615 $\pm$ 25	0.33 $\pm$ 0.2	865 $\pm$ 32	15.0 $\pm$ 0.7	7.34 $\pm$ 0.3	6.14 $\pm$ 0.8	2.41 $\pm$ 2	0.16 $\pm$ 0.1
GI-Treated	Week 8	8.80 $\pm$ 0.1	783 $\pm$ 16	901 $\pm$ 23	272 $\pm$ 0.5	685 $\pm$ 5	7.09 $\pm$ 7	958 $\pm$ 7	14.1 $\pm$ 0.4	7.79 $\pm$ 0.1	6.67 $\pm$ 0.3	BDL	0.24 $\pm$ 0.2
GI-Treated	Week 20	8.82 $\pm$ 0.1	704 $\pm$ 1	808 $\pm$ 3	265 $\pm$ 1	672 $\pm$ 2	7.75 $\pm$ 0.2	852 $\pm$ 2	13.0 $\pm$ 0.1	6.46 $\pm$ 0.1	7.70 $\pm$ 0.1	BDL	0.14 $\pm$ 0.2
GI-Treated	Week 34	8.83 $\pm$ 0.1	678 $\pm$ 2	777 $\pm$ 2	268 $\pm$ 1	665 $\pm$ 10	4.50 $\pm$ 1	871 $\pm$ 5	13.2 $\pm$ 0.1	6.28 $\pm$ 0.1	9.22 $\pm$ 0.1	BDL	0.10 $\pm$ 0.2
GI-Treated	Week 52	8.83 $\pm$ 0.1	436 $\pm$ 2	500 $\pm$ 5	178 $\pm$ 4	419 $\pm$ 17	BDL	568 $\pm$ 9	8.37 $\pm$ 0.1	3.76 $\pm$ 0.1	5.65 $\pm$ 0.4	BDL	0.23 $\pm$ 0.1
STP Oxidic FFT													
Non-GI	Week 2	8.63 $\pm$ 0.1	764 $\pm$ 10	895 $\pm$ 10	275 $\pm$ 16	675 $\pm$ 10	BDL	880 $\pm$ 16	14.4 $\pm$ 0.8	6.93 $\pm$ 0.1	8.83 $\pm$ 0.4	4.44 $\pm$ 0.4	1.10 $\pm$ 0.3
Non-GI	Week 4	8.61 $\pm$ 0.1	751 $\pm$ 5	881 $\pm$ 10	295 $\pm$ 17	685 $\pm$ 20	BDL	868 $\pm$ 16	16.2 $\pm$ 0.5	7.57 $\pm$ 0.2	9.51 $\pm$ 0.3	4.11 $\pm$ 0.5	1.74 $\pm$ 0.2
Non-GI	Week 8	8.71 $\pm$ 0.1	714 $\pm$ 5	825 $\pm$ 18	309 $\pm$ 23	708 $\pm$ 19	BDL	871 $\pm$ 26	15.8 $\pm$ 0.9	7.12 $\pm$ 0.4	8.69 $\pm$ 0.2	2.42 $\pm$ 1	1.85 $\pm$ 0.3
Non-GI	Week 20	8.45 $\pm$ 0	692	822	394	726	BDL	952	20	9.4	11.1	0.26	5.42 $\pm$ 0
Non-GI	Week 34	8.42 $\pm$ 0	718 $\pm$ 22	854 $\pm$ 26	412 $\pm$ 5	751 $\pm$ 16	0.76 $\pm$ 0.6	991 $\pm$ 30	18.6 $\pm$ 0.3	9.15 $\pm$ 0.1	11.2 $\pm$ 0.2	BDL	3.73 $\pm$ 0.1
Non-GI	Week 52	8.57 $\pm$ 0.1	638 $\pm$ 11	752 $\pm$ 15	338 $\pm$ 24	820 $\pm$ 69	0.21 $\pm$ 0.1	990 $\pm$ 60	13.6 $\pm$ 0.5	5.85 $\pm$ 0.3	6.03 $\pm$ 0.1	BDL	0.72 $\pm$ 0.3
GI-Treated	Week 2	8.59 $\pm$ 0.1	763 $\pm$ 9	897 $\pm$ 14	259 $\pm$ 4	643 $\pm$ 21	BDL	867 $\pm$ 10	15.0 $\pm$ 0.5	7.15 $\pm$ 0.2	9.20 $\pm$ 0.3	4.70 $\pm$ 0.3	1.59 $\pm$ 0.3
GI-Treated	Week 4	8.60 $\pm$ 0.1	746 $\pm$ 19	876 $\pm$ 27	297 $\pm$ 27	675 $\pm$ 12	BDL	850 $\pm$ 6	15.1 $\pm$ 6	7.32 $\pm$ 0.1	9.21 $\pm$ 0.4	3.67 $\pm$ 0.7	1.55 $\pm$ 0.3
GI-Treated	Week 8	8.62 $\pm$ 0.1	718 $\pm$ 4	842 $\pm$ 4	290 $\pm$ 12	683 $\pm$ 10	BDL	860 $\pm$ 7	15.6 $\pm$ 0.3	7.26 $\pm$ 0.2	8.87 $\pm$ 0.4	3.42 $\pm$ 1	2.40 $\pm$ 0.3
GI-Treated	Week 20	8.44 $\pm$ 0.1	695 $\pm$ 4	827 $\pm$ 8	360 $\pm$ 3	677 $\pm$ 2	BDL	917 $\pm$ 8	22.0 $\pm$ 5	10.3 $\pm$ 2	11.3 $\pm$ 0.5	0.41 $\pm$ 1	3.53 $\pm$ 0
GI-Treated	Week 34	8.44 $\pm$ 0.1	687 $\pm$ 15	816 $\pm$ 17	365 $\pm$ 6	690 $\pm$ 14	1.40 $\pm$ 0.1	937 $\pm$ 9	18.8 $\pm$ 0.1	8.95 $\pm$ 0.2	10.8 $\pm$ 0.2	BDL	5.95 $\pm$ 0.1
GI-Treated	Week 52	8.57 $\pm$ 0.1	646 $\pm$ 6	761 $\pm$ 5	346 $\pm$ 7	835 $\pm$ 40	0.52 $\pm$ 0.4	990 $\pm$ 20	15.0 $\pm$ 0.3	6.48 $\pm$ 0.2	7.23 $\pm$ 0.3	BDL	0.85 $\pm$ 0.3

Table 2.5 STP anoxic OSPW and FFT water chemistry (mean $\pm$ SE (n=2))

Treatment	Time	pH	Total Alkalinity mg/L CaCO ₃ eq.	HCO ₃ mg/L	SO ₄ mg/L	Cl mg/L	NO ₃ mg/L	Na mg/L	K mg/L	Mg mg/L	Ca mg/L	NH ₄ mg/L	Fe mg/L
STP Anoxic OSPW													
Non-GI	Week 2	8.73 $\pm$ 0.1	736 $\pm$ 2	854 $\pm$ 3	249 $\pm$ 1	640 $\pm$ 0	0.74 $\pm$ 0.1	890 $\pm$ 11	14.2 $\pm$ 0.4	7.19 $\pm$ 0.1	5.82 $\pm$ 0.2	4.74 $\pm$ 0.2	0.40 $\pm$ 0.2
Non-GI	Week 4	8.75 $\pm$ 0.1	627 $\pm$ 120	726 $\pm$ 136	212 $\pm$ 37	530 $\pm$ 110	0.27 $\pm$ 0.1	744 $\pm$ 147	11.0 $\pm$ 2	5.74 $\pm$ 1	4.84 $\pm$ 0.6	4.36 $\pm$ 0.7	0.16 $\pm$ 0.1
Non-GI	Week 8	8.70 $\pm$ 0.1	870 $\pm$ 379	870 $\pm$ 431	249 $\pm$ 134	650 $\pm$ 270	0.22 $\pm$ 0.1	858 $\pm$ 383	12.6 $\pm$ 5	6.75 $\pm$ 4	5.39 $\pm$ 0.9	4.72 $\pm$ 2	0.11 $\pm$ 0.1
Non-GI	Week 20	8.71 $\pm$ 0.1	868 $\pm$ 2	868 $\pm$ 7	243 $\pm$ 0.5	661 $\pm$ 3	1.05 $\pm$ 0.7	897 $\pm$ 7	12.6 $\pm$ 0.4	6.83 $\pm$ 0.1	7.59 $\pm$ 0.9	4.35 $\pm$ 0.1	BDL
Non-GI	Week 34	8.67 $\pm$ 0.1	842 $\pm$ 2	842 $\pm$ 6	237 $\pm$ 0.5	669 $\pm$ 5	0.94 $\pm$ 0.4	913 $\pm$ 25	11.9 $\pm$ 0.1	6.44 $\pm$ 0.1	9.13 $\pm$ 0.7	2.90 $\pm$ 0.1	0.06 $\pm$ 0
Non-GI	Week 52	8.56 $\pm$ 0.1	551 $\pm$ 13	551 $\pm$ 20	153 $\pm$ 6	496 $\pm$ 24	0.43 $\pm$ 0.3	611 $\pm$ 18	8.87 $\pm$ 0.4	3.47 $\pm$ 0.1	4.55 $\pm$ 0.1	2.33 $\pm$ 0	BDL
GI-Treated	Week 2	8.78 $\pm$ 0	733 $\pm$ 2	846 $\pm$ 2	245 $\pm$ 7	620 $\pm$ 20	BDL	899 $\pm$ 6	14.8 $\pm$ 1	7.78 $\pm$ 0.3	5.29 $\pm$ 0.4	4.68 $\pm$ 0.2	2.76 $\pm$ 0.7
GI-Treated	Week 4	8.77 $\pm$ 0.1	751 $\pm$ 0.5	868 $\pm$ 0.5	253 $\pm$ 2	640 $\pm$ 0	0.20 $\pm$ 0.1	908 $\pm$ 6	13.4 $\pm$ 0.7	7.47 $\pm$ 0.2	7.39 $\pm$ 0.1	4.99 $\pm$ 0.1	1.02 $\pm$ 0.5
GI-Treated	Week 8	8.72 $\pm$ 0.1	585 $\pm$ 160	679 $\pm$ 184	194 $\pm$ 56	525 $\pm$ 115	BDL	879	10.2 $\pm$ 2	5.29 $\pm$ 2	5.70 $\pm$ 1	4.41 $\pm$ 0.6	0.96 $\pm$ 0.5
GI-Treated	Week 20	8.70 $\pm$ 0.1	729 $\pm$ 6	849 $\pm$ 11	254 $\pm$ 4	672 $\pm$ 1	0.27 $\pm$ 0.1	948 $\pm$ 3	14.2 $\pm$ 0.1	7.34 $\pm$ 0.2	9.35 $\pm$ 0.3	4.75 $\pm$ 0	1.51 $\pm$ 0.4
GI-Treated	Week 34	8.70 $\pm$ 0.1	697.5 $\pm$ 14	813 $\pm$ 18	245 $\pm$ 2	676 $\pm$ 6	0.28 $\pm$ 0.1	878 $\pm$ 21	13.4 $\pm$ 0.4	6.77 $\pm$ 0.4	9.31 $\pm$ 0.4	4.43 $\pm$ 0	1.61 $\pm$ 0.6
GI-Treated	Week 52	8.68 $\pm$ 0.1	462 $\pm$ 12	539 $\pm$ 15	178 $\pm$ 8	453 $\pm$ 10	BDL	598 $\pm$ 18	8.02 $\pm$ 0.3	3.81 $\pm$ 0.1	5.27 $\pm$ 0.4	BDL	1.16 $\pm$ 0
STP Anoxic FFT													
Non-GI	Week 2	8.64 $\pm$ 0.1	741 $\pm$ 11	867 $\pm$ 14	258 $\pm$ 3	663 $\pm$ 3	BDL	856 $\pm$ 15	14.5 $\pm$ 0.5	6.65 $\pm$ 0.1	8.62 $\pm$ 0.4	5.20 $\pm$ 0.2	1.45 $\pm$ 0.2
Non-GI	Week 4	8.60 $\pm$ 0.1	722 $\pm$ 26	849 $\pm$ 32	286 $\pm$ 10	665 $\pm$ 5	BDL	875 $\pm$ 5	16.0 $\pm$ 0.4	7.15 $\pm$ 0.2	8.37 $\pm$ 1	4.80 $\pm$ 0.3	2.52 $\pm$ 0.7
Non-GI	Week 8	8.68 $\pm$ 0.1	703 $\pm$ 9	819 $\pm$ 12	280 $\pm$ 7	675 $\pm$ 6	BDL	865 $\pm$ 10	15.7 $\pm$ 1	7.02 $\pm$ 0.3	8.38 $\pm$ 0.9	4.25 $\pm$ 0.3	2.64 $\pm$ 0.7
Non-GI	Week 34	8.56 $\pm$ 0	708 $\pm$ 1	834 $\pm$ 1	320 $\pm$ 0.5	673 $\pm$ 8	0.73 $\pm$ 0.1	898 $\pm$ 3	17.1 $\pm$ 0.4	7.92 $\pm$ 0.3	10.1 $\pm$ 0.5	4.55 $\pm$ 0.1	2.92 $\pm$ 0.4
Non-GI	Week 52	8.49 $\pm$ 0.1	606 $\pm$ 18	718 $\pm$ 25	232 $\pm$ 12	687 $\pm$ 8	0.72 $\pm$ 0.6	822 $\pm$ 22	11.7 $\pm$ 0.4	4.46 $\pm$ 0.2	4.83 $\pm$ 0.2	2.85 $\pm$ 0.9	0.68 $\pm$ 0.1
GI-Treated	Week 2	8.59 $\pm$ 0.1	755 $\pm$ 12	888 $\pm$ 16	269 $\pm$ 11	655 $\pm$ 5	BDL	886 $\pm$ 6	16.1 $\pm$ 0.4	7.51 $\pm$ 0.2	9.50 $\pm$ 0.5	5.01 $\pm$ 0.2	2.41 $\pm$ 0.6
GI-Treated	Week 4	8.62 $\pm$ 0.1	713 $\pm$ 24	848 $\pm$ 28	285 $\pm$ 6	655 $\pm$ 5	BDL	846 $\pm$ 27	15.5 $\pm$ 0.5	7.10 $\pm$ 0.3	8.24 $\pm$ 1	4.68 $\pm$ 0.4	2.70 $\pm$ 0.4
GI-Treated	Week 8	8.72 $\pm$ 0.1	710 $\pm$ 7	819 $\pm$ 14	290 $\pm$ 5	670 $\pm$ 7	BDL	829 $\pm$ 3	15.8 $\pm$ 0.4	7.54 $\pm$ 0.2	8.19 $\pm$ 0.6	4.25 $\pm$ 0.2	3.83 $\pm$ 0.6
GI-Treated	Week 20	8.52 $\pm$ 0.1	715 $\pm$ 5	845 $\pm$ 5	318 $\pm$ 13	679 $\pm$ 4	BDL	867 $\pm$ 4	17.4 $\pm$ 0.7	8.31 $\pm$ 0.1	10.4 $\pm$ 0.2	3.50 $\pm$ 1	4.35 $\pm$ 1
GI-Treated	Week 34	8.53 $\pm$ 0.1	689 $\pm$ 1	814 $\pm$ 1	343 $\pm$ 14	676 $\pm$ 10	0.22 $\pm$ 0.1	969 $\pm$ 17	24.6 $\pm$ 4	9.10 $\pm$ 0.4	10.9 $\pm$ 0.6	4.14 $\pm$ 1	6.02 $\pm$ 0.2
GI-Treated	Week 52	8.61 $\pm$ 0.1	593 $\pm$ 8	696 $\pm$ 10	260 $\pm$ 1	682 $\pm$ 2	BDL	830 $\pm$ 8	12.4 $\pm$ 0	4.69 $\pm$ 0.1	5.04 $\pm$ 0.2	0.59 $\pm$ 0.1	1.42 $\pm$ 0.3

Dissolved oxygen flux within oxic FFT as a function of GI treatment

No DO was detected in either the OSPW or FFT of any of the anoxic microcosms regardless of material type or treatment condition. Under oxic conditions, the DO concentration in the surface water ranged from 5-7 mg L⁻¹ over the 52-week period, and dropped to below limits of detection in sediments within the first few mm below the OSPW-FFT interface. During weeks 4 to 52 DO_{flux} (O₂ depletion rate) increased across the OSPW-FFT interface in oxic GI-treated P1A_{GI} microcosms (Figure 2.15) compared to the oxic P1A_{non-GI} treatments indicating stimulation in aerobic respiration. The DO_{flux} reached a maximum at week 8 in P1A_{GI} (14.3 mmol m⁻² d⁻¹) and then declined over time at weeks 20 (8.96 mmol m⁻² d⁻¹), 36 (8.26 mmol m⁻² d⁻¹) and 52 (5.04 mmol m⁻² d⁻¹), respectively (Figure 2.15). In the P1A_{non-GI} microcosms, the maximum DO_{flux} was reached at the end of week 8 (12.2 mmol m⁻² d⁻¹) and then decreased at weeks 20 (5.10 mmol m⁻² d⁻¹), 36 (5.62 mmol m⁻² d⁻¹) and 52 (3.29 mmol m⁻² d⁻¹). Based on these observations, the GI treatment resulted in net aerobic respiration stimulus of 17%, 76% and 47% across weeks 8, 20 and 36, respectively in P1A_{GI} compared to P1A_{non-GI} for the aged system. In contrast, the DO_{flux} in the STP_{GI} reached a maximum at week 8 (11 mmol m⁻² d⁻¹), which is higher than the values for STP_{non-GI} (7.73 mmol m⁻² d⁻¹) for the same time period. Overall, a 42% increase in DO flux was observed for the GI-treated STP materials during the early weeks of the experiment. From weeks 20 to 52 no difference in DO_{flux} was observed between STP_{GI} and STP_{non-GI} treatments. After week 20, the system appeared to reach steady state with respect to oxygen

diffusion across the interface at $\sim 4 \text{ mmol m}^{-2} \text{ d}^{-1}$. The greater DO_{flux} observed in the GI-treated materials could reflect the presence of greater amounts of bioavailable intermediate organic products, enabling increased microbial productivity, thus directly stimulating aerobic microbial respiration. Or the increase in DO_{flux} could be due to an increase in chemical oxidation of reduced species diffusing towards the sediment surface, reacting chemically with DO. However, no HS^- was detected in any of the oxic microcosms so the observed difference is more likely to be associated with increased aerobic respiration. In some lake environments, more than 80% of hypolimnetic oxygen depletion is attributed to the degradation of organic matter in the water column and the upward diffusion of reduced substances originating from the anaerobic decomposition of organic matter in the most recently deposited layer of sediment (Matzinger et al., 2010). Short-term stimulation of bacteria that fosters increased aerobic respiration is desirable from a bioremediation perspective because it leads to faster biodegradation of persistent organic toxins and the intermediates produced through GI-treatment, compared to anaerobic respiration processes (Clement and Fedorak, 2005). The increase in DO flux that we observed following GI treatment is consistent with the hypothesis put forward by Martin et al., (2010), who observed increased biodegradability of NAs following ozonation. These authors implied that the accelerated rate of biodegradation might be linked to increased biological oxygen demand caused by more rapid microbial consumption of labile intermediate products, allowing the microbial population to grow more rapidly.

Figure 2.15 Time series of DO_{flux} (A) denotes oxic P1A DO_{flux} (B) oxic STP DO_{flux} . No oxygen was detected within the OSPW or FFT of the anoxic microcosms.

Long-term DO_{flux} stimulation (increased SOD) can lead to short-term hypoxia in wetlands and shallow water bodies, and hypolimnetic anoxia in stratified reclaimed freshwater landscapes. In coastal estuaries, anoxia can cause direct mortality to benthic macro-invertebrates or indirect mortality through the release of toxic by-products of anaerobic mineralization such as hydrogen sulfide (Wang and Chapman, 1999). In the oxic P1A microcosms, we also observed the greatest rate of ammonia oxidation, alkalinity consumption and iron oxidation (based on visual evidence) during weeks 8-34, when DO_{flux} was greatest. Greater DO_{flux} in GI-treated materials did not appear to significantly influence the chemistry of bulk OSPW or FFT pore water between those treatments in either fresh or aged materials.

However, the greater DO_{flux} observed in GI-treated materials at specific time points suggests there is accelerated aerobic respiration of organic matter within the aerobic surface water/anoxic sediment interface, combined with increased oxidation of reduced substances diffusing upwards from anaerobic mineralization occurring below the sediment water interface. Regardless of treatment or source material, peak DO_{flux} was reached at week 8 after which it declined progressively until week 52. The magnitude of DO_{flux} is determined by both the DO concentration and supply rate of labile C to the sediment source (Archer and Devol, 1992). The greatest DO_{flux} is expected to occur at the time that the most bioavailable NA residuals become degraded. The DO_{flux} was enhanced in GI-treated materials at particular time-points, but presumably as the most biodegradable NA residuals become oxidized and only the persistent fractions remain, we then observe a drop in DO_{flux} to below $5 \text{ mmol m}^{-2} \text{ d}^{-1}$ regardless of treatment and source material as the system reaches equilibrium with respect to oxygen diffusion at week 52. Therefore, it appears that GI stimulates the short-term kinetics of the system, speeding up the degradation rate of NAs and reducing the concentration of residual NA after GI. However, GI did not stimulate a persistent long-term (52 week) increase in DO_{flux} , which would be beneficial for the sustainability of reclaimed ecosystems.

The peak DO_{flux} values recorded at week 8 (Figure 2.15) are comparable to DO_{flux} values reported in natural systems. Elberling et al., (2011) reported a DO_{flux} of $18 \text{ mmol m}^{-2} \text{ d}^{-1}$ in a northern Danish fen. Fen peatlands cover up to 65% of the surface of natural landscapes in the oil sands region prior to

extraction and will be a fundamental option in the reclamation of post-mined landscapes (Price et al., 2010). Similar to oil sands FFT, marine sediments represent an environment with high organic carbon inputs reflecting the carbonate buffering of the system, circumneutral pH and high salinity (Sanz Lazaro et al., 2011), providing a novel comparison for the DO_{flux} values. Marine environments are subject to elevated sulfate levels in the overlying water and the diffusion of sulfate into anoxic sediments, therefore SRB play a major role in the degradation of organic matter and are a key driver to the overall biogeochemical cycling within those systems. In both oil sands and marine environments, HS^- accumulation is buffered by Fe^{2+} -driven precipitation, which can sequester up to 63% of HS^- in the system (Chen et al., 2013; Sanz Lazaro et al., 2011). In coastal marine sediments from the Golfe de Fos station of the Mediterranean Sea, the DO_{flux} ranged from 12.7 to 16.5 $mmol\ m^{-2}\ d^{-1}$ (Rabouille et al., 2003), while in the Arhus Bay of Denmark DO_{flux} ranged from 9.7 to 46 $mmol\ m^{-2}\ d^{-1}$ (Nielson et al., 2010). In subaqueous sulfide tailings Elberling and Damgaard, 2001 observed a DO flux of 35 $mmol\ m^{-2}\ d^{-1}$. Comparing DO fluxes to natural environments will help modelers to predict what factors influence DO flux over time so that a sustainable rate can be attained that avoids anoxia in the surface waters of reclaimed aquatic landscapes. Towards the end of the study the DO_{flux} in fresh and aged tailings ($\sim 4\text{-}5\ mmol\ m^{-2}\ d^{-1}$) was considerably lower than natural systems mentioned above, which is beneficial for reducing risk of anoxia. However, the DO_{flux} in FFT is expected to increase as the biological productivity increases in reclaimed aquatic landscapes, as this will

provide more bioavailable organic matter for microbial respiration processes (Matzinger et al., 2010).

Hydrogen sulfide flux within anoxic FFT as a function of GI treatment

Sulfate reduction is a dominant terminal electron accepting process in many organic carbon-rich environments, accounting for more than half of organic C mineralization (Sturman et al., 2008). Sulfate reduction appears to be a defining process in these FFT materials. Determination of the $\text{HS}^-_{\text{flux}}$ at a high spatiotemporal scale provides a proxy for the rate of organic matter mineralization in the sediment (Jørgensen et al., 2001). The retention of HS^- is regulated by bacterial or chemical oxidation, precipitation as iron sulfides and the formation of organic sulfides (Brüchert et al., 2003). In tailings ponds, the concentration of acid soluble metal sulfide precipitates can be up to 10-fold higher than any dissolved sulfide (Ramos-Padrón et al., 2010). Although there was evidence of localized zones of SRB activity within oxic P1A microcosms, no HS^- was detected in any of the oxic STP or P1A microcosms regardless of GI treatment over the course of the study. This behavior contrasts with the study of Chen et al., (2013), in which the authors observed HS^- concentrations up to $150 \mu\text{mol L}^{-1}$ in microcosms kept under atmospheric conditions using FFT obtained from Syncrude Canada Ltd's active West-In Pit tailings pond.

In anoxic microcosms, no HS^- was detected within the overlying OSPW, suggesting that the HS^- was oxidized by bacterial or chemical reaction into

oxidized S intermediates prior to reaching the OSPW-FFT interface. The HS⁻ flux was higher in P1A_{non-GI} over weeks 2 (37.7 $\mu\text{mol m}^{-2} \text{d}^{-1}$), 4 (11.7 $\mu\text{mol m}^{-2} \text{d}^{-1}$), 16 (7.38 $\mu\text{mol m}^{-2} \text{d}^{-1}$) and 20 (10.9 $\mu\text{mol m}^{-2} \text{d}^{-1}$) than in P1A_{GI} microcosms at weeks 2 (24.4 $\mu\text{mol m}^{-2} \text{d}^{-1}$), 4 (9.2 $\mu\text{mol m}^{-2} \text{d}^{-1}$), 16 (5.02 $\mu\text{mol m}^{-2} \text{d}^{-1}$) and 20 (9.78 $\mu\text{mol m}^{-2} \text{d}^{-1}$) (Figure 2.16). Therefore, GI-treatment did not stimulate HS⁻_{flux} in P1A over the first 20 weeks, resulting in an average HS⁻_{flux} increase of 24% over the first 20 weeks in the P1A_{non-GI} treatment compared to the P1A_{GI}. The greater flux observed in P1A_{non-GI} at week 2 over P1A_{GI} may in part be due to the reduced biomass of the inoculate (e.g. 10%) at the start of the experiment. Also methane was observed during weeks 2, 4, and 8 in the headspace of P1A_{GI} (Figure 2.14). Therefore the increased HS⁻_{flux} in the P1A_{non-GI} FFT may be due to an increase in AOM processes, coupling the oxidation of methane to sulfate reduction as discussed in chapter 2. Alternatively, the impact of GI on the organic carbon source may have stimulated methanogenic conditions through the breakdown of NA isomers. GI did not alter the long-term HS⁻_{flux} as similar values were observed in P1A_{GI} (7.17 $\mu\text{mol m}^{-2} \text{d}^{-1}$) and P1A_{non-GI} (7.12 $\mu\text{mol m}^{-2} \text{d}^{-1}$) at week 52. At week 52 there is no CH₄ detected in the headspace of both P1A microcosms, therefore the AOM processes are not occurring because all of the residual CH₄ has been consumed. Suggesting that the sulfate reduction rate contributing to the HS⁻_{flux} may be limited by the availability of sulfate at this time point in both P1A treatments as the observed sulfate concentration has dropped from a maximum of ~200 ppm down to 80 ppm and 25 ppm in the FFT of the P1A_{GI} and P1A_{non-GI} microcosms respectively (Table 2.3).

The STP $\text{HS}^-_{\text{flux}}$ remained $<3 \mu\text{mol m}^{-2} \text{d}^{-1}$ over the first 8 weeks in both STP_{GI} and STP_{non-GI} (Figure 2.15). By week 12, a 50% increase in $\text{HS}^-_{\text{flux}}$ was observed in STP_{GI} ($10.3 \mu\text{mol m}^{-2} \text{d}^{-1}$) compared to STP_{non-GI} ($6.87 \mu\text{mol m}^{-2} \text{d}^{-1}$) and at week 36 a 34% increase was observed in STP_{GI} ($8.37 \mu\text{mol m}^{-2} \text{d}^{-1}$) compared to STP_{non-GI} ($6.27 \mu\text{mol m}^{-2} \text{d}^{-1}$). GI did not stimulate long-term $\text{HS}^-_{\text{flux}}$ as the flux decreased to $3.17 \mu\text{mol m}^{-2} \text{d}^{-1}$ in STP_{GI} and to $2.61 \mu\text{mol m}^{-2} \text{d}^{-1}$ in STP_{non-GI} at week 52. Long-term $\text{HS}^-_{\text{flux}}$ stimulation is not desirable as sulfide oxidation can lead to rapid depletion of dissolved oxygen in the overlying water. In organic-rich marine sediments HS^- oxidation can account for up to 90% of total oxygen uptake (Ferdeman et al., 1999). At 52 weeks the $\text{HS}^-_{\text{flux}}$ was similar between GI and non-GI treatments for each source material, with values being higher in P1A ($\sim 7 \mu\text{mol m}^{-2} \text{d}^{-1}$) than STP ($\sim 3 \mu\text{mol m}^{-2} \text{d}^{-1}$). At this time there was less sulfate available for reduction and the most residual labile NA would have already been oxidized.

Regardless of treatment, $\text{HS}^-_{\text{flux}}$ was much greater in P1A than in STP microcosms over the initial weeks of the experiment, even though the initial sulfate concentrations were higher in STP treatments. This also coincided with the observed iron monosulfide formation on the outer surface of the microcosms, which developed more quickly in P1A than in STP microcosms. The delayed onset of HS^- in STP sediments is likely due to differences in the microbial community structure. The physical and chemical observations from chapter 2 suggest P1A FFT has a more diverse microbial community, possibly as a result of its greater successional stage

compared to STP. The magnitude of DO and HS⁻ flux was greater in P1A, and microbial activity developed more quickly as was evident by the greater HS⁻ flux and the development of iron monosulfide and iron hydroxide deposits in the anoxic and oxic environments, respectively.

Figure 2.16 Time series of HS⁻ flux within anoxic FFT (A) denotes P1A (B) denotes STP microcosms. No HS⁻ was detected within oxic microcosms.

Chapter 2: References

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CHAPTER 3: EFFECTS OF GAMMA IRRADIATION TREATMENT ON THE BREAKDOWN OF NAPHTHENIC ACIDS AND TOXICITY IN OIL SANDS PROCESS WATER AND FLUID FINE TAILINGS

Introduction

In recent years a great deal of research has been conducted on a group of compounds known as naphthenic acids (NAs) which are found in the process waters of oil sands from Alberta, Canada and biodegraded petroleum sources around the world. Although over 50% of the extracts being considered as NAs in recent literature do not fall under the classical definition of NAs according to the International Union of Pure and Applied Chemistry (Grewer et al., 2010) it is this group of oil sands acid-extractable organics that is of major concern due to their toxicity, persistence, corrosion to processing equipment and elevated concentrations in OSPW. The goal of this chapter is to determine the efficacy of GI-treatment followed by biodegradation on the breakdown of NAs in fresh and aged OSPW and FFT; and to determine if GI-treatment results in decreased toxicity immediately after treatment and following periods of biodegradation.

Methods

Collection and Preservation of OSPW and FFT Samples

OSPW and FFT samples were collected from the small microcosms used for destructive sampling as described in chapter 2. The OSPW surface water from each microcosm was collected with a syringe and frozen in Nalgene containers. The

microcosm with remaining FFT was placed directly in the freezer. Prior to shipping the FFT was allowed to thaw slightly so that the frozen FFT could be removed and was transferred into wide-mouth Nalgene containers for shipping. Samples were shipped to Syncrude Canada Ltd. – Research and Development Centre (Edmonton, AB) for NA determination. Pore water was extracted by centrifugation from the FFT prior to analysis.

NA Determination by Fourier Transform Infrared Spectroscopy

The industry standard for quantifying NA concentrations by Fourier Transformed Infrared Spectroscopy (FTIR) was developed by Syncrude Canada Ltd. (Clemente and Fedorak, 2005). Using this method, the acids are extracted from acidified water sample into methylene chloride, the absorbance of monomeric and dimeric forms of carboxylic acids are measured using FTIR spectroscopy at wave-lengths of 1743 and 1706 cm^{-1} (Yen et al., 2004). The sum of absorbance is compared to commercially available NA preparations for quantitative analyses. To correct for the 10% untreated OSPW and FFT inoculum added to GI-treated materials to stimulate microbial growth, a factor of 10% of the NA concentration in non-GI treatment was subtracted from the NA concentration within its respective GI treated alternative for that material source and incubation period to calculate the total percent reduction as a result of GI. Some instrumentation errors were encountered with the initial OSPW results. Therefore, another subset of OSPW samples that had been set aside and

aged for 0, 56 and 180 days was used for the determination of NA concentrations.

MicroTox 7® (Vibrio fischeri) Bioassay

The MicroTox 7® toxicity assay is conducted using the luminescent bacterium test organism *Vibrio fischeri*. Because this method is relatively inexpensive, rapid and gives reproducible results compared to trout and *Daphnia magna* assays, this assay has commonly been used to monitor the toxicity of OSPW in environmental samples and NA solutions (Clemente and Fedorak, 2005). MicroTox analysis was performed by HydroQual Laboratories Ltd. Calgary, AB. using standard protocols for 15 minute MicroTox on 1-mL aliquots (MicroTox Manual, 1991). Materials had been allowed to age for 2, 4, 8 and 52 weeks following GI treatment, and were then stored at -20°C. Each 30 mL subsample was thawed and filtered through a 0.22-µm filter prior to shipping. The toxicity is expressed as the inhibitory concentration resulting in x percent decrease in luminescence (respiration) of *V. fischeri* 15 minutes after exposure, expressed as IC_x. For example, a 50 percent decrease during 15-minute exposure is referred to as the 15-minute IC₅₀.

Results and Discussion

Efficacy of GI-treatment on NA Reduction in Aged and Fresh OSPW and FFT

GI treatment resulted in an immediate reduction of NA concentrations in the OSPW of P1A from an initial value of 60 mg L⁻¹ in P1A_{non-GI} to 9 mg L⁻¹ in the P1A_{GI}

treatment at day 0 - a reduction of 85% (Table 3.1). The concentration of NAs within the P1A_{non-GI} treatment declined to 48 mg L⁻¹ following 180 days of incubation. Interestingly, 180 days incubation did not appear to further reduce the concentration of NAs within the P1A_{GI} OSPW, this may be due to the microbes degrading the most bioavailable carbon source. In the case of P1A_{GI}, this is assumed to be the short-chain organics produced by irradiating NAs. Alternatively, the NA isomers remaining are persistent and not able to undergo biodegradation in the conditions that are present in the microcosm and with the microbial community that was active during that time interval. In the extracted FFT pore water from P1A, GI resulted in an initial NA reduction of 52% in the P1A_{GI} (36 mg L⁻¹) compared to P1A_{non-GI} (75.3 mg L⁻¹) (Table 3.1). The OSPW from STP_{GI} resulted in an initial reduction of 97% NAs in the STP_{GI} (2 mg L⁻¹) compared STP_{non-GI} (52 mg L⁻¹). In contrast to P1A_{non-GI}, incubation did not further reduce the NA concentrations in either STP_{GI} or STP_{non-GI} OSPW. Since the STP microbial community is presumably not as developed as the aged P1A microbial community, it appears to be less effective in biodegrading NAs. Within the FFT, there was an 80% reduction in STP_{GI} (8 mg L⁻¹) compared to STP_{non-GI} (39.5 mg L⁻¹).

Table 3.1 *Naphthenic acid concentrations within OSPW and FFT of P1A and STP*

Incubation Time (days)	NA concentration (mg L ⁻¹)		Percent Reduction
	P1A_{non-GI} OSPW	P1A_{GI} OSPW	
0	60	9	85%
56	50	9	82%
180	48	10	79%
	P1A_{non-GI} FFT	P1A_{GI} FFT	
0	75 ± 0.71	36 ± 8.87	52%
56	74 ± 0.71	33 ± 3.46	56%
180	70 ± 9.19	38 ± 1.41	45%
	STP_{non-GI} OSPW	STP_{GI} OSPW	
0	52	2	97%
56	48	2	95%
180	52	4	93%
	STP_{non-GI} FFT	STP_{GI} FFT	
0	40 ± 4.95	8 ± 3.40	80%
56	44 ± 5.64	6 ± 2.22	85%
180	46 ± 1.41	10 ± 6.36	74%

P1A has not received fresh FFT for >35 years, leaving only the persistent NA fractions in the FFT following the long-term biodegradation of NA congeners (Quagraine et al., 2005). This is presumably why we observe less reduction of NA concentrations within the P1A_{GI} (45-56%) FFT compared to STP_{GI} (74-85%), which contains the full suite of NA congeners because as an active tailings pond it continuously receives freshly produced OSPW and FFT. Alternatively, the reduced efficacy in degrading NAs in P1A may be related to the physical properties of the FFT. The FFT from P1A has undergone settling and densification for over 40 years and therefore consists of increased % solids (36.28) than the FFT from STP (32.37). The OSPW in P1A is still recycled with recently produced OSPW and is therefore also

expected to contain the full suite of NA congeners. However, we still observed a higher reduction of NAs within the OSPW of STP (93-97%) compared to P1A (79-85%) (Table 3.1). GI treatment was slightly less effective at reducing NA concentrations in FFT compared to OSPW for both material sources, possibly due to decreased penetration from the solids within the FFT. Gamma rays do easily penetrate water and liquid sludge. However, the half value thickness of Co-60 rays decrease from 28 cm in water to no less than 25 cm in normal liquid sludge (Wang and Wang, 2007).

GI has shown potential as an effective treatment option to reduce NA concentrations up to 97% in OSPW and up to 85% in FFT (Table 3.1). The observed NA reduction is similar values observed using ozonation; another treatment option that can effectively reduce the concentration of NA in fresh OSPW by 70% and 95% after 50 and 130 min ozonation, respectively (Scott et al. 2008). We assume that GI is able to degrade the full range of NA congeners, including oxidized NAs and sulfur/nitrogenated NAs in a manner similar manner to ozonation (Hwang et al., 2013) because both mechanisms rely on oxidative degradation through the production of OH[•] radicals. Currently, the NA degradation byproducts created during GI treatment remain unknown because the FTIR method used to quantify NAs in this study does not provide information on the characterization of NAs. OSPW and FFT from this study have been preserved for future characterization using high-resolution methods analytical techniques. An advantage of GI over other treatment options is its ability to penetrate turbid fluids and sediments, making

pre-treatment filtration unnecessary, and allowing for the treatment of NAs present within FFT sediments. As FFT densifies and settles over time, contaminated water is continuously released to groundwater sources and into the overlying OSPW intended for eventual use in reclaimed landscapes. A treatment such as GI, which can treat FFT before its release to the environment or as a pump-and-treat method for already released FFT would be beneficial to oil sands operators in accelerating the detoxification of OSPW and FFT for ultimate release into the environment.

Toxicity of GI-treated OSPW

In the P1A_{non-GI}, aging alone had minimal effect on toxicity reduction, which must be diluted to 45% of its original concentration (95% CI 38-53%) (Figure 3.1) to reduce toxicity to the point that causes a 50% inhibition of luminescence (15-min MicroTox assay – IC₅₀). GI treatment of P1A_{GI} OSPW significantly reduced toxicity, although the effect was not immediate. By day 28, the IC₅₀ ($\pm$ 95% CI) was 80% (68 to >91) and remained at this level for the duration of the 52-week study (Figure 3.2). The toxicity reduction was apparently achieved through enhanced microbial degradation, presumably acting on less persistent (but apparently toxic) compounds generated by the radiolysis of refractory NAs. Although the total concentration of NAs in the P1A_{GI} OSPW determined by FTIR did not appear to decrease with aging (Table 3.1), an increase in the concentration of lower molecular weight NAs produced through GI may be responsible for the initial toxicity, as studies suggest that lower molecular weight NAs are more toxic than

high molecular weight NAs (Holowenko et al., 2002; Frank et al., 2008). Ozonation treatment of OSPW also did not immediately remove toxicity to *Vibrio fischeri*. However, complete removal of toxicity was achieved following inoculation and incubation with the OSPW microbial community (Martin et al., 2010; Hwang et al., 2013).

Aging STP_{non-GI} OSPW for one year resulted in reduced inhibition of luminescence in MicroTox assays. Water aged for 52 weeks had an IC₅₀ ($\pm 95\%$ CI) of over 91% (68 to >91%) (Figure 3.3). Minimal reduction in toxicity was observed over aging periods of 8 weeks or less (14-day IC₅₀ ($\pm 95\%$ CI) was 45% (42-49)). GI treatment of STP water immediately reduced inhibition of luminescence. The fourteen-day IC₅₀ ($\pm 95\%$ CI) was 78% (42 to >91) in OSPW from the STP_{GI} treatment (Figure 3.4). The effect on STP water was additionally manifested through enhanced microbial degradation. In this case, toxicity changes appeared to be biphasic. Materials of intermediate age (8 weeks) were anomalously more toxic than younger and older materials. This is likely a consequence of different byproducts being produced through microbial community succession in the microcosms, as parallel trends were observed in both STP_{non-GI} and STP_{GI}. Both GI treated and non-GI STP water treatments were nontoxic as ascertained by MicroTox assays after 52 weeks. The immediate reduction in toxicity to *Vibrio fischeri* is was also observed with 5- photocatalysis treatment of OSPW NA extract (Mishra et al., 2010).

Figure 3.1 MicroTox results for $P1A_{non-GI}$ OSPW

Figure 3.2 MicroTox results for P1A_{GI} OSPW

Figure 3.3 MicroTox results for *STP_{non-GI} OSPW*

Figure 3.4 MicroTox results for STP_{GI} OSPW

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CHAPTER 4: IMPLICATIONS OF GAMMA IRRADIATION TREATMENT FOR TAILINGS POND MANAGEMENT AND RECLAMATION

To our knowledge, this is the first study to examine the potential of GI to amend and detoxify OSPW and FFT for reclamation purposes and sustainable management. Gamma Irradiation reduces NA concentrations in OSPW as effectively as other existing technologically feasible treatment options such as ozonation (Martin et al. 2010) catalyzed photo-oxidation with UV light (McMartin et al. 2004). Additionally, this research demonstrated that GI is the first treatment that can degrade NAs within the FFT sediments as well as OSPW. The long-term risk of pore water release during FFT compaction containing elevated NA concentrations is a major concern for proponents of reclaimed wet-landscapes, as this will contaminate previously treated surface water, potentially leading to a buildup or increased concentrations of toxic compounds within the surface water of reclaimed wetlands and end-pit lakes. The fact that GI can penetrate through FFT and degrade NAs within the pore water will provide oil sands operators with new opportunities to treat FFT either prior to its initial release into settling basins or as a pump-and-treat method during the transfer of overlying layer of FFT to remediation systems. The surficial layer of FFT exerts the greatest influence on the quality of the surface water due to diffusive processes at the OSPW-FFT interface, which, if treated, will serve as a buffer between the surface water and untreated FFT with elevated pore water NA concentrations deeper below the tailings interface.

GI treatment of both fresh and aged OSPW reduced toxicity to *Vibrio fischeri*. The toxicity of fresh OSPW, which contains the full suite of NA isomers, was immediately reduced following GI treatment. The toxicity of aged OSPW was reduced following GI treatment and several weeks of incubation with that source materials native microbial community. These findings suggest that GI degrades the persistent NA isomers; that contain larger alkyl-branched side chains and heterocyclic groups, into lower molecular weight organics that are more readily biodegraded. If the OSPW in settling basins is GI-treated, a period of incubation should follow to allow for the biodegradation of the remaining NA fractions and further reducing toxicity before those materials are incorporated into reclaimed landscapes.

Aerobic microbial respiration was stimulated in GI treated materials as indicated by the increase in DO_{flux} , resulting from increased bioavailability of intermediate organic products. This is useful from a remediation perspective as the DO_{flux} serves as a proxy for measuring the sediment oxygen demand, which includes the aerobic organic carbon mineralization (biochemical oxygen demand) and the chemical oxidation of reduced chemical species (chemical oxygen demand). Aerobic microbial degradation processes serve as an important detoxification process in aerobic OSPW surface water and the uppermost FFT layer where DO has not yet been consumed. However, GI did not result in a long-term increase in DO_{flux} , which is beneficial for the long-term

management of DO concentrations in reclaimed freshwater ecosystems. Long-term increases to DO_{flux} are a concern as they can lead to hypolimnetic anoxia, causing direct mortality to benthic macro-invertebrates or indirect mortality through the release of toxic by-products of anaerobic mineralization such as hydrogen sulfide (Wang and Chapman, 1999). However, at this stage in the remediation of OSPW and FFT materials before they are incorporated into reclaimed landscapes an increase in DO_{flux} (aerobic respiration) is beneficial because many of the organics present are harmful petroleum byproducts.

Hydrogen sulfide flux was stimulated by GI treatment of the fresh materials collected from STP. This was not observed in the GI-treated materials from the aged P1A FFT. However, the lower HS^- flux values observed in the P1A_{GI} FFT are explained by the microbial biomass starting with 10% of the biomass than the P1A_{non-GI} FFT which allowed the sulfate reduction coupled to AOM processes to establish more rapidly in those materials. After one year the HS^-_{flux} was similar between GI and non-GI treatments for both fresh and aged materials. In P1A, no methane was detected in the headspace of GI and non-GI treatments at week 52, suggesting that the increased HS^-_{flux} at the earlier time points was due to increased AOM processes. GI treatment did not result in long-term (52 week) HS^-_{flux} stimulation, which is desirable as the oxygen demand from continued sulfide oxidation can ultimately deplete oxygen in surface waters. These finding suggests that SRBs are not limited by the availability of organic carbon. This information will permit improved predictive capacity over

existing biogeochemical models that are important in developing environmentally acceptable reclaimed ecosystems (Chen et al. 2013).

Future Research

Our findings suggest that GI has promise as treatment option to accelerate the detoxification and reclamation of settling basins. However, several unknowns remain regarding the microbial detoxification processes occurring following GI. Additional research is needed to characterize the residual NA congeners created by GI-treatment of OSPM and the biodegradation products produced, using high resolution mass spectrophotometric methods. This will provide insight into the direct effects that GI is having on NA structures and the biodegradation pathways taking place following GI. A combination of the data presented in the current study with the NA characterization and microbial community analysis (VanMensel et al., in preparation) will allow researchers to better understand the biodegradation mechanisms taking place. With a greater understanding of the specific mechanisms of biodegradation occurring for specific NA congeners the biodegradation efficiency can be enhanced through bioaugmentation or biostimulation. Bioaugmentation involves the addition of microbial consortia that are specifically formulated to treat a waste solution. For example, the DO and HS⁻ fluxes were higher in the aged P1A materials (chapter 3) and the physical observations of the microcosms (chapter 2) suggest that the microbial community is more diverse and may be composed of a higher proportion of

facultative anaerobes that are more resilient to changes in oxygen concentrations. Therefore, treating the fresh STP material with GI and inoculating with the microbial community from P1A could result in faster detoxification and NA degradation than inoculating with the microbial community from the fresh materials. Biostimulation involves the addition of rate limited nutrients and electron acceptors. For example if a specific consortia which may include SRB's are determined to be a integral component in NA degradation but become limited by the availability of sulfate, then sulfate amendments could be added to FFT materials to further stimulate the activity of SRB's.

This study has shown the potential of GI treatment during a short-term (>52 weeks) study using laboratory microcosms. However, the long-term impact of GI on OSPM materials is unknown. Specifically, GI treatment may or may not affect longer-term biogeochemical processes, microbial community succession and further detoxification of NAs and other toxic constituents in OSPW as a function of dose. An assessment of these issues requires a multi year study using field mesocosms of GI-treated and untreated OSPM materials began in May 2014 at the Suncor lease area north of Fort McMurray, AB. (Weisener and Ciborowski).

There is also need for toxicity testing of GI-treated OSPW using more ecologically representative test organisms to assess the risk associated with GI-

treatment and ensure the safe release of treated OSPM into the environment before GI can be applied to real-world application in the oil sands industry. The MicroTox assay used in this study has become the standard toxicity assay used in studies on the treatment of NAs because it is rapid and gives reproducible results. However, it is considered to be of ecologically limited relevance and may not necessarily predict toxicity to the aquatic biota expected to colonize reclaimed ecosystems. Research in progress will contrast the relative toxicity of fresh vs. aged samples of GI-treated OSPW using standard planktonic (*Ceriodaphnia dubia*) and benthic (*Chironomus riparius*) invertebrate bioassays (Ciborowski et al., in preparation).

Scale-up studies are needed to determine what GI doses, exposure times, additives and catalysts can most effectively degrade NAs in OSPW and FFT. When testing the efficacy of GI for the treatment of sewage effluent, GI effectively oxidized 70% of organic materials into CO₂, while the remaining organics were catabolized into smaller organic acids (Jung et al., 2002). When the authors compared GI treatment with and without the presence of a catalyst (titanium dioxide), decomposition of organics was significantly enhanced, leading to three times the removal of COD and improved removal of TOC and BOD (Jung et al., 2002). Enhanced decomposition in the presence of TiO₂ has been attributed to increased catalysis of OH[•] combined with greater adsorption of organics materials onto the TiO₂ surface. As well, a higher concentration of hydrogen peroxide in aqueous solution has been found to be more effective in the decomposition, mineralization and nitrogen release

of *p*-nitrophenol, a toxic, biorefractory organic compound used in the manufacture of pesticides, pharmaceuticals and synthetic dyes (Yu et al., 2010). Such improvements may enhance the efficiency of GI treatment producing a greater reduction of NAs.

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APPENDIX

Declaration of Co-Authorship for Thesis

Inbox x

Ryan Boudens <boudens@uwindsor.ca> Jan 25 (2 days ago) ☆

to Chris, Thomas, Danielle, Sabari, Jan ▾

Hi everyone,

I will also require your permission as co-author for the following publication that will be included in my thesis.

Boudens, R., Reid, T., VanMensel, D., Prakasan M. R., S., Ciborowski, J., Weisener, C. 2015 Bio-Physicochemical Effects of Gamma Irradiation Treatment for Naphthenic Acids in Oil Sands Fluid Fine Tailings

Could you please respond to this e-mail with your name giving permission to publish the following results in chapter 2 and 3 of my thesis.

Thanks,
Ryan Boudens

Thomas Reid Jan 25 (2 days ago) ☆

to me, Chris, Danielle, Sabari, Jan ▾

You have my permission to publish the work in your thesis.

- Thomas Reid

Jan Ciborowski Jan 25 (2 days ago) ☆

to boudens ▾

Hi Ryan,

You have my permission.

Cheers, Jan

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Sabari Mullapulli Raveendran Jan 26 (1 day ago) ☆

to me, Chris, Thomas, Danielle, Jan ▾

Dear Ryan Boudens

You have my permission

Thanks

Sabari Prakasan M.R

Danielle Vanmensel 11:22 AM (23 hours ago) ☆

to Sabari, me, Chris, Thomas, Jan ▾

Hi Ryan,

You have my permission as well.

Christopher Weisener Jan 24 (3 days ago) ☆

to Thomas, me, Danielle, Sabari, Ciborowski ▾

you have my permission

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