Biodegradation of organic compounds in OSPW with microbial community indigenous to MFT

by

Miao Yu

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Environmental Engineering

Department of Civil and Environmental Engineering
University of Alberta

ABSTRACT

Tailings ponds contain significant amounts of organic contaminants that cannot be released to the environment without further treatment. The use of mature fine tailings (MFT) was proposed as a potential source of microorganisms for biological treatment to remove dissolved organic compounds from oil sands process-affected water (OSPW). In order to test the capacity of microorganisms indigenous to MFT for organic compounds removal in OSPW and determine whether they could be extracted from MFT to form biofilm on biofilm carriers, two groups of batch bioreactors were established (1) one treating acetic-acid-supplemented OSPW and (2) one treating high pressure oxidation (HiPOx)-treated OSPW. In addition, several bioreactors that contained no MFT but MFT-originated biofilm were set up to test the feasibility of using MFT-originated biofilm to biodegrade organic compounds. The bioreactors supplemented with acetic acid yielded a rapid depletion of sulfate and nitrate with partial removal of COD. The COD was reduced from 600 mg/L to a minimum residual COD of 200 mg/L. This is lower than the COD in the original OSPW before acetic acid addition, indicating possible co-metabolic biodegradation of recalcitrant organic compounds. HiPOx-treated OSPW contained larger amounts of sulfate and less readily biodegradable organic compounds compared to acetic-acid-supplemented OSPW. With longer reaction times, sulfate could be depleted and the residual COD could be further reduced to 150 mg/L. The bioreactors that contain MFT-extracted biofilms could remove 20% of the NAs from the acetic-acid-supplemented OSPW and 50% of the COD from the HiPOx-treated OSPW.

Further confirmation was obtained from bioreactors using acclimatized biofilms, which could remove 30% of the NAs from the OSPW without HiPOx treatment. This study demonstrated the feasibility of seeding biofilm reactor with indigenous microorganisms from MFT. The results provide insights on biodegradation of toxic and recalcitrant organic compounds and help the design of continuous bioreactor for OSPW treatment.

Dedication

I would like to dedicate this thesis to my family. Thank you for supporting my studying abroad in order for me to further my education.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisor Dr. Tong Yu for his consistent guidance and unwavering support throughout my course work and research. To this day he continues to introduce me to new academic and research experiences, which has been crucial to my development within the engineering field. His expertise and experience was essential for the accomplishment of this work. I am deeply grateful to Dr. Ania Ulrich, my co-supervisor for her patience and guidance and for reviewing and correcting my thesis. She provided me valuable inputs and kind support throughout this work

I would also like to acknowledge financial support from the Natural Science and Engineering Research Council (NSERC). I would like to thank the Oil Sands Research and Information Network (OSRIN) for their funding, support, and resources available during my research.

Finally, I would like to extend my appreciation to the technicians in the Civil and Environmental Engineering Department at the University of Alberta: Jela Burkus and Elena Dlusskaya. Their assistance, experience, suggestions, availability and support contributed substantially to the success of my many experiments.

TABLE OF CONTENT

ABSTRACT ii
Table of Content vi
List of Tables viii
List of Figures ix
CHAPTER 1: Introduction 1
1.1 Background 1
1.1.1 The Oil Sands Tailings 1
1.1.2 Biological Removal of Toxic and Recalcitrant Organic Compounds Using Biofilm Reactor 2
1.1.3 Advanced Oxidation 4
1.2 Research Objectives 5
1.3 Thesis Outline 5
CHAPTER 2: Literature review 6
2.1 Oil Sands Process-affected Water (OSPW) 6
2.2 Naphthenic Acids (NAs) 8
2.3 MFT and Indigenous Microbial Community 10
2.4 Biodegradation of Petroleum Hydrocarbon 11
2.5 Biofilm Reactor 14
2.6 Advanced Oxidation 15
CHAPTER 3: Experimental approach, Materials and Analytical Methods 18
3.1 Experimental Approach 18
3.2 OSPW and HiPOx-treated OSPW 18
3.3 MFT and Development of Biofilm 19
3.4 Batch Bioreactors for Biodegradation Studies 19
3.4.1 Batch Bioreactors Treating OSPW Using MFT and Using Biofilms Originated from MFT 19
3.4.2 Batch Bioreactors Treating HiPOx-treated OSPW Using MFT and Using Biofilms Originated from MFT 23
3.4.3 Batch Bioreactors Treating OSPW Using Acclimatized Biofilms from a bioreactor 26
3.4.4 Sampling and Analysis Methods 27
CHAPTER 4: Results and Discussion 31
4.1 Anaerobic Biodegradation of OSPW with MFT and with Biofilms Originated from MFT 31

4.1.1 Characterization of the OSPW 31
4.1.2 Anaerobic Biodegradation of OSPW with MFT 32
4.1.3 Anaerobic Biodegradation of OSPW with Biofilms Originated from MFT 45
4.2 Anaerobic Biodegradation of HiPOx-treated OSPW with MFT and Biofilms Originated from MFT 49
4.2.1 Characterization of the HiPOx-treated OSPW 49
4.2.2 Anaerobic Biodegradation of HiPOx-treated OSPW with MFT 50
4.2.3 Anaerobic Biodegradation of HiPOx-treated OSPW with Biofilms Originated from MFT 60
4.3 Anaerobic Biodegradation of OSPW with Acclimatized Biofilms from a Bioreactor 64
4.3.1 Characterization of OSPW 64
4.3.2 Anaerobic Biodegradation of OSPW with Acclimatized Biofilms From a Bioreactor 66
CHAPTER 5: Conclusions 69
5.1 Anaerobic Biodegradation of OSPW with MFT and Biofilms Originated from MFT 69
5.2 Anaerobic Biodegradation of HiPOx-treated OSPW with MFT and Biofilms Originated from MFT 70
5.3 Anaerobic Biodegradation of OSPW with Acclimatized Biofilms from a Bioreactor 70
5.4 Recommendations 71
References 72
CHAPTER 6: Appendices 83

LIST OF TABLES

Table 2-1. Categorization of treatment technologies (AECOM 2010) 7
Table 2-2. Current methods for naphthenic acids measurement 9
Table 2-4. Summary of ozonation studies on oil sands tailings water 16
Table 3-1. Experimental matrix and amendments in biodegradation study of OSPW with MFT and with biofilm originated from MFT 22
Table 3-2. Experimental matrix and amendments in biodegradation study of OSPW with MFT and with biofilm originated from MFT 25
Table 3-3. Experimental matrix and amendments in biodegradation study of OSPW with MFT and with biofilms originated from MFT 27
Table 4-1. Characterization of OSPW obtained for the anaerobic biodegradation tests with MFT 32
Table 4-2 Characterization of HiPOx-treated OSPW obtained for the tests conducted with MFT 50
Table 4-3. Characterization of OSPW obtained for the anaerobic biodegradation tests with MFT 65

LIST OF FIGURES

Figure 3-1. Schematics of the batch bioreactors and photo of a bottle 20

Figure 3-2. Correlation between NAs concentration measured with HPLC and fluorescence intensity 30

Figure 4-1. Biodegradation of dissolved organic compounds in batch bioreactors with OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L..... 34

Figure 4-2. Utilization of sulfate in the biodegradation tests in batch bioreactors with 250 mL OSPW/L MFT, 500 mL OSPW/L MFT, and 1000 mL OSPW/L MFT..... 35

Figure 4-3. Biodegradation of dissolved organic compounds in the batch bioreactors treating OSPW using MFT at OSPW to MFT ratios of 1000 mL/L, 3000 mL/L and 7000 mL/L 37

Figure 4-4. Utilization of sulfate in the biodegradation tests in the batch bioreactors treating OSPW using MFT at OSPW to MFT ratios of 1000 mL/L, 3000 mL/L, and 7000 mL/L 38

Figure 4-5. Nitrate and sulfate concentration profiles in the batch bioreactors with OSPW to MFT ratios of (a) 250 mL/L, (b) 500 mL/L, (c) 1000 mL/L, (d) 3000 mL/L, and (e) 7000 mL/L 40

Figure 4-6. Biodegradation of NAs in batch bioreactors treating OSPW with MFT at OSPW to MFT ratios of 250 mL/L, 500 mL/L, 1000 mL/L, 3000 mL/L, and 7000 mL/L 43

Figure 4-7. Biodegradation of dissolved organic compounds in OSPW with biofilms originated from MFT at OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2 45

Figure 4-8. Utilization of sulfate in the biodegradation tests in the batch bioreactors with OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2 47

Figure 4-9. Nitrate and sulfate concentration profiles in the batch bioreactor treating OSPW using MFT-originated biofilms at OSPW loadings of (a) 8000 mL/m^2 of surface area and (b) 16000 mL/m^2 of surface area..... 48

Figure 4-10. Biodegradation of NAs in OSPW with MFT-originated biofilms in the batch bioreactor with OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2 49

Figure 4-11. Biodegradation of dissolved organic compounds in HiPOx-treated OSPW at OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L 52

Figure 4-12. Utilization of sulfate in the biodegradation tests with OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L 53

Figure 4-13. Biodegradation of dissolved organic compounds in HiPOx-treated OSPW in the batch bioreactors with OSPW to MFT ratios of 1000 mL/L, 3000 mL/L, 7000 mL/L 54

Figure 4-14. Utilization of sulfate in the biodegradation tests in the batch bioreactors with OSPW to MFT ratios of 1000 mL/L, 3000 mL/L, and 7000 mL/L 55

Figure 4-15. Nitrate and sulfate concentration profiles in the biodegradation tests of HiPOx-treated OSPW with MFT in the batch bioreactors with OSPW to MFT ratios of (a) 250 mL/L, (b) 500 mL/L, (c) 1000 mL/L, (d) 3000 mL/L, and (e) 7000 mL/L 57

Figure 4-16 Biodegradation of NAs in HiPOx-treated OSPW in the batch reactors with OSPW to MFT ratios of 250 mL/L, 500 mL/L, 1000 mL/L, 3000 mL/L and 7000 mL/L 59

Figure 4-17. Biodegradation of dissolved organic compounds in OSPW with biofilms originated from MFT at OSPW loadings of 8000 mL OSPW/m^2 and 16000 mL OSPW/m^2 61

Figure 4-18. Utilization of sulfate in the biodegradation tests at OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2 62

Figure 4-19. Nitrate and sulfate concentration profiles in the biodegradation tests of OSPW with biofilms originated from MFT at OSPW loadings of(a) 8000 mL/m^2 and (b) 16000 mL/m^2 63

Figure 4-20. Biodegradation of NAs in HiPOx-treated OSPW with biofilms originated from MFT in the batch reactors with loadings of 8000 mL OSPW/m^2 of surface area and 16000 mL OSPW/m^2 of surface area..... 64

Figure 4-21. Biodegradation of dissolved organic compounds in OSPW with acclimatized biofilms at OSPW loadings of 8000 mL OSPW/m^2 of surface area and 16000 mL OSPW/m^2 of surface area..... 67

Figure 4-23 Biodegradation of NAs in OSPW with acclimatized biofilms at OSPW loadings of 8000 mL/m^2 of surface area and 16000 mL/m^2 of surface area 68

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 The Oil Sands Tailings

Athabasca oil sands industry produces 77,900 m^3/d of synthetic crude oil in 2011(ERCB 2012). As the world largest unconventional oil reserve, Athabasca oil sands expanded rapidly to meet the increasing global demand for oil and gas in recent 20 years. The recovery and conversion of such an unconventional crude oil utilizes water-based extraction processes producing large amount of tailings containing 70 to 80 wt% water, 20 to 30 wt% solids and 1-3 wt% bitumen (Kasperski 1992).

Since oil sands tailings contain residual bitumen and large amount of suspended solids, it cannot be discharged to the environment without further treatment. Oil sands producers build numerous settling basins called tailings ponds to store and dewater primary and secondary extraction tailings onsite. Currently, oil sands tailings ponds occupy a land area of more than 170 km^2 containing approximately 840 million m^3 of oil sands tailings (Siddique et al. 2011).

Upon delivery to tailings ponds, a clarified surface water layer would be developed in the tailings ponds due to gravity settling. With total suspended solids (TSS) of 15–70 mg/L (MacKinnon and Sethi 1993), the clarified water was reused during extraction operations to reduce fresh water intake for oil sands industry. Consequently, many contaminants became concentrated within the recycled water (Allen 2008a), including organic acids, inorganic cations and anions, and heavy metals. Zero liquid discharge (ZLD) policy was implemented to minimize potential environmental impacts of discharging this water stream due to its toxicity caused by the concentrated contaminants. Tailings ponds also collect surface runoff, connect water, and basal water produced from the mining area. The water accumulated in tailings ponds for recycle and reuse is called oil sands process-affected water (OSPW).

An aqueous suspension of fine particles called fine tailings (FT) was also formed in the tailings ponds. Dewatering FT in the settling basin is very time-consuming. The FT settle to 20 wt% solids within a few weeks, but require several years to reach 30–35 wt% solids, at which point consolidation slows considerably and the sludge is designated as mature fine tailings (MFT) (Don Scott et al. 1985). The slow settling process results in large volume of slurry waste stored onsite and presenting an issue for land reclamation and environmental protection.

Oil sands industry is reducing its environmental footprint by reclaiming existing tailings ponds and discontinuing new ponds construction leading to needs for releasing excessive oil sands tailings that requires rapid consolidation of MFT and detoxification of the pore water released from consolidated tailings. One of the major contributors to toxicity was found to be a group of organic acids called naphthenic acids (NAs) (Allen 2008a), which are naturally occurred within ores and present in heavy crude oil at concentrations up to 4 wt % (Barrow et al. 2009). Continuous recycling OSPW during bitumen extraction concentrates NAs in the OSPW. The organic contamination due to concentrated NAs would cause membrane fouling in filtration process when OSPW was treated for reuse, decreasing the efficiency of oil production and refining (Ku et al. 2012).

With the rapid increase in world demand for fossil fuels, the oil sands mining operations expand throughout western Canada. As a result, the production of oil sands tailings will increase as well. Therefore, an understanding of the characteristics and nature of the OSPW and MFT may allow for the potential safe discharge of excessive water stored on site while providing a mean of reclaiming existing tailings ponds and proper disposal of oil sands tailings.

1.1.2 Biological Removal of Toxic and Recalcitrant Organic Compounds Using Biofilm Reactor

One of the contaminants of major concern is the soluble organic compounds present in the OSPW due to the toxicity caused. In early research, physical and chemical treatments were tested to investigate the cost-effectiveness of these processes. Recent research has focused on biological process since the high

organic compounds removal efficiency demonstrated in the applications in municipal wastewater treatment indicates a great potential of being a solution to oil sands tailings treatment.

Biological degradation refers to the use of microorganisms to transform contaminants, including inorganic nutrients and organic pollutants into acceptable end products (Metcalf 2002). Typical biological process that has been applied in commercial scale includes suspended growth and attached growth processes. In attached growth processes, the microorganisms responsible for the conversion of organic material or nutrients are attached to an inert packing material. The consortia of microorganisms of one or more species that adhere to a surface and are enveloped in an extra-polymeric substance are called biofilms (Nicolella et al. 2000). For industrial wastewater, especially the OSPW, because some of the contaminants are toxic to microorganisms, the application of conventional suspended growth process is often hindered by the sensitivity of microorganisms to feed water salinity and toxic organic chemicals (Allen 2008b). In order to reduce susceptibility to the environmental stressors such as salts, heavy metals or hydrocarbons, biofilms are preferred over suspended growth process (Hall-Stoodley et al. 2004, Harrison et al. 2007).

The presence of key microorganisms that could metabolize target pollutants is crucial to successfully develop a biological process removing the toxic and recalcitrant organic compounds (Metcalf 2002). MFTs harbor diverse methanogenic community that could be used as a readily available source of key microorganisms (Penner and Foght 2010). The indigenous microbial community could be cultured in bioreactors with primary substrate and degrade contaminants through potential co-metabolism. In addition, the recalcitrance of organics in OSPW observed in vitro could be due to insufficient acclimation time that is required to induce and sustain enzymes for degradation (Riser-Roberts 1992). This indicates that with the presence of readily biodegradable organic compounds, the toxic and recalcitrant organic compounds could be degraded through biological process.

The full potential of biodegradation of dissolved organic compounds in OSPW in a bioreactor has yet to be demonstrated. Therefore, further understanding of the biological removal of toxic and recalcitrant organic compounds in OSPW is necessary in order to gain insights on potential biological process performance in OSPW treatment.

1.1.3 Advanced Oxidation

Advanced oxidation processes (AOPs) degrade pollutants through a series of ionic or radical reactions involving an oxidant compound that either accepts electrons or donates an electron-accepting group (Allen 2008b). AOPs are commonly tested as a pretreatment methods to enhance biological treatment efficiency because it could break down the long chain and aromatic organic compounds, and thus is well suited to treat dissolved organic compounds in oil field produced water (Gogate and Pandit 2004). The toxic and recalcitrant organic compounds that are resistant to biodegradation are transformed into smaller molecules that are more biodegradable after AOP treatment. Consequently, the efficiency of biodegradation could be increased (Kannel and Gan 2012).

As one of the strongest commercially available oxidant, ozone was found very effective in oxidizing recalcitrant organic compounds in the OSPW (Stanford et al. 2011). Through two decomposition pathways, ozone could destruct the recalcitrant organic compounds with either hydroxyl radicals or ozone molecules directly (Staehelin and Hoigne 1982, Tomiyasu et al. 1985). Traditionally, hydroxyl radical pathway is preferred in advanced oxidation because it could non-selectively degrade almost all of the organic compounds in wastewater completely (Wang 2011).

Though AOP could completely mineralize recalcitrant organic compounds, it has not become an economically viable option for OSPW treatment due to its high operating cost and intensive energy consumption (Ince and Apikyan 2000). Further treatment; more specifically, biological treatment, of chemically-oxidized OSPW is critical. It can be expected that biodegradation of chemically-oxidized OSPW would differ from original OSPW. Therefore, further understanding of the

microbial metabolism of chemically-oxidized organic compounds in the OSPW is necessary in order to develop a proper engineering application for OSPW treatment.

1.2 Research Objectives

The goal of the research is to investigate whether indigenous microorganisms in MFT have the ability to biodegrade organic compounds in OSPW and, if they do, whether they can be utilized in development of engineered bioreactors for OSWP treatment. The specific objectives include:

1. Characterize the biodegradation of organic compounds in OSPW by indigenous microorganisms in MFT and on the biofilm carriers.
2. Characterize the biodegradation of organic compounds in chemically-oxidized OSPW by indigenous microorganisms in MFT.
3. Investigate the feasibility of developing biofilm on the carriers from indigenous microbial communities in the MFT to treat OSPW in a biofilm reactor.

1.3 Thesis Outline

This thesis consists of five chapters, each of which will contribute to the overall main objectives of the research. A review of the field of study and research conducted in this area completed thus far is presented in Chapter 2. Chapter 3 concentrates on the experimental design of batch bioreactors for biodegradation tests. The biological removal of organic compounds and leaching potential from MFT are addressed and discussed in Chapter 4. Chapter 5 presents the final conclusions as well as the engineering significance of the biodegradation of OSPW with indigenous microorganisms in MFT.

CHAPTER 2: LITERATURE REVIEW

2.1 Oil Sands Process-affected Water (OSPW)

The Atabasca oil sands deposit, located in Northern Alberta contains over 174 billion barrels of bitmen that could be extracted through Clark hot water extraction process (Allen 2008a). During oil processing, large quantity of water is used to separate bitumen from clay particles and the mixture of water and fine clays as well as the residue bitumen are sent to tailings ponds to settle sands particles out from the aqueous phase and release pore water for recycle and reuse (Chalaturnyk et al. 2002). Due to ZLD policy, numerous tailings ponds are built to contain the slurry waste and allow the oil sands producers to recycle 80 to 95% of the clarified water (CAPP, 2009). Continuous reusing and recycling the clarified water within bitumen extraction concentrates a range of pollutants of varying concentrations in the tailings ponds (Vlasopoulos et al. 2006). The concentrations of the contaminants in the tailings ponds differ among oil sands mining sites across the Athabasca region due to the unique geology of the mining area as well as ore quality. However, the contaminants of major concern are similar, including coarse and fine sediments, dissolved inorganic and organic byproducts, and fractions of unrecoverable bitumen (Small 2011).

Oil sands producers recognize the big environmental footprint of tailings ponds and increasing public concerns about oil sands industrial pollution. In order to reduce the environmental impacts, reclaiming oil sands tailings ponds is necessary. Accelerating the densification of FT to a mechanically stable, soil-like material is the first step toward reclamation of tailings ponds (Voordouw 2013). Current technologies of tailings dewatering such as composite/ consolidated tailings (CT) and thickened tailings (TT) employ certain chemicals to form desired sediments without significant differences in the particle size distribution across the FT layer in vertical directions (Zhu et al. 2011). In CT technologies, gypsum ($CaSO_4$) and tailings sands were added to accelerate consolidation and the CT will consolidate enough to provide a reclaimable surface (Pollock et al. 2000). However, this will

result in elevated calcium and sulfate concentration in aqueous phase that requires further water treatment. TT adds polymer and utilizes thickener to treat the fine fraction of tailings (Chalatumyk et al. 2002). After tailings drying, the pore water released will be sent back into tailings ponds for reuse.

After tailings are dewatered, dried tailings are reclaimed from the tailings ponds leaving large quantify of water containing dissolved inorganic and organic contaminants (List and Lord 1997). With successful reclamation of current tailings ponds, the containment capacity of tailings ponds is reducing leading to urgent needs for elimination of the excessive amount of water stored in the tailings ponds known as OSPW. Oil sands producers tested various water treatment technologies to evaluate their cost-effectiveness in treating OSPW. Categorized by their treatment capacity and required influent water quality, water treatment technologies are grouped and listed in table 2-1 for comparison. Life cycle analysis (LCA) results indicate that a combination of two or more water treatment technologies may provide more effective solutions toward safe discharge of the OSPW (Vlasopoulos et al. 2006).

Table 2-1. Categorization of treatment technologies (AECOM 2010)

Technologies (removal objectives)	**Examples**	**Stages**			
		I	**II**	**III**	**IV**
Suspended Solids	Sedimentation (With or without coagulants)	■			
	Air Flotation (With or without coagulants)	■			
	Filtration	■	■		
	Membrane Filtration			■	■
Oil & Grease	Sedimentation (Without Coagulants)	■			
	Air Flotation (With or without coagulants)	■			
	Biological Treatment		■		
Dissolved Organics	Biological Treatment			■	
	Chemical Oxidation			■	
	Adsorption			■	
Dissolved Solids	Reverse Osmosis				■
	Ion Exchange				■

2.2 Naphthenic Acids (NAs)

Among all the contaminants found in OSPW, a group of endogenous organic carboxylic acids called NAs that is present in 50 mg/L to 100 mg/L are identified as a major composition of organic matters (Anderson et al. 2012b). It is originated from undissociated naphthenic amphiphiles that are present in bitumen at concentrations up to 4 wt %. The naphthenic amphiphiles could promote oil-water phase separation under acidic formulation conditions (Barrow et al. 2009). But the alkaline treatment required in oil sands bitumen recovery process would result in a pH greater than the pKa of naphthenic amphiphiles (Brandal and Sjoblom 2005). Consequently, the water-soluble form of naphthenic amphiphiles, naphthenate salts are produced and dissolved in the aqueous phase (Kiran et al. 2011).

The composition of NAs in the OSPW is not clearly identified and expected to differ across the Athabasca oil sands mining area (Small 2011). Generally speaking, NAs represent a class of complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids (Yen et al. 2004). Many literatures consider NAs in the OSPW to be only classical NAs that could be represented by general formula $C_nH_{2n+z}O_2$ (Clemente et al. 2003). However, this is not an accurate interpretation of chemical analysis results since the presence of non-classical NAs including oxynaphthenic acids and heterocyclic carboxylic acids was common in the OSPW (Grewer et al. 2010). Due to the ambiguity of structural information of NAs, there is no standardized procedure for NAs quantification. Current methods for NAs measurement found in literature are tabulated in table 2-2.

Table 2-2. Current methods for naphthenic acids measurement

Methods	Identification	Quantification	References
FABMS	Molecular weight distribution	Relative ion intensity	(Fan 1991)
FTIR	Carboxyl group	Calibrated to Kodak, Merichem NAs	(Holowenko et al. 2001)
GC	Methyl esters derivatization	Calibrated to an internal standard	(Herman et al. 1994, Jones et al. 2001)
GC / MS	*tert*-butyldimethylsilyl esters derivatization	Mass spectra	(St John et al. 1998) (Merlin et al. 2007)
HPLC / UV	2-nitrophenylhydrazine derivatization	Calibrated to Kodak and Merichem NAs	(Clemente and Fedorak 2003, Yen et al. 2004)
HPLC / MS	Retention time correction by internal standards	Negative ion mass spectra	(Han et al. 2008, Wang and Kasperski 2010)
HPLC / Fluorometry	Excitation emission matrix (EEM)	Fluorescence spectra	(Lu et al. 2013)
ESI / MS	Retention time correction	Negative ion mass spectra	(Purves and Guevremont 1999, Scott et al. 2009)
ESI / FAIMS / MS	Retention time correction	Negative ion mass spectra	(Gabryelski and Froese 2003)

For routine analysis in process monitoring, fluorescence detector could rapidly generate a fingerprinting of NAs (Beltran et al. 1998). Fluorescence spectroscopy utilizes the fluorescing nature of PHCs when illuminated with ultraviolet (UV) light to directly quantify NAs in aqueous sample without any pre-separation or other preparation steps (Alostaz 2008). In spite of its limitations on NAs speciation, the fluorescence emission spectroscopy can still be used to monitor the relative change of NAs concentrations (Lu et al. 2013).

Not only NAs are present in large amount in the OSPW produced from Athabasca oil sands mining area, the presence of NAs are also found to be closely related to

OSPW toxicity (Clemente and Fedorak 2005). The fresh OSPW was found to be acutely toxic to bacteria while after NAs in the OSPW were destructed by ozonation, the toxicity was eliminated (Gamal El-Din et al. 2011). Previous research also revealed that fresh OSPW was acutely and chronically toxic to benthic invertebrates (Anderson et al. 2012a). Sub-chronic exposure to commercial NAs reduced immunity of goldfish (Hagen et al. 2012). Further studies showed the organic fraction in OSPW exhibited higher lever of toxicity to mammal cells than commercial NAs (Garcia-Garcia et al. 2011b). And the immunotoxicity was abolished after ozone treatment (Garcia-Garcia et al. 2011a) indicating NAs may be the main contributor to the toxicity.

Biological treatment such as reclamation ponds demonstrated the capability of decreasing toxicity by reducing the concentration of NAs in the aged OSPW after biodegradation (Anderson et al. 2012a). A decrease in toxicity of process-affected waters accompanied an increase in the proportion of NAs in the "C22+cluster" (Holowenko et al. 2002). Studies on a combination of ozonation and biodegradation processes also revealed mild ozonation accelerated microbial remediation of OSPW (Martin et al. 2010). Though biological treatment itself could not completely remove or destruct NAs from the OSPW, it could largely reduce the OSPW toxicity cost-effectively. Therefore, insights on biodegradation of NAs in a bioreactors would be valuable for process development and OSPW remediation.

2.3 MFT and Indigenous Microbial Community

Operating under zero-discharge policy, the oil sands producers must hold all slurry waste on site (Fedorak et al. 2003). Sedimentation basin was utilized to retain tailings and consolidate tailings to separate clay and fine particles from froth. A complete separation of solids and water and formation of clear water-sedimentation interface would take 125 years based on modeling simulation results (Eckert et al. 1996). During the slow settling period, an unsettled layer of suspended silt and clay was developed, namely MFT.

Recently, microbial metabolism of residue hydrocarbons in MFT causing methane and carbon dioxide emissions were reported in many literatures. Exposure to NAs could induce the microorganisms capable of biodegrading commercial NAs while aged NAs derived from OSPW appeared to be recalcitrant to microbial degradation (Del Rio et al. 2006). Bacterial cultures enriched from oil sands tailings could utilize both commercial and OSPW-extracted NAs as sole carbon source and mineralize 20 to 50% of model compounds (Herman et al. 1994). Enumeration studies found methanogenic population was robust within the fine tailings zone of various oil sands waste settling basins(Holowenko et al. 2000). Based on rate of naphtha metabolism measured in laboratory incubation, a kinetic model was developed and predicted production of 8.9–400 million liters of CH4 per day from Mildred Lake Settling Basin (MLSB) (Siddique et al. 2008).

Sulfate-reducing bacteria (SRB) was also present with elevated numbers when sulfate was available. Penner and Foght (2010) thoroughly examined the indigenous methanogenic microbial communities by constructing clone libraries of amplified archaeal and bacterial 16S rRNA genes. The results showed MFT harbor a diverse community of prokaryotes presumptively responsible for producing methane from substrates indigenous to the MFT. Further studies compared microbial communities in the bioreactor fluid fine tailings (FFT) and reference tailings samples taken form west in pit (WIP) tailings pond. Bacteria was enriched in sulfidic zone and after 300 days operations, archaeal became predominant in bioreactors (Chi Fru et al. 2013). No significant dissimilarities were observed between bioreactors and reference tailings samples.

2.4 Biodegradation of Petroleum Hydrocarbon

Most oil sands tailings contain petroleum hydrocarbon (PHC), which is a group of recalcitrant substrates for microorganisms. Generally, non-biodegraded oil contains a relatively larger amount of nonpolar compounds and less saturated carboxylic acids (Borgund et al. 2007). With organic supplements and inorganic electron acceptor amendments, the in-situ biodegradation of PHC was well investigated in many studies. Benzene biodegradation occurred currently with

nitrate reduction at constant ratio of 10 mol of nitrate consumed per mol of benzene degraded (Burland and Edwards 1999). Under anaerobic conditions, BTEX degradation was observed under sulfate and ferric reducing conditions (Coates et al. 1996a, Coates et al. 1996b). With anaerobic sulfide-reduced defined mineral medium supplemented with 20 mM sulfate, up to 200 μM of benzene was depleted and more than 90% was mineralized to CO_2 (Edwards and Grbicgalic 1992).

Radioactively labeled benzene, naphthalene and phenanthrene is incubated with sediments collected from San Diego Bay. Anaerobic benzene oxidation coupled to the reduction of sulfate is evident in the 80-days biodegradation process. And the addition of Fe(III) did not switch the terminal electron-accepting process (TEAP) from sulfate reduction to Fe(III) reduction.

Mueller et al. (1991) examined the biotreatability of an extensive range of polycyclic aromatic hydrocarbons (PAHs) with indigenous microbes. The sediments and surface soils collected from contamination site were incubated with and without inorganic nutrients amendments. It was found the sediments carried more contaminants and microorganisms, while the stimulatory effect of nutrients addition is less obvious in the composite sample of sediments. Ozonated PAH-contaminated municipal sewage sludge was treated with anaerobic digester without recirculation and 405 μg/L of PAH was removed when the HRT was 40 days (Bernal-Martinez et al. 2009).

The anaerobic BTEX biodegradation was also examined within a BTEX-ethanol mixture (Da Silva et al. 2005) and ethanol-blended gasoline (gasohol) (Chen et al. 2008). BTEX-ethanol mixtures biodegradation efficiencies were compared under sulfate, ferric and nitrate reducing conditions in a simulated aquifer column with a flow velocity of 3×10-6 m/s. It was observed the addition of ethanol could impair BTEX utilization significantly. However, with the addition of electron acceptors, BTEX biodegradation could be recovered except benzene, which could be removed only with controlled oxygen intrusion. Moreover, BTEX biodegradation was essentially shut down when the mixture culture was supplemented with

5000mg/L of ethanol. In conclusion, as an organic supplement, ethanol biodegradation could outcompete BTEX utilization when electron acceptor is limited. Stimulation with sulfate, ferric nitrate and oxygen could increase PAHs-ethanol mixture biodegradation effectively, while benzene biodegradation was inhibited once oxygen was purged out of system. Field studies found the addition of sulfate to an anaerobic petroleum contaminated aquifer resulted in removal of benzene from the groundwater and the benzene degradation could account for 53% of sulfate depletion (Anderson and Lovley 2000).

Other PHC components degradation was studied as well. Musat et al. (2010) established a model to predict cyclic saturated hydrocarbons biodegradation and anaerobic ammonium oxidation. The ammonium and nitrate were stoichiometrically consumed in the first fifteen days of incubation along with a continuously consumption of cyclohexane. 1000 mg/kg of phenanthrene could be removed from a solid phase reactor using indigenous bacteria isolated from petroleum-contaminated site in 8 weeks (Arbabi et al. 2009). The combination of bioaugmentation with inorganic nutrient and surfactant amendments could improve the removal rate of PHC to 50% after a 9-month period of experiment conducted on refinery wastewater contaminated soil (Couto et al. 2010). Furthermore, with a nitrate supplement and adequate buffering, 56% of crude oil was removed from the bacterial consortium (Foght et al. 1999). Sulfate reducing bacteria of phylogenetically different groups might be involved in the degradation of petroleum hydrocarbons at low temperature (Higashioka et al. 2011).

NAs biodegradation was also extensively studied in Alberta to develop a mitigation measures to reduce the environmental risks of oil sands tailings. Some surrogate NAs including 3-cyclohexylpropanoic acid at 400–800 mg/L, 5-cyclohexylpentanoic acid at 200 mg/L or 6-phenylhexanoic acid at 200 and 400 mg/L could stimulate methanogenesis in sewage sludge (Holowenko et al. 2001). Tailings water microorganisms in Syncrude tailings ponds preferentially deplete the least alkyl-substituted fraction (Bataineh et al. 2006). Increased cyclization in OSPW NAs would lead to a slower biodegradation rate comparing to commercial

NAs (Han et al. 2008). Up to 40% of NAs could be removed by laboratory cultures during 30 to 40 days incubation periods (Clemente and Fedorak 2003). 100 mg/L of commercial NAs could be biodegraded within 10 days of incubation (Clemente et al. 2004).

2.5 Biofilm Reactor

Biofilms are preferred in the biological removal of toxic and recalcitrant organic compounds because it is more sustainable under harsh environment. The diesel degradation rates by immobilized mixed diesel degrading bacteria cells and free cells are similar (Lee et al. 2010). Immobilized *Pseudoxanthomonas* sp. RN402 cells showed higher efficacy of diesel oil removal than free cells, achieving a removal rate of 1050 mg/L•day (Nopcharoenkul et al. 2013). Immobilized cells of oil-degradation microorganisms are able to remove 2000 mg/L oil and 2000 mg/L COD within 50 h at 30 °C (Wu et al. 2009). Immobilized cell bioreactors using microbial culture originated from an oil reservoir could remove sulfate at a maximum rate of 1.7 g/L•h through sulfate reduction (Baskaran and Nemati 2006).

With certain inoculum medium, the biofilm need to be developed on the growth media before added into the biofilm reactor for OSPW treatment. Alcohol could promote adhesion of the bacterial cells to the oil-water interface (Abbasnezhad et al. 2011). Mixed species biofilms were developed on the Calgary Biofilm Device (CBD) in an inoculum experiments (Golby et al. 2012). A circulating packed bed bioreactor seeded with indigenous microorganisms in OSPW was used to treat a mixture of three model NAs. Experimental results revealed that the maximum biodegradation rate was significantly higher than suspended growth process (Huang et al. 2012). This indicates that seeding biofilm reactors from indigenous microbial community is feasible. After the biofilm was established in the reactor, the biodegradation could be carried out in the biofilm reactor with batch or continuous operation.

2.6 Advanced Oxidation

Defined as the process that generates hydroxyl radicals to destroy complex chemicals present in the aqueous phase, AOPs have been studied extensively in water and wastewater treatment for the removal of recalcitrant organic compounds (Gogate and Pandit 2004). As one of the common oxidants, ozone has demonstrated great potential in removing naphthenic acids and eliminating toxicity in the OSPW. When treated with 20 mg/L of ozone, 60mg/L of commercial NAs were destroyed completely while the toxicity measured by Microtox Bioassays was not affected (Martin et al. 2010). After the OSPW was treated with 30 mg/L, the concentration of NAs was reduced from 23.6 mg/L to 12.1 mg/L and the survival of *Chironomus dilutes* exposed to ozonated-OSPW was increased to 95% (Anderson et al. 2012b). 76% of NAs (referred as acid-extractable organics) were removed when the utilized ozone dose was 150 mg/L. With utilized ozone dose of 150 mg/L, the toxicity towards *Vibrio fischeri* was completely eliminated (Gamal El-Din et al. 2011). The relations between treatment efficiency and ozonation time was also investigated. Onzonation for 130 min could reduce the naphthenic acids concentration to 2 mg/L while the total organic carbons (TOCs) remain unchanged (Scott et al. 2008). After 180 minutes of ozonation, the diesel-contaminated soil was incubated for 9 weeks for bioremediation in which 25.4% of TPH was removed suggesting that appropriate ozonation and indigenous microorganisms survived ozonation could enhance remediation (Ahn et al. 2005).

The ozone demand can be extrapolated from previous test results obtained on tailings water. An extensive research work has been done to determine the ozone demand of oil sands tailings water. Table 2-4 shows a summary of test results obtained from ozonation studies on tailings water.

Gamal El-Din et al. (2011) found Microtox toxicity (EC20) was completely removed with utilized ozone dose of 35 mg/L. With utilized ozone dose of 150 mg/L, chemical oxygen demand (COD) in tailings water was reduced from 250 mg/L to 200 mg/L when alkalinity is 690 mg/L as $CaCO_3$.

Table 2-4. Summary of ozonation studies on oil sands tailings water

Water Source	Target Parameter	Influent Water Quality	Utilized Ozone Dose	Removal Efficiency
MLSB WIP (2011)[a]	COD	250 mg/L	150 mg/L	20%
	Toxicity (EC20)	24%	35 mg/L	Completely eliminated
Suncor OSPW (2010)[b]	COD	336 mg/L	200 mg/L	36%
			300 mg/L	50%
	Toxicity (EC50)	17%	200 mg/L	Completely eliminated
Suncor Pond 2/3 (2012)[c]	COD	343.8 mg/L	200 – 300 mg/L	17%
	Toxicity (EC20)	2.7%	200 – 300 mg/L	86%
	Toxicity (EC50)	11.6%	200 – 300 mg/L	75%

[a](Gamal El-Din et al. 2011)

[b](AECOM 2010)

[c](EPCOR 2011)

The ozone transfer efficiency (OTE) also needs to be estimated to determine applied ozone dose and operating cost. However, there is no literature available to evaluate OTE when ozone dose is as high as 577 mg/L. Most ozone transfer devices were tested under lower ozone dose. Multi-stage ozone transfer may be needed to achieve OTE of 95 to 99%.

In Mazzei's Pipeline Flash Reactors, the OTE could achieve 100% when ratio of gas to liquid flow is 0.04 (Bale et al. 2003), which is 7.16 mg/L in applied ozone dose. Increasing the flow ratio would lead to a decrease on OTE. Multiple stage pipeline injection and liquid flow circulation within the injection system could reduce the ratio of gas to liquid flow and increase OTE. Circulation would lead to an increase on pipeline size and the OTE promotion effects are limited by the maximum pipeline size available. Multiple stage pipeline injection would results in large plant foot print and vast capital investment. Further investigation is necessary to optimize ozone transfer system design under high ozone scenario.

Other AOPs that could generate hydroxyl radicals were also investigated in removing NAs. UV/H_2O_2 process could destroy cyclohexanoic acid (CHA)

selected as a model NA in less than 20 min in ultrapure water, while the presence of real OSPW matrix would cause a decrease of up to 82% in degradation rate (Afzal et al. 2012a). Further study has found the reactivity favors OSPW NAs with higher carbon number (Afzal et al. 2012b).

HiPOx process has been tested in tailings water treatment pilot plant (EPCOR 2012). COD is reduced from 343 mg/L to 284 mg/L with applied ozone dose of 200 to 300 mg/L. The toxicity is reduced by 86% (EC20) However, due to lack of ozone concentration data in off-gas, it is hard to determine OTE of the HiPOx serpentine reactor. The vendor expects the OTE in HiPOx could achieve 95%.

The experiments are to investigate the feasibility of engineering application of biological treatment in oil sands tailings disposal. The microorganisms in MFTs are to be extracted and incubated to multiply into a large population. As a result, the microorganisms obtained from the lab can be applied in water treatment plant to initiate the process and the information about optimum growth condition can provide experiences in water treatment process control.

CHAPTER 3: EXPERIMENTAL APPROACH, MATERIALS AND ANALYTICAL METHODS

3.1 Experimental Approach

In order to test the capacity of microorganisms indigenous to mature find tailings (MFT) for organic compounds removal in oil sands process-affected water (OSPW) and determine whether they could be extracted from MFT to form biofilms on biofilm carriers, two groups of batch bioreactors were established (1) one treating acetic-acid-supplemented OSPW and (2) one treating advanced oxidized OSPW. Acetic acid amendment provided sufficient readily biodegradable organic compounds to activate the indigenous microbial community and adjusted the pH in the bioreactors to neutral level offering an environment that is more favorable for microbial growth.

3.2 OSPW and HiPOx-treated OSPW

OSPW was sampled from one of the major oil sands mining sites located in northern Alberta. Oil barrels were used for sampling, storage and transport of OSPW samples. Approximately 200 L of fresh OSPW samples were obtained from primary extraction tailings ponds. OSPW was transferred into plastic pails upon arrival at University of Alberta and preserved at 4 °C until batch bioreactor startup. The DO concentration was measured to be 6.0 mg/L in clarified water indicating aerobic environment in OSPW.

The sampling site also has a pilot plant having been running for six months to test treatment efficiency of different advanced water treatment technologies, including high pressure oxidation (HiPOx) treatment and moving bed bioreactor (MBBR). Both water treatment technologies were treating the fresh OSPW pumped from a secondary extraction tailings pond with no pretreatments.

HiPOx-treated OSPW and MBBR carriers with acclimatized biofilm were collected and shipped to University of Alberta after demolition of the OSPW treatment pilot plant. Approximately 200 L of fresh HiPOx-treated OSPW

samples were contained in ten plastic pails when transported from the sampling site to lab and stored in 4 °C upon arrival. MBBR carriers were transported with MBBR water content that contains biomass minimizing loss of microbial activity and transferred into batch bioreactor once it was received by the lab.

3.3 MFT and Development of Biofilm

The sampling site for MFT is one of the major oil sands mining sites located in northern Alberta. As a primary extraction tailings pond, the sampling pond contains less organic compounds and more clay particles compared to typical tailings ponds. Monitoring data showed depletion of oxygen in sediments indicating anaerobic environment in MFT.

The biofilm was established in a bioreactor by intermittently mixing 20 L of MFT with 3 L of high-density polyethylene (HDPE) media (Peenox™ media, Mabarex Inc. QC, CA) that could provide solid surface for cells adhesion over six months. Following this period a substantial amount of biofilm was formed on the carriers. The carriers were then transferred into 2L batch bioreactors in order to investigate the biodegradation of organic compounds in the OSPW.

3.4 Batch Bioreactors for Biodegradation Studies

3.4.1 Batch Bioreactors Treating OSPW Using MFT and Using Biofilms Originated from MFT

The batch bioreactor designed for this study is illustrated in Figure 3-1. Each batch bioreactor used a 2L Pyrex® reusable media/solution bottle (CORNING 1395-2L, Tewksbury MA, USA). Each bottle was capped with a PTFE stopper. The batch bioreactors were kept in anaerobic atmosphere generation bags (Sigma-Aldrich 68061-10SACHETS-F, St. Louis, MO, USA) during the testing period, which allowed sampling of water content from the bioreactors while maintaining anaerobic conditions. Anaerobic conditions were achieved by flushing the anaerobic bags and OSPW with nitrogen gas prior to and during the testing periods.

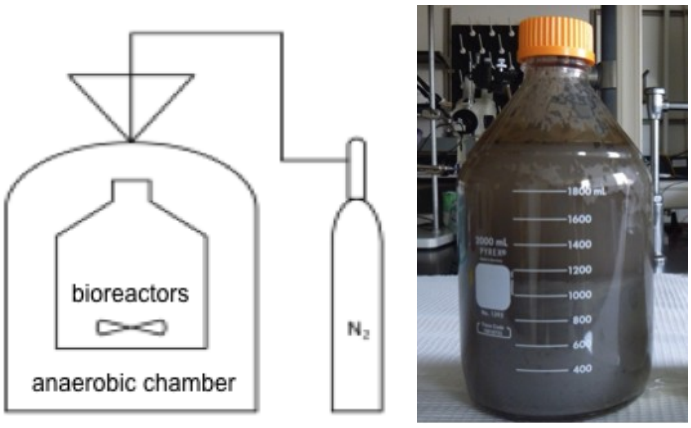

Figure 3-1. Schematics of the batch bioreactors and photo of a bottle

Adding MFT to the batch bioreactor would introduce excessive hydrocarbons to the system inevitably. However, biofilm bioreactor could eliminate this leaching effect by selecting microorganisms that could attach to the surface of carriers to form biofilms and removing bitumen from microbial communities to achieve better performance. The biodegradation of OSPW with biofilm originated from MFT was tested to examine the feasibility of culturing biofilm from indigenous microbial community and use of the biofilms for OSPW treatment.

Fourteen batch bioreactors were set up to investigate the effects of different substrate-to-biomass ratios on biodegradation and determine the optimal amount of MFT to be used for reactors start-up. The experimental matrix and amendment concentrations are summarized in Table 3-1. Acetic acid addition was used to adjust the pH in OSPW to 7.0 while supplying readily biodegradable organic compounds as primary substrate.

All batch bioreactors were operated for 2 weeks for sulfate depletion then amended with nitrate and phosphate. The intended amendment concentrations were achieved by injecting 5 mL of each amendment solution. A 7.2 M solution of KNO_3, and a 6.4 mM solution of K_2HPO_4 were used for nitrate and phosphate amendment to achieve a final concentration of 1.8 mM (KNO_3) and 0.16 mM (K_2HPO_4), respectively. All chemicals were purchased from Fisher Scientific (Nepean, ON, CA).

The initial substrate-to-biomass ratios were adjusted to the range of 250 to 7000 mL OSPW/L MFT for batch bioreactors using MFT and 8000 to 16000 mL OSPW/m^2 of surface area for bioreactors using biofilm. Once prepared, all batch bioreactors were operated at 22°C for ten weeks.

Table 3-1. Experimental matrix and amendments in biodegradation study of OSPW with MFT and with biofilm originated from MFT

Notation	Constituents					Initial substrate to biomass ratio
	OSPW (mL)	MFT (mL)	MFT-originated biofilm (mL)	DI water (mL)	Acetic Acid (mg/L)	
OSPW-MFT250	250	1000	-	750	75	250 mL OSPW/L MFT
OSPW-MFT500	500	1000	-	500	150	500 mL OSPW/L MFT
OSPW-MFT1000	1000	1000	-	-	300	1000 mL OSPW/L MFT
OSPW-MFT3000	1500	500	-	-	300	3000 mL OSPW/L MFT
OSPW-MFT7000	1750	250	-	-	300	7000 mL OSPW/L MFT
OSPW-biofilm8000	1000	-	250	1000	150	8000 mL OSPW/$m^2$of surface area
OSPW-biofilm16000	2000	-	250	-	300	16000 mL OSPW/$m^2$of surface area

Scenarios OSPW-MFT250, OSPW-MFT500, and OSPW-MFT1000 composed of the same amount of MFT (1000 mL) with various concentration of substrate (expressed as mL OSPW/L MFT) to investigate the effects of different substrate-to-biomass ratios on the biodegradation of dissolved organic compounds. The acetic acid addition acts as buffering solution and primary substrate supplements. To maintain a consistent pH in all scenarios, concentrations of acetic acid added were proportionated to the OSPW amount. Scenarios OSPW-MFT3000 and OSPW-MFT7000 composed of the same concentrations of COD with various amounts of MFT to investigate the effects of different initial substrate-to-biomass ratios on the biodegradation of organic compounds in OSPW with indigenous microorganisms in the MFT.

Scenarios OSPW-biofilm8000 and OSPW-biofilm16000 used MFT-originated biofilms as an alternative source of microorganisms to replace MFT and examine whether there is viable biofilms formed on the carriers after six months' incubation with MFT. In order to be comparable with bioreactors using MFT, 250mL of HDPE media was added to the batch reactor.

3.4.2 Batch Bioreactors Treating HiPOx-treated OSPW Using MFT and Using Biofilms Originated from MFT

HiPOx-treated OSPW was collected after the tailings water pilot plant demolition. As an AOP, HiPOx uses ozone and hydrogen peroxide to break down long chain organic compounds into smaller molecule that could be completely mineralized during bacteria metabolism. Consequently, the organic compounds could be removed from the OSPW.

AOP would substantially increase the biodegradable organic fraction in OSPW that could support microbial growth in MFT. Consequently, the addition of acetic acid as primary substrate would not be necessary. The readily biodegradable organic compounds in OSPW needed to support microbial growth was the oxidation product of original organic matrix in OSPW and the COD concentration in HiPOx-treated OSPW is lower than the OSPW supplemented with acetic acid.

The experimental design and methods of sampling were adopted from biodegradation study of acetic acid supplemented OSPW and have been described in details in Section 3.2.1. The experimental matrix and amendment concentrations are summarized in Table 3-2. As with the biodegradation study of acetic acid supplemented OSPW, the NO^{3-} and PO_4^{3-} were amended at 7.2 mM and 6.4 mM to achieve a final concentration of 1.8 mM (KNO_3) and 0.16 mM (K_2HPO_4), respectively.

Table 3-2. Experimental matrix and amendments in biodegradation study of OSPW with MFT and with biofilm originated from MFT

Notation	Constituents				Initial substrate to biomass ratio
	HiPOx-treated OSPW (mL)	MFT (mL)	MFT-originated biofilm (mL)	DI water (mL)	
HiPOx-MFT250	250	1000	-	750	250 mL OSPW/L MFT
HiPOx-MFT500	500	1000	-	500	500 mL OSPW/L MFT
HiPOx-MFT1000	1000	1000	-	-	1000 mL OSPW/L MFT
HiPOx-MFT3000	1500	500	-	-	3000 mL OSPW/L MFT
HiPOx-MFT7000	1750	250	-	-	7000 mL OSPW/L MFT
HiPOx-biofilm8000	1000	-	250	1000	8000 mL OSPW/m^2 of surface area
HiPOx-biofilm16000	2000	-	250	-	16000 mL OSPW/m^2 of surface area

Scenarios HiPOx-MFT250, HiPOx-MFT500 and HiPOx-MFT1000 contained the same amount of MFT with various concentration of HiPOx-treated OSPW. Lower initial COD concentration was achieved by diluting the HiPOx-treated OSPW with DI water. After HiPOx treatment, the pH decreased to 7, which is preferred for mixed microbial growth. Consequently, the pH adjustment with acetic acid is not necessary and the needs for primary substrate for microbial growth could be met by breaking down long-chain organic compounds into smaller molecules that are less persistent to biodegradation.

Scenarios HiPOx-MFT3000 and HiPOx-MFT7000 contained different amount of MFT with the same initial concentration of substrate to investigate the effects of different biomass concentration to the biodegradation process. This would provide insight on biodegradation kinetics as well as the leaching effects due to the concentrated bitumen carried by MFT. By comparing the biodegradation in both scenarios with previous batch reactors, the leaching of naphthenic acids from suspended bitumen and solids would be revealed.

Scenarios HiPOx-biofilm8000 and HiPOx-biofilm16000 utilized HDPE media with MFT-originated biofilm to eliminate the leaching effects and examine the efficiency of MFT-originated biofilms in treating HiPOx-treated OSPW.

3.4.3 Batch Bioreactors Treating OSPW Using Acclimatized Biofilms from a bioreactor

The tailings water treatment pilot plant tested MBBR to investigate the feasibility of OSPW biological treatment. After acclimated for three months, the aerobic biofilm developed on the carriers were collected from the pilot plant. A visible layer of biofilm was developed on the carriers. Since the MBBR was running under nitrification conditions, it is expected that nitrifiers would be dominant in the biofilms.

OSPW supplemented with acetic acid was used in the bioreactors study. Providing sufficient readily biodegradable organic compounds serving as primary growth substrate, the biofilms on carriers could sustain and support growth and

aggregate in large amount to form thicker biofilms and utilize naphthenic acids as secondary growth substrate removing them from the aqueous phase.

Table 3-3. Experimental matrix and amendments in biodegradation study of OSPW with MFT and with biofilms originated from MFT

Notation	Constituents				Initial substrate to biomass ratio (mL OSPW/m^2 of surface area)
	OSPW (mL)	Acclimatized biofilm (mL)	DI water (mL)	Acetic Acid (mg/L)	
OSPW-MBBR8000	1000	250	1000	150	8000
OSPW-MBBR16000	2000	250	-	300	16000

Scenarios OSPW-MBBR8000 and OSPW-MBBR16000 used biofilms taken from an engineering biofilm reactor that has been treating OSPW for nine months to treat original organic matrix from OSPW. Lower initial COD concentration was achieved by diluting the origin OSPW with DI water. A thin layer of biofilm was developed on the carriers. Though the reactor was operated under aerobic condition in bulk phase, both aerobic and anaerobic biofilms could be developed on the carriers because of the advantageous structure of biofilms. These two scenarios were set up to examine the viability of biofilms developed from aerobic reactors and the biodegradation of OSPW.

3.4.4 Sampling and Analysis Methods

The water content in the bioreactors was sampled for chemical analysis at prescribed time intervals to monitor the depletion of the organic compounds and electron acceptors. Fresh OSPW and the filtrate water samples were analyzed for: pH, total alkalinity, major cations and anions with ion chromatography (IC) [Dionex IC 2500 and ICS 2000], and organic compounds concentration using chemical oxygen demand (COD). Alkalinity values were determined through

potentiometric titrations with 0.02 N H_2SO_4 using arbitralry pH end-point of 4.5 for industrial waste or complex system. The alkalinity was determined relative to a particular pH from the curve.

IC was calibrated using a serial dilution of 1x, 2x, 5x, 10x, and 20x from Seven Anions Standard II (Dionex). Cations calibration curve were plotted in 2x, 10x, 20x, 100x, and 200x using six Cation II standard (Dionex). Anions instrumentation required an eluent stream of 8.0 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ (1mL/min), whereas the cations instrumentation required an eluent of ultra-pure water (1mL/min). Both systems were pressurized using nitrogen in 60 kPa.

Chemical oxygen demand (COD) is used as a universal, nonspecific method for organic matter content monitoring in water and wastewater sample. COD could be related to BOD, or total organic carbon for a certain type of wastewater, which enables it a fast and convenient determination method for organic contents measurement. OSPW contains a wide range of dissolved organic compounds that is difficult to be identified and quantified. Current analytical methods could only determine the concentration of certain group of organic compounds that has similar structure, which is a partial quantification of organic matter content in OSPW. As a nonspecific analytical method, COD could provide us a general idea of organic compounds level in the water sample, which is more meaningful in wastewater treatment process monitoring. Meanwhile, COD is widely adapted as a standard parameter in wastewater discharge guideline. Using COD to quantify treatment efficiency could generate research results that are more comparable with current engineering applications.

COD was measured according to the Standard Methods for the Examination of Water and Wastewater. The determination of COD utilizes the ability of most organic materials, with the exception of some aromatics such as benzene, to be completely oxidized by a strong chemical oxidant (potassium dichromate), under acidic conditions. The COD of a sample is then measured in terms of the amount

of potassium dichromate that is reduced during a 2 h reflux in the presence of 50% sulfuric acid, and a silver sulfate catalyst.

Filtered water samples were scanned with a fluorescence spectrometer (Varian Cary Eclipse) at excitation wavelengths ranging from 260 to 600 nm, where emission wavelengths were obtained from 250 to 600 nm in 1 nm increments. Fluorescence spectrometry is a screening tool used to quantify NAs within OSPW (Mohamed et al. 2008). No sample preparation was necessary. Afterwards, sample absorbance was collected at 290 and 300 nm using a UV-VIS spectrometer (Biochrom Ultrospec® 500/1100) in order to correct for inner and outer filtering effects caused by solute self absorption (Tucker et al. 1992). Primary inner filtering can be described as the absorbance of excitation UV light by other molecules within the sample, where only a fraction of the light reaches the specific molecules of interest. The primary filtering effect was considered to be more significant. Mathematical correction factors have been established in order to adjust the fluorescence signal intensities based on the assumption of monochromatic excitation and emission beams for simplification (Tucker et al. 1992). These factors were used to correct for primary and secondary filtering, and was described by the following equations (4-1 to 4-3) adapted from (Tucker et al. 1992):

$$f_{prim} = \frac{2.303A(y-x)}{10^{-Ax}-10^{-Ay}} \quad (4\text{-}1)$$

$$f_{sec} = \frac{(v-u)(1/b)lnT}{T_{at\ v/b}-T_{at\ u/b}} \quad (4\text{-}2)$$

$$f_{corrected} = f_{prim}f_{sec}Intensity \quad (4\text{-}3)$$

Where,

- A = Absorbance per centimeter obtained experimentally (ex. UV-VIS Spectrometer)

- y,v and u, x = Represent the width of the excitation and emission beams

- b = Path length of the beam through the sample cell

- T = Transmittance related to the absorbance through the cell (10^{-A})

- Intensity = Measured through the fluorescence spectrometer in auxiliary units

Fluorescence spectrometry was calibrated with high performance liquid chromatography – Mass Spectrum (HPLC-MS). Aqueous samples contain 10%, 20%, 50%, and 100% of OSPW were sent to Axys (Victoria, BC, CA) for HPLC-MS analysis. The concentration determined through HPLC-MS analysis were used to establish a standard curve to calculate relative concentration based on fluorescence intensity.

Figure 3-2 showed the calibration curve used to calculate NAs concentration.

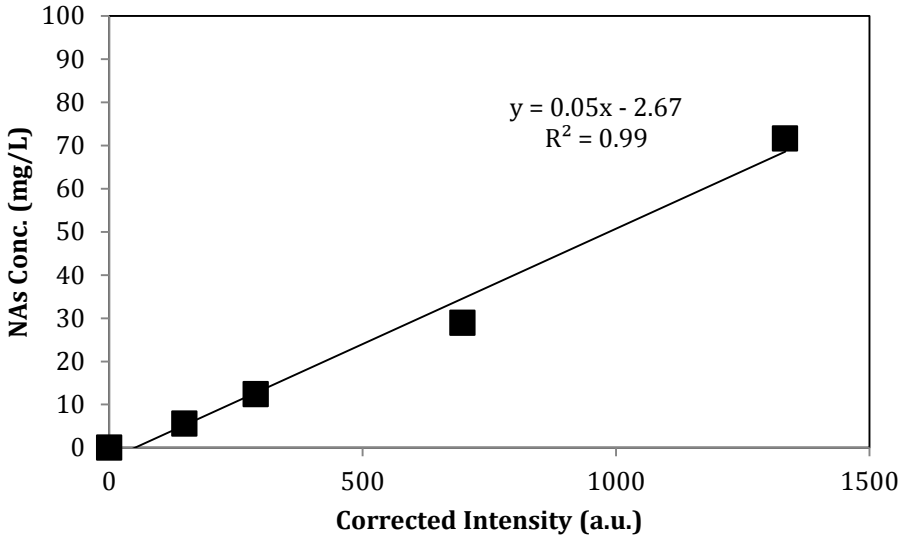

Figure 3-2. Correlation between NAs concentration measured with HPLC and fluorescence intensity

As it is shown in the calibration curve, the correlation coefficient is 0.99 indicating a strong linear correlation between the NAs concentration and fluorescence intensity. The range of NAs detected using this curve is from 0 mg/L to 70 mg/L. This calibration curve could be used to calculate the NAs concentration measured with fluorescence spectrometry.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Anaerobic Biodegradation of OSPW with MFT and with Biofilms Originated from MFT

4.1.1 Characterization of the OSPW

Table 4-1 characterizes original OSPW without acetic acid amendment before anaerobic biodegradation with MFT. The OSPW is highly saline with alkalinity of 863.0 mg/L, indicating high total dissolved solids (TDS) concentration. Having been determined to be 341.2 mg/L after filtration to 0.45 μm, the COD value is quite similar to municipal wastewater. As two major contributors to toxicity, naphthenic acids and ammonium concentrations are measured to be 71.6 and 68.7 mg/L, respectively. Nitrate in OSPW is under detection limit, which is expected since the oil sands extraction process does not involve nitrate addition. Therefore, no residue nitrate was found in the tailings water produced from the extraction process. Sulfate is determined to be 140 mg/L by ion chromatography (IC), indicating the presence of significant amount of electron acceptors. The other ion of concern, chloride concentration is measured to be 358.7 mg/L in ion chromatography.

Table 4-1. Characterization of OSPW obtained for the anaerobic biodegradation tests with MFT

Parameter	Units	OSPW	River (2011[a])
pH	-	8.7 ± 0.1	6.5 - 9.0
Alkalinity	mg $CaCO_3$/L	863.0 ± 1.9	-
Sodium	mg/L	68.7	-
Ammonium	mg/L	43.5 ± 4.4	0.1
Chloride	mg/L	358.7	120
Sulfate	mg/L	140.9 ± 6.6	-
Nitrate	mg/L	ND	13
COD	mg/L	341.2 ± 20.4	-
NAs	mg/L	71.6	-

[a] Canadian aquatic water quality guidelines (CCME, 2011)

According to CCME water quality guidelines for the protections of aquatic life, ammonium concentration in river water are limited to 0.1 mg/L, which is much lower than OSPW, indicating potential needs for biological nutrients removal treatment. COD has been commonly used to measure the amount of organic compounds in water, but they are not regulated by CCME water quality guidelines. Further study on the environmental impacts of releasing the organic compounds in OSPW is necessary to develop a water discharge guideline for oil sands industry.

4.1.2 Anaerobic Biodegradation of OSPW with MFT

To observe the substrate utilization in aqueous phase, sacrificial samples were obtained from bench bioreactors and measured by COD test. COD is a common measurement method to monitor the concentration of organic compounds and

regulated for safe discharge of many other industrial wastewaters. COD concentration in aqueous phase was monitored for 8 weeks until the concentration change became minimal. The results indicate that the biodegradation of dissolved organic compounds was initially fast, but the original organic matrix in OSPW could not be completely mineralized biologically in 8 weeks. Since the HRT in a bioreactor is usually within 24 hours, the initial rapid degradation is more critical for engineering applications.

The sterile control was achieve by autoclaving the batch bioreactors for 3 consecutive days. However, huge amount of water was lost during the autoclaving process leading to a deviated concentration profile in the aqueous phase and insufficient moisture content in the reactor for sampling. The data obtained from the sterile control is not comparable with the monitoring data. As a result, the data is not shown on the reactor performance graph.

Figure 4-1 shows the results obtained from the batch bioreactors treating OSPW using MFT at OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L during the period of sulfate reducing. Each data point represents the average concentration measured in two duplicate batch bioreactors. The error bars were set up for one standard deviation, which covers 68% of probabilities. The COD of original OSPW is around 300 mg/L. After supplemented with acetic acid, aqueous phase contains 600 mg/L of COD. Lower initial COD concentration in aqueous phase was achieved by diluting the acetic acid supplemented OSPW. As a readily biodegradable substrate, the acetic acid was consumed rapidly in the first several days of incubation, resulting in a rapid and significant decrease on COD.

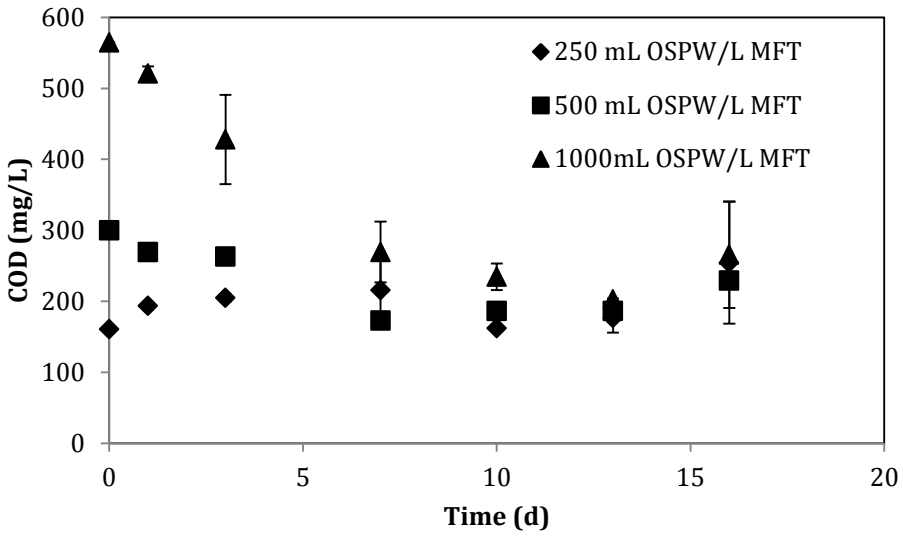

Figure 4-1. Biodegradation of dissolved organic compounds in batch bioreactors with OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L

As it is shown in the graph, the biodegradation in the batch bioreactors with OSPW to MFT ratio of 1000 mL/L showed a significant decrease on COD concentration in first 10 days incubation. The loss of dissolved organic compounds in OSPW indicates there is an active microbial community in MFT that could utilize readily biodegradable organic compounds as primary substrate to sustain the biological activity.

When the OSPW was diluted to reduce the OSPW to MFT ratio, the COD decreased from 300 mg/L to 200 mg/L in 5 days indicating a loss of readily biodegradable organic compounds. Comparing to the batch bioreactors with OSPW to MFT ratio of 1000 mL/L, the biodegradation rate and removal rate decreased after the acetic acid supplemented OSPW was diluted. Further dilution leads to an initial COD of 150 mg/L. Consequently, COD fluctuated intensively between 150 to 200 mg/L during the anaerobic incubation. Moreover, no rapid decrease on COD was observed in the beginning of the incubation. This may be because that along with the dilution of acetic acid amended OSPW the amount of biodegradable organic compounds was largely reduced and may not be sufficient

to sustain biological activity in the MFT. Organic compounds leached out from excessive bitumen carried by MFT may also interfere COD concentration measured in aqueous phase.

Sulfate is present in significant concentration in OSPW and it can serve as electron acceptors for sulfate-reducing bacteria, which consumes organic compounds under anaerobic conditions and produce hydrogen sulfite. The amount of COD used for sulfate reduction is 0.67 g COD/g sulfate (Arceivala 1999). Figure 4-2 shows the results of sulfate utilization in the batch bioreactors treating OSPW using MFT at OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L. The amount or COD consumed during sulfate reduction was around 3 mg COD/mg sulfate, which is larger than the number found in the literature, indicating that other COD biodegradation mechanisms may also exist in the system.

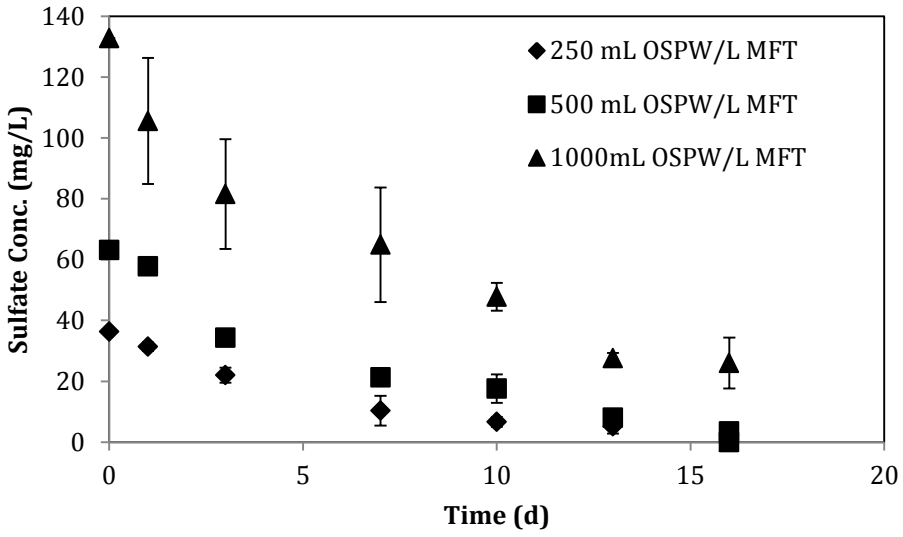

Figure 4-2. Utilization of sulfate in the biodegradation tests in batch bioreactors with 250 mL OSPW/L MFT, 500 mL OSPW/L MFT, and 1000 mL OSPW/L MFT

In all cases, sulfate was depleted in 15 days along with the COD removal. The rapid depletion of sulfate indicates that biodegradable organic compounds

provided sufficient organic carbon source for sulfate reducing process and the indigenous microbial activity was promoted by adding supplementary carbon source. It also suggests that the indigenous microbial community from MFT could be a ready source of microorganisms for bioreactor start up in oil sands tailings water treatment.

In order to investigate the whether the excessive amount of bitumen would cause fluctuation on tests results, MFT was diluted with OSPW to reduce the amount of bitumen introduced into the system. The amount of MFT added in the batch bioreactors was reduced to 500 mL and 250 mL. As a result, the OSPW to MFT ratios were further increased to 3000 mL/L and 7000 mL/L respectively. Though this may reduce the initial amount of indigenous microorganisms introduced to the system, with the presence of readily biodegradable organic the microbial community should be able to utilize the substrate and develop sufficient biomass to biodegrade the organic compounds in OSPW.

Figure 4-3 shows the biodegradation of dissolved organic compounds in batch bioreactors with OSPW to MFT ratios of 1000 mL/L, 3000 mL/L and 7000 mL/L. In all three trails, COD concentrations were reduced from 600 mg/L to 200 mg/L approximately, indicating the microbial community in 250 mL or more of MFT could develop sufficient biomass to biodegrade the dissolved organic compounds in OSPW. In addition, 1000 mL of MFT carries more microorganisms initially resulting in the rapidest depletion of biodegradable organic compounds in the beginnings of three biodegradation trials. It indicates that biodegradation rate is positively correlated to the amount of microorganisms and with increasing biomass concentration the reaction time could be largely shortened. Cultivating MFT with supplemented OSPW in a continuously running bioreactor could increase biomass concentration in the system where the critical microorganisms in MFT could utilize primary substrate to support growth and transform the recalcitrant organic compounds.

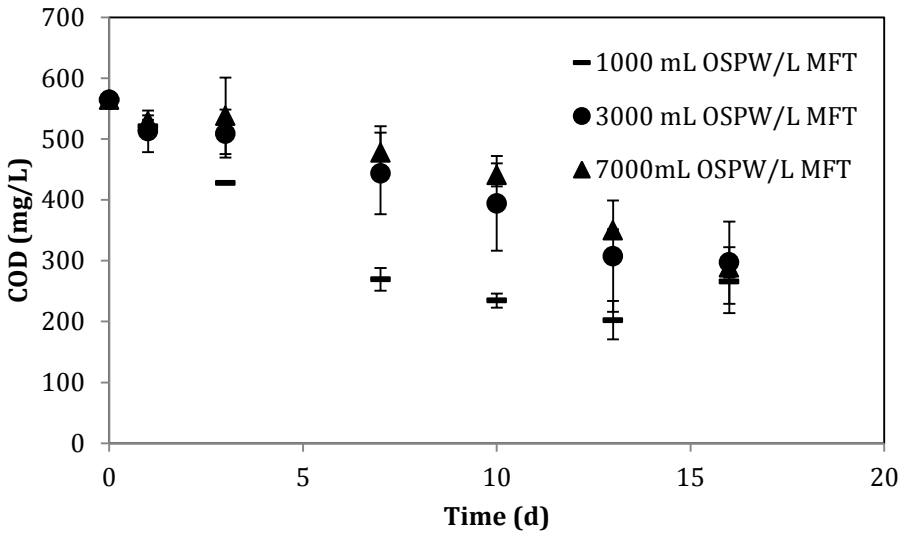

Figure 4-3. Biodegradation of dissolved organic compounds in the batch bioreactors treating OSPW using MFT at OSPW to MFT ratios of 1000 mL/L, 3000 mL/L and 7000 mL/L

Figure 4-4 shows that sulfate was depleted in 18 days along with the removal of COD under anaerobic conditions. Similarly to the COD results, when the MFT was reduced, the sulfate utilization slowed down, indicating the sulfate utilization rate may be affected by biomass concentration.

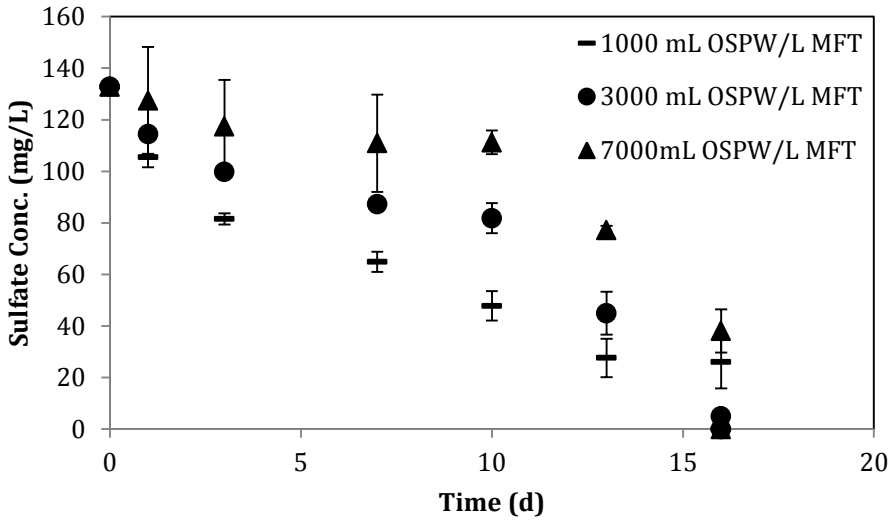

Figure 4-4. Utilization of sulfate in the biodegradation tests in the batch bioreactors treating OSPW using MFT at OSPW to MFT ratios of 1000 mL/L, 3000 mL/L, and 7000 mL/L

Bioreactors could enhance biodegradation process and increase utilization rate through concentrating microorganisms. With initial sulfate concentration of 140 mg/L, it took 3 days to remove 35% of sulfate in the batch bioreactor indicating a very low sulfate utilization rate. Baskaran and Nemati (2006) has found the sulfate reducing rate in a continuous immobilized cell bioreactor with sulfate reducing bacteria (SRB) from oil sands tailings ponds could achieve as high as 1.7 g/L h with corresponding conversion of 35.4% when the reactor is fed with modified Coleville Synthetic Brine (m-CSB). It indicates that with sufficient biodegradable organic compounds and continuously running bioreactor the sulfate reducing rate could be further increased.

Under anaerobic conditions, nitrate could be utilized as electron acceptor for biological oxidation of organic compounds or as nutrient for microbial growth. In order to determine whether the organic compounds in OSPW could be further biodegraded with nutrients supplementation, 1.8 mM of nitrate was added to the system to yield a C:N ratio of 10:1. The results show that the COD reduction could be promoted by nitrate utilization but due to lack of information of nitrate

utilization pathway, it is hard to determine whether nitrate was utilized as growth nutrients or electron acceptors.

Figure 4-5 shows the nitrate, sulfate and COD concentration profiles in the batch bioreactors with OSPW to MFT ratios of (a) 250 mL/L, (b) 500 mL/L, (c) 1000 mL/L, (d) 3000 mL/L, and (e) 7000 mL/L. The OSPW to MFT ratio could be viewed as an indication of substrate to biomass ratio and with increased OSPW to MFT ratio, the concentration profiles demonstrated the effects of different substrate to biomass ratios.

Figure 4-5 shows that once added, in most of the batch bioreactors, nitrate was depleted in 10 days suggesting that the presence of acetate not only promoted sulfate reduction process but also activated the denitrifiers that could utilize nitrate as electron acceptors and consume organic compounds. It is clearly shown in the graph that when the OSPW to MFT ratio was increased to 7000 mL/L, 10 mg/L of nitrate was not still remained in the aqueous phase indicting that reducing the substrate to biomass ratio may result in slower nitrated utilization and less active microbial community in the batch reactors.

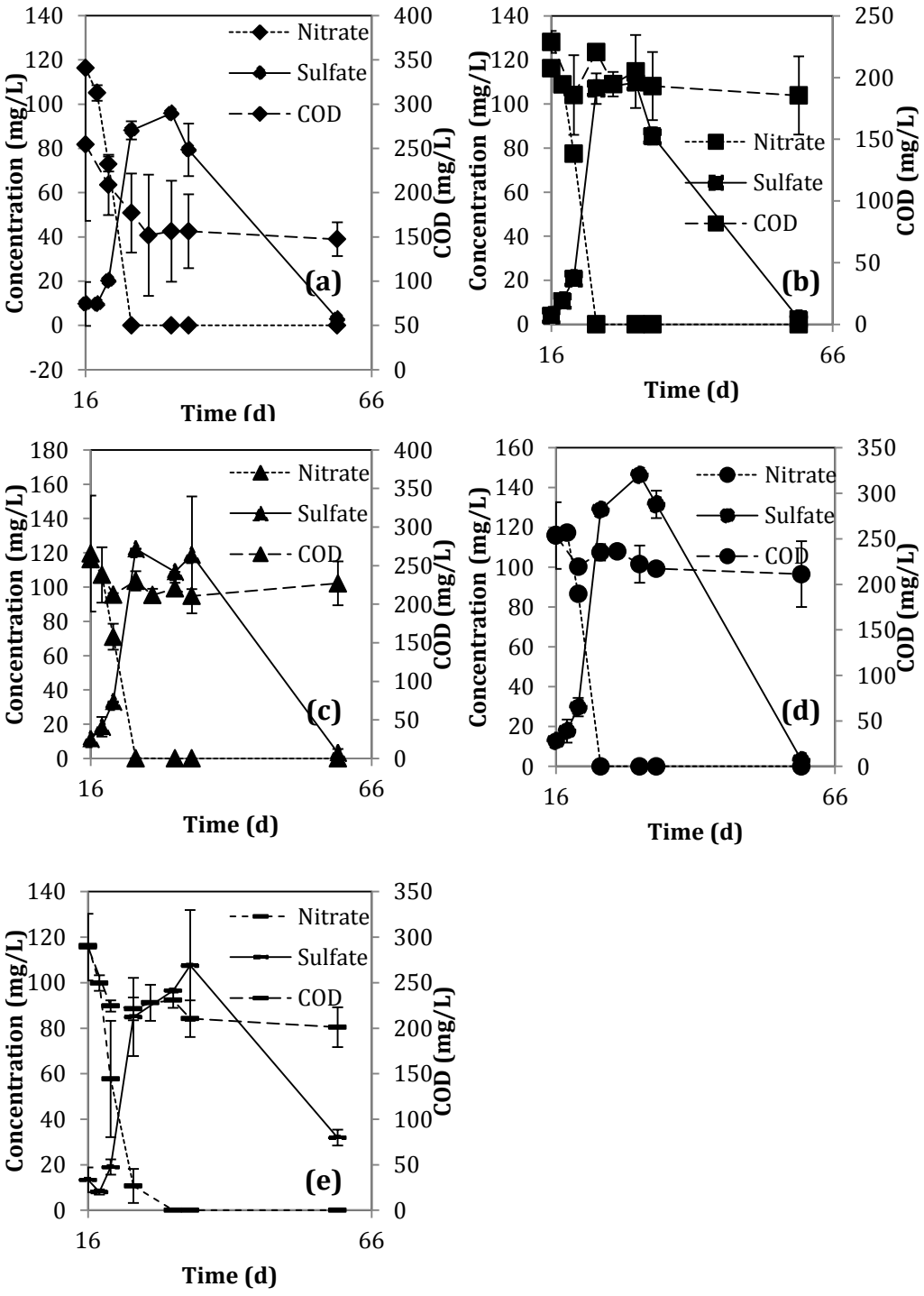

Figure 4-5. Nitrate and sulfate concentration profiles in the batch bioreactors with OSPW to MFT ratios of (a) 250 mL/L, (b) 500 mL/L, (c) 1000 mL/L, (d) 3000 mL/L, and (e) 7000 mL/L

As it is shown in Figure 4-5, heterotrophic denitrifiers (hNRB) may compete with SRB for biodegradable organic electron donors and potentially inhibit sulfate reduction. Consequently, further depletion of sulfate was not observed. The sudden increase on sulfate concentration may result from nitrate reducing sulfate oxidizing bacteria (NR-SOB) metabolism, where sulfide was utilized as electron donors in denitrification process. Hubert and Voordouw (2007) isolated several NRBs from packed-bed reactors inoculated with oil field produced water and investigated their denitrification process that coupled with oxidation of organic and inorganic electron donors in pure cultures. The results showed that both NR-SOB activity and hNRB activity existed in the bioreactors and the microorganisms that capable of both NR-SOB and hNRB activity dominated in the bioreactors suggesting that utilization of organic and inorganic electron donors occurred in anaerobic biodegradation trials.

Utilization of organic electron donors when no primary substrate was present in aqueous phase could further reduce COD concentration. After acetate was depleted (data not shown), the rate of COD decrease was higher in denitrification period than sulfate reduction period indicating biodegradation of toxic and recalcitrant organic compounds may be promoted by denitrification rather than sulfate reduction. The microbial metabolism of denitrification coupled with oxidation of inorganic and organic electron donors was not thoroughly investigated in petroleum-contaminated water. Further study may be helpful to identify the critical factor that affects rate of biodegradation of recalcitrant organics coupled with biological anaerobic oxidation.

After nitrate was depleted, SRBs started to utilize sulfate as electron acceptors and potentially consume organic electron donors. The second sulfate depletion took longer time primarily because after acetate was depleted (data not shown) the organic electron donors left in OSPW are not readily available for SRBs. The biodegradation rate and removal rate of dissolved organics coupled with sulfate reduction were also lower than that coupled with denitrification. Further research

on biodegradation pathway may reveal whether the microorganisms in MFT could biodegrade more recalcitrant organic compounds through denitrification.

To observe whether NAs were consumed along with the utilization of primary substrate or through biological anaerobic oxidation, water samples were obtained and scanned with fluorescence spectrometry. Fluorescence spectrometry has been recently used to detect petroleum hydrocarbon contaminants utilizing their fluorescing nature under UV light excitation. It could also identify aromatic hydrocarbon based on their fluorescence spectral signatures. No clear evidence of NAs biodegradation was observed. Contrarily, results from batch bioreactors with OSPW to MFT ratios of 250 mL/L and 500 mL/L indicate that the NAs concentration in aqueous phase was increasing during the experimental period. When the amount of MFT was reduced, NAs concentration decreased slightly to a final concentration of 25 mg/L during 8 weeks of experiments. The results were unexpected and further investigation was carried out with increased OSPW to MFT ratio to examine whether the NAs concentration could be reduced when the MFT was reduced.

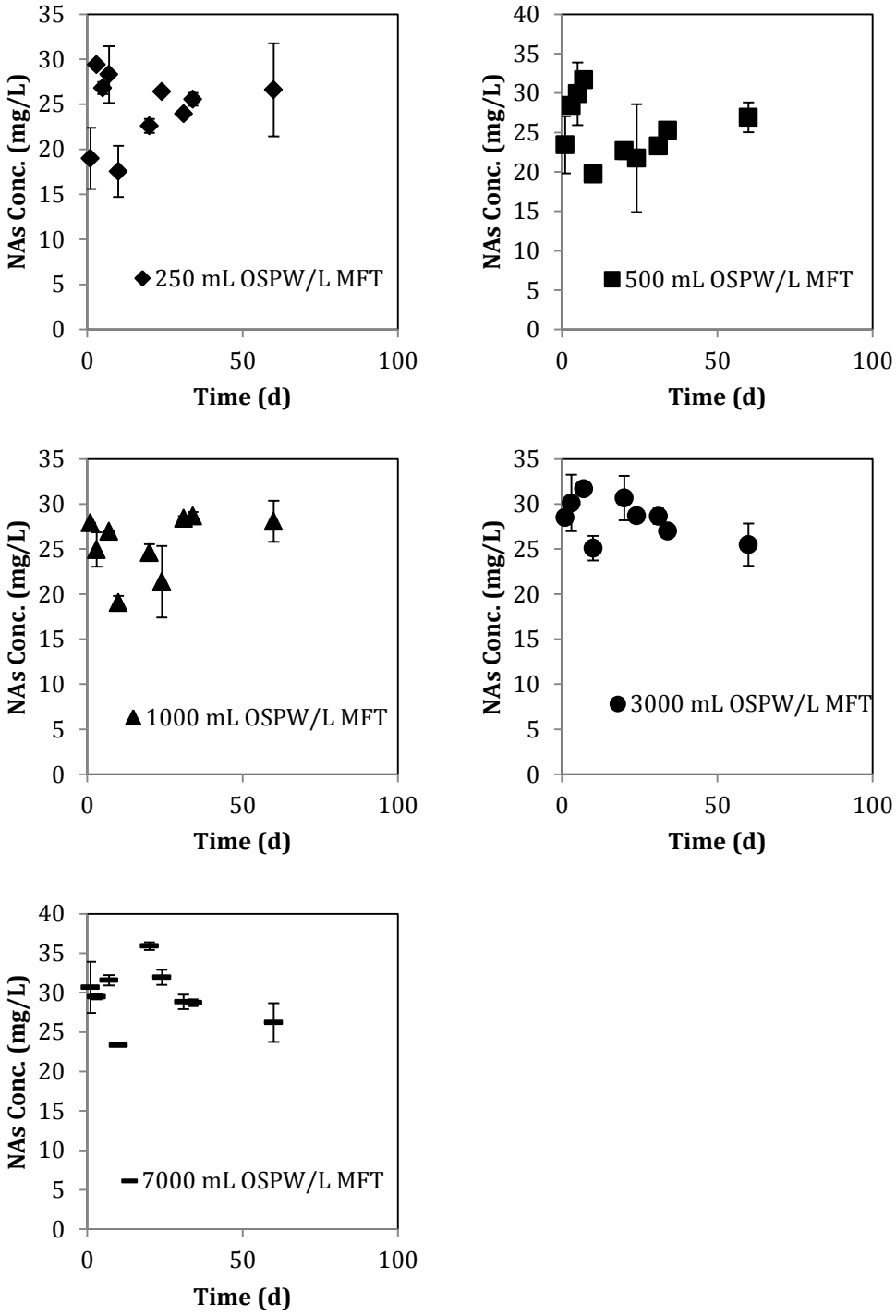

Figure 4-6. Biodegradation of NAs in batch bioreactors treating OSPW with MFT at OSPW to MFT ratios of 250 mL/L, 500 mL/L, 1000 mL/L, 3000 mL/L, and 7000 mL/L

As it is shown in Figure 4-6, the NAs concentration increased from 19 mg/L and 23 mg/L to 26 mg/L at the end of the experiments resulting in a negative removal rate for batch bioreactors with OSPW to MFT ratios of 250 mL/L and 500 mL/L. In addition, regardless of the initial NAs concentration, the final concentrations of NAs in these two batch bioreactors were around the same level, which is unexpected. When the amount of MFT in the batch bioreactors was reduced and the OSPW to MFT ratios were increased, slight NAs removal started to appear in the results, indicating that the amount of MFT added in the batch bioreactors significantly affects the final NAs concentration.

As a group of organic acids naturally originated from naphthenic amphiphiles that are present in Athabasca heavy crude oil at concentrations up to 4 wt% (Kiran et al. 2011), NAs may slowly dissolve into aqueous phase from the bitumen carried by MFT during the incubation leading to an increase on aqueous concentration. When the amount of MFT was reduced, the bitumen introduced to the system was reduced at the same time. As a result, the continuous dissolving of NAs could be eliminated leading to a lower final concentration of NAs.

NAs did not decrease during the experimental period, which may be due to the interference from bitumen. Among all batch bioreactors, the one with OSPW to MFT ratio of 250 mL/L shows the most significant increase, resulting in a 40% elevation in NAs concentration at the end points. This may be due to the lack of biological activity and presence of excessive amount of MFT, because it contains the least amount of readily biodegradable organic compounds and most amount of MFT. The increase was slightly mitigated when OSPW to MFT ratio is increased. Since the acetic acids amendments were added proportionate to the amount of OSPW, increasing the OSPW to MFT ratio would increase the acetic acids supplementation amount resulting in a elevated concentration of available readily biodegradable organic compounds in the aqueous phase leading to a more active microbial community.

4.1.3 Anaerobic Biodegradation of OSPW with Biofilms Originated from MFT

Since the continuous dissolving of NAs into aqueous phase may cause fluctuation in tests results, indigenous biofilm originated from MFT was used as sole source of microorganisms to eliminate the interference of bitumen. Golby et al. (2012) successfully cultivated mixed species biofilms from MFT under aerobic, microaerobic, and anaerobic growth conditions. The anaerobic indigenous biofilms used in these biodegradation tests were cultivated on HDPE biofilms carriers that are commonly used in MBBR. A layer of thin black biofilms was developed on the carrier before they were incubated with the acetic acids amended OSPW.

Figure 4-7 shows the biodegradation of dissolved organic compounds in OSPW with indigenous biofilms originated from MFT at loadings of 8000 mL OSPW /m^2 of surface area and 16000 mL OSPW/m^2 of surface area. Acetic acids contribute to 50% of the total COD, which provided sufficient readily biodegradable organic compounds.

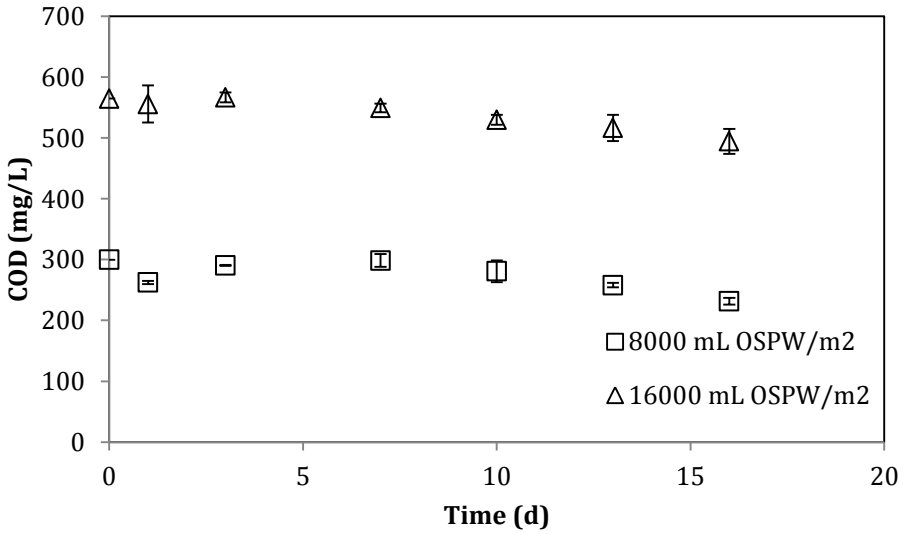

Figure 4-7. Biodegradation of dissolved organic compounds in OSPW with biofilms originated from MFT at OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2

As it is shown in figure 4-7, in the beginning of the incubation, the COD concentration slowly decreased suggesting that the microbial community is not active enough to utilize the readily biodegradable organic compounds rapidly. Since the MFT-originated biofilms was developed without acclimatization, the biofilms established on the carriers would be less active and contain insufficient biomass to biodegrade substantial amount of organic compounds in the beginning. The COD removal was gradually accelerated after 8 days of biodegradation trials indicating that the biofilms established on the carriers would need to acclimatize to the new environment to sustain the biodegradation process.

Sulfate reduction would consume organic compounds, so sulfate utilization rate is positively related to COD reduction rate. Similar to the biodegradation of COD, sulfate utilization is relatively slow compared to the biodegradation tests with MFT. Figure 4-8 shows that during the first 16 days of experiments, the sulfate was not depleted. This may be due to the low biomass concentration present in the system. Meanwhile, the indigenous biofilms originated from MFT may need an acclimation time after isolated from MFT environment. The low sulfate and COD reduction rate suggests that the indigenous biofilms cultivated on MBBR carriers would need a long term exposure to the OSPW after isolated from MFT to induce and sustain the enzymes and bacteria required for degradation.

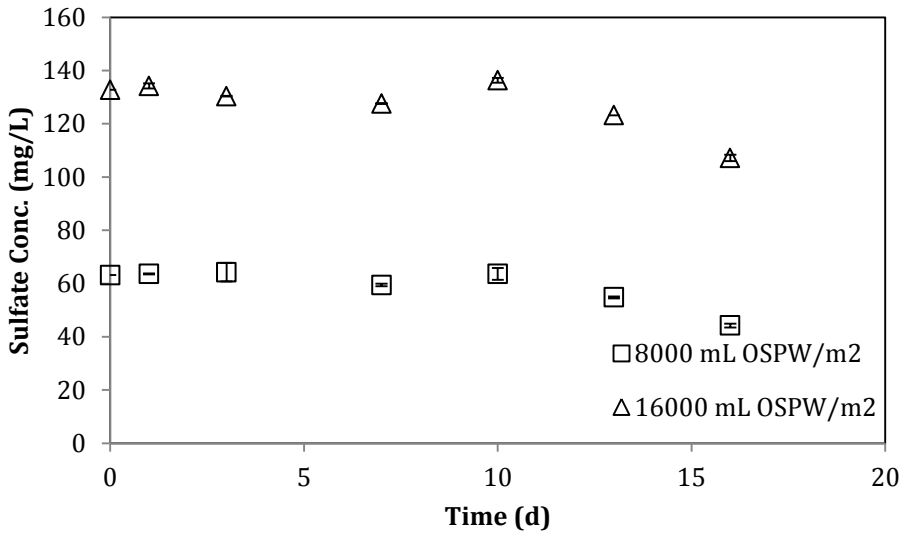

Figure 4-8. Utilization of sulfate in the biodegradation tests in the batch bioreactors with OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2

As it is shown in Figure 4-8, the sulfate concentration started to decrease after seven days of incubation indicating the SRB in the indigenous biofilms started to utilize electron acceptors and carry out anaerobic biological oxidation metabolism. A continuous running bioreactor would be necessary to isolate more indigenous biofilms and provide sufficient biomass to enhance the biodegradation process.

Figure 4-9 shows that once added, nitrate was depleted along with a sudden drop on COD concentration suggesting nitrate may have provided nutrients necessary for microbial growth. Nitrate addition clearly accelerated the COD reduction and promoted microbial activity suggesting that during the long-term acclimation, nutrients may be critical to support the growth of indigenous biofilms.

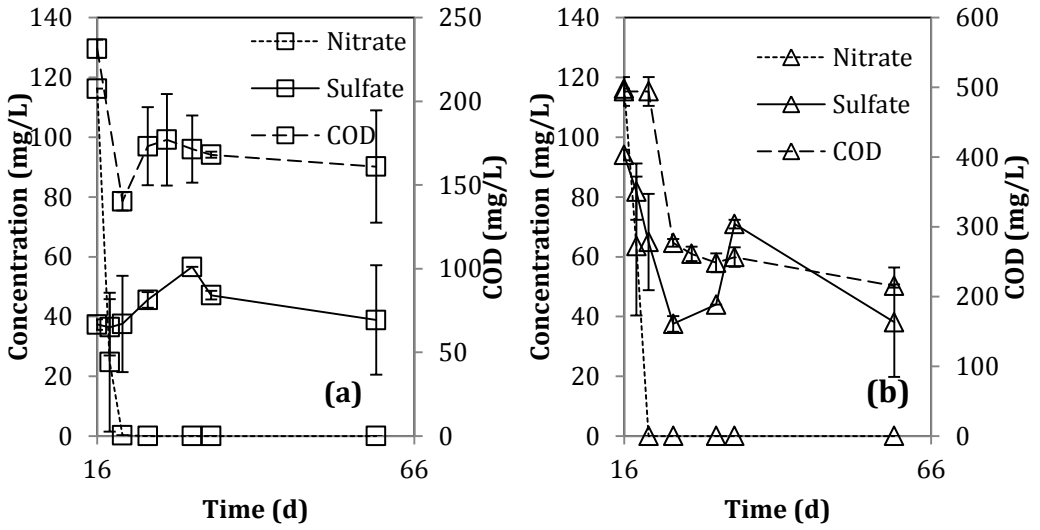

Figure 4-9. Nitrate and sulfate concentration profiles in the batch bioreactor treating OSPW using MFT-originated biofilms at OSPW loadings of (a) 8000 mL/m^2 of surface area and (b) 16000 mL/m^2 of surface area

Figure 4-9 also shows that a delayed increase on sulfate concentration occurred after nitrate was depleted. As discussed previously, the sulfate could be oxidized when the nitrate was reducing. And in the biofilm reactor, the substrate need to be diffused into the biofilms and utilized for the biological process. The mass transfer process may cause delay in concentration changes in the aqueous phase. In addition, the MFT-originated biofilms used in the batch bioreactors have not gone throught a acclimatization process. The low activity may also contribute the delay of concentration changes in the aqueous phase. Further study on mixed species biofilms that are cultivated from MFT would help to understand the microbial growth during bioreactor startup period.

Figure 4-10 shows the biodegradation of NAs during primary substrate utilization, denitrification and sulfate reduction process. The OSPW was diluted with demineralized water to reduce the initial COD concentration. As a result, the NAs concentration was reduced to 17.9 mg/L while the undiluted acetic acid supplemented OSPW contains 32.8 mg/L of NAs. After 8 weeks of incubation, the NAs concentrations in these two systems are 20.0 mg/L and 25.7 mg/L

respectively. Notably, the NAs concentration increased when the OSPW was diluted indicating reducing the OSPW amount may decrease the treatment efficiency since the acetic acids amendment would be reduced.

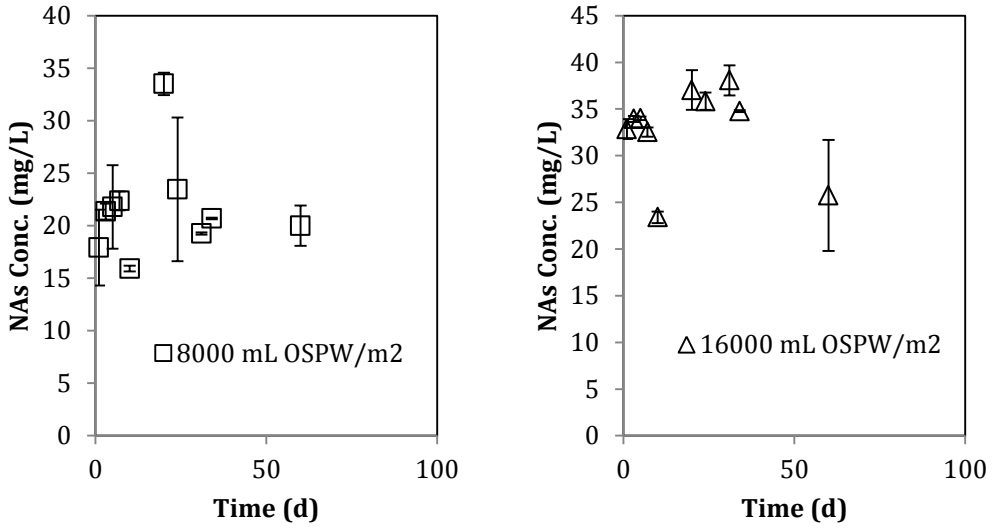

Figure 4-10. Biodegradation of NAs in OSPW with MFT-originated biofilms in the batch bioreactor with OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2

4.2 Anaerobic Biodegradation of HiPOx-treated OSPW with MFT and Biofilms Originated from MFT

4.2.1 Characterization of the HiPOx-treated OSPW

Table 4-2 characterizes the HiPOx-treated OSPW before the anaerobic biodegradation with MFT. HiPOx treatment utilizes ozone and hydrogen peroxide to break down long chain organic compounds that the microorganisms could not biodegrade. However, the organic compounds in OSPW are present in such a large amount that it is not economically viable to use HiPOx treatment for a complete destruction. Therefore, only partial COD reduction was achieved in the HiPOx-treated OSPW. Compared to the raw OSPW, NAs concentration was significantly reduced indicating the long chain organic compounds were broke down after HiPOx treatment. COD was slightly reduced suggesting that HiPOx

treatment did not achieve complete mineralization. It likely transformed long chain organic compounds into smaller molecules that are more biodegradable. Biological process is to be used for further reduction on COD.

Table 4-2 Characterization of HiPOx-treated OSPW obtained for the tests conducted with MFT

Parameter	Units	OSPW	River (2011[a])
pH	-	7.2 ± 0.0	6.5 - 9.0
Alkalinity	mg $CaCO_3$/L	674.3 ± 1.5	-
Sulfate	mg/L	390.9 ± 20.3	-
Nitrate	mg/L	ND	13
COD	mg/L	241.2 ± 17.0	-
NAs	mg/L	1.2 ± 0.2	-

[a]Canadian aquatic water quality guidelines (CCME, 2011)

As it is listed in Table 4-2, the pH was reduced to 7.2 along with a loss of 150 mg $CaCO_3$/L in alkalinity indicating that alkalinity may be consumed during reaction with hydroxyl radicals resulting in a neutral pH value. Sulfate concentration was elevated to 390.9 mg/L in HiPOx-treated OSPW. This may be due to oxidation of organic and inorganic sulfides. Sulfate removal is critical. Nitrate is under detection limit suggesting potential nutrients deficiency during anaerobic incubation.

4.2.2 Anaerobic Biodegradation of HiPOx-treated OSPW with MFT

In order to observe the biodegradation of recalcitrant organic compounds in OSPW after advanced oxidation, water samples were obtained from aqueous phase and measured with COD. In order to observe whether the biodegradation of oxidized recalcitrant organic compounds in OSPW has been completed, the

HiPOx-treated OSPW was mixed with MFT under anaerobic conditions for 30 weeks. The results indicate that the decrease of COD was initially fast, but the COD value fluctuated significantly in the end of the experiments. This may be due to the lack of biodegradation activity and interference from MFT. Compared to biodegradation of original organic matrix supplemented with acetic acid, the initial biodegradation rate is much slower. This may be caused by the different organic compositions in two types of OSPW. Acetic acid contributes to 50% of COD in the amended OSPW and the rapid depletion of COD was a result of the utilization of readily biodegradable organic compounds, while HiPOx-treated OSPW contains oxidized products of original organic matrix from OSPW that is less biodegradable than acetic acid. Consequently, the utilization of organic substrate in HiPOx-treated OSPW is much slower.

Figure 4-11 shows the loss of dissolved organic compounds in anaerobic biodegradation tests with OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L. COD was used to quantify the amount of dissolved organic compounds remaining in the aqueous phase. The results indicate that with increasing OSPW to MFT ratio, the initial rate of biodegradation of dissolved organics increased. The amount of COD removed for the batch bioreactors with 1000 OSPW mL/L MFT is the greatest among these three batch bioreactors. This is attributed to the greater amount of biodegradable organic compounds present in the batch bioreactors starting with highest OSPW to MFT ratio.

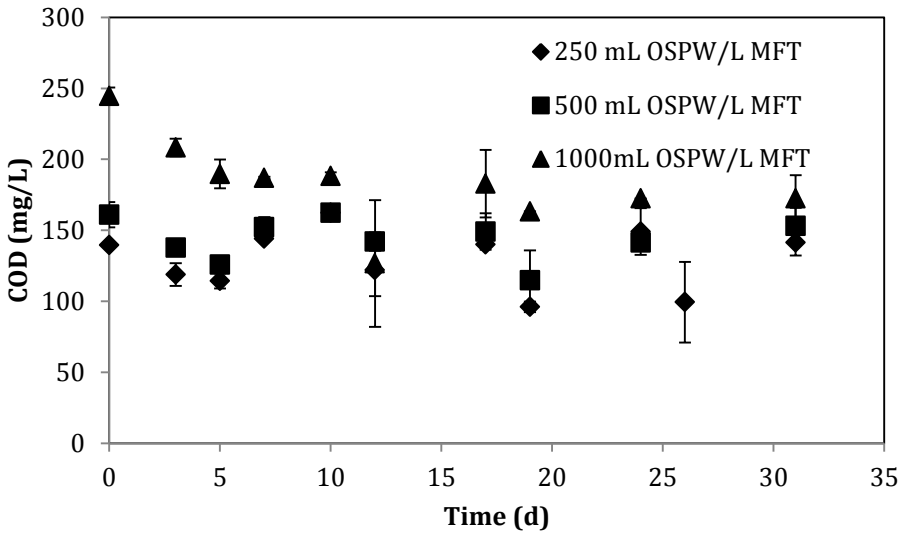

Figure 4-11. Biodegradation of dissolved organic compounds in HiPOx-treated OSPW at OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L

Sulfate reduction under anaerobic conditions consumes organic compounds. The elevated sulfate concentration in HiPOx-treated OSPW needs more COD for complete sulfate reduction. Figure 4-12 indicates that with increasing initial sulfate concentration, sulfate depletion time increased.

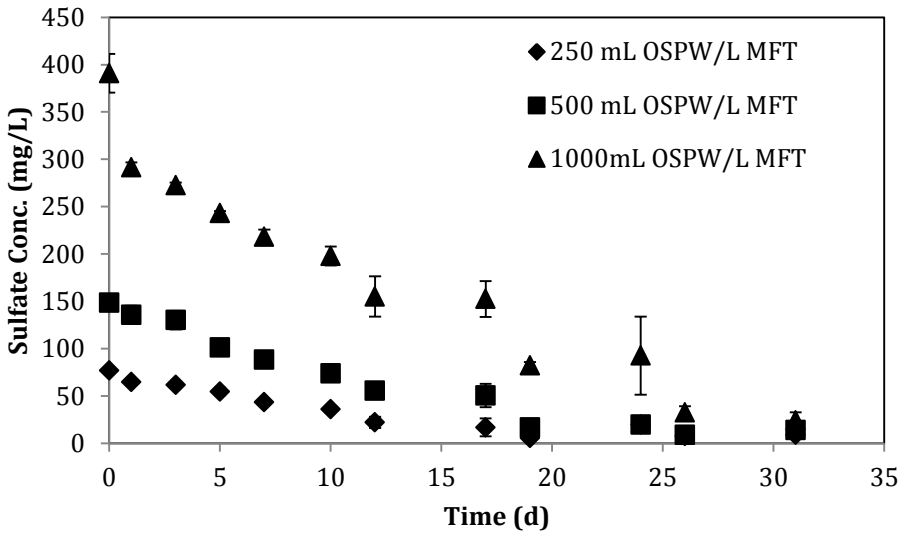

Figure 4-12. Utilization of sulfate in the biodegradation tests with OSPW to MFT ratios of 250 mL/L, 500 mL/L and 1000 mL/L

As it is shown in Figure 4-12, the rate of sulfate reduction is almost constant throughout each biodegradation test. Along with sulfate serving as electron acceptor, the organic compounds served as the electron donors. Compared to COD consumption shown in Figure 4-11, COD removed to sulfate is not consistent with that found in literature. This is attributed to the dynamic equilibrium existed between MFT and OSPW, in which the organic compounds would continuously dissolve into aqueous phase making up the loss of soluble organics. Compared to the results in biodegradation of original organic matrix with presence of readily biodegradable substrate, the sulfate reduction rate is slightly reduced. This is attributed to the change on composition of organic compounds in HiPOx-treated OSPW. The oxidization products of original organic matrix may not be as readily biodegradable organic compounds as acetic acid and the microbial activity would be affected consequently.

Figure 4-13 shows the biodegradation of dissolved organic compounds in HiPOx treated OSPW with in the batch bioreactors with OSPW to MFT ratios of 1000 mL/L, 3000 mL/L and 7000 mL/L. In all three batch bioreactors, COD concentration was reduced to 150 mg/L in spite of the MFT amount indicating

250 mL of MFT could provide sufficient biomass to biodegrade the dissolved organic compounds in ozonated OSPW. In addition, since 1000 mL of MFT carries more microorganisms, the biodegradation rate is the highest among the three batch bioreactors. In other words, the biodegradation rate is positively correlated to the MFT amount indicating increasing the biomass concentration could increase the reaction rate.

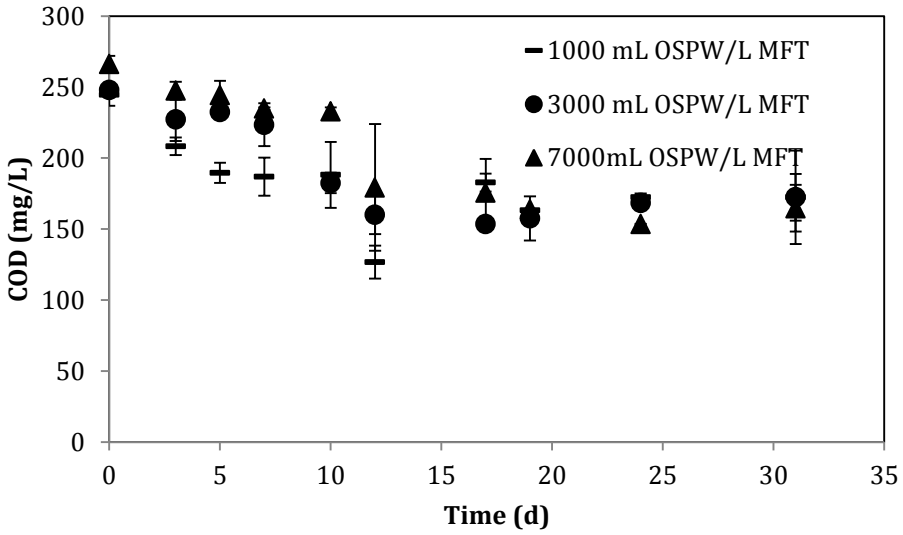

Figure 4-13. Biodegradation of dissolved organic compounds in HiPOx-treated OSPW in the batch bioreactors with OSPW to MFT ratios of 1000 mL/L, 3000 mL/L, 7000 mL/L

Notably, the overall COD removal rates are around 45% for the three batch bioreactors in spite of different OSPW to MFT ratios. Though many researchers have found removal of recalcitrant organic compounds by partitioning onto the biomass could be a significant factor in COD removal, this is not the case. In all three experimental conditions, a consistent removal rate of 45% was achieved at the end of experimental period, though the biomass concentration was different, indicating that the biomass concentration may not affect the overall removal rate of COD. Since the biomass concentration does not affect the overall removal rate, it indicates that the organic compounds in OSPW was biodegraded rather than adsorbed.

HiPOx treatment also increased BOD and transform recalcitrant organic compounds into more biodegradable organics. The residue COD in the batch bioreactors treating HiPOx-treated OSPW was further reduced to 160 mg/L. This is 70 mg/L less than the residue COD in the batch bioreactors treating acetic acid supplemented OSPW, indicating that HiPOx process partially converted the recalcitrant organic compounds to biodegradable organic compounds leading to a lower residue COD concentration.

Figure 4-14 shows the results of sulfate utilization in the batch reactors treating HiPOx-treated OSPW with MFT at OSPW to MFT ratios of 1000 mL/L, 3000 mL/L and 7000 mL/L. As it is shown in the figure, sulfate utilization rate was largely reduced, indicating both primary substrate and biomass concentrations could affect sulfate utilization and sulfate reducing rate.

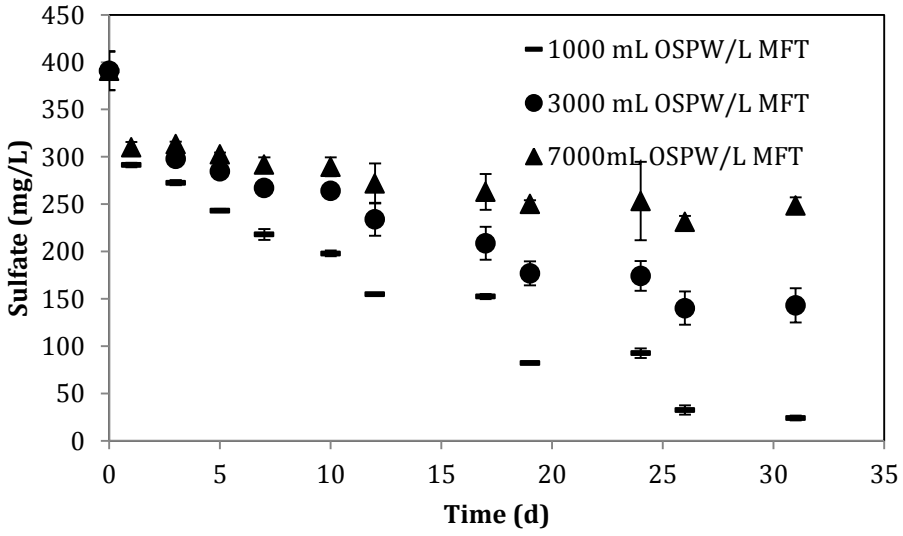

Figure 4-14. Utilization of sulfate in the biodegradation tests in the batch bioreactors with OSPW to MFT ratios of 1000 mL/L, 3000 mL/L, and 7000 mL/L

Figure 4-15 shows the interaction between denitrification and sulfate reduction process in the batch reactors treating HiPOx-treated OSPW with MFT. The nitrate was added to the OSPW after sulfate was depleted. As it is shown in the graph,

the nitrate was utilized once added to the OSPW and depleted in the first eight days. At the same time, sulfate concentration increased during nitrate depletion indicating potential oxidation of sulfide.

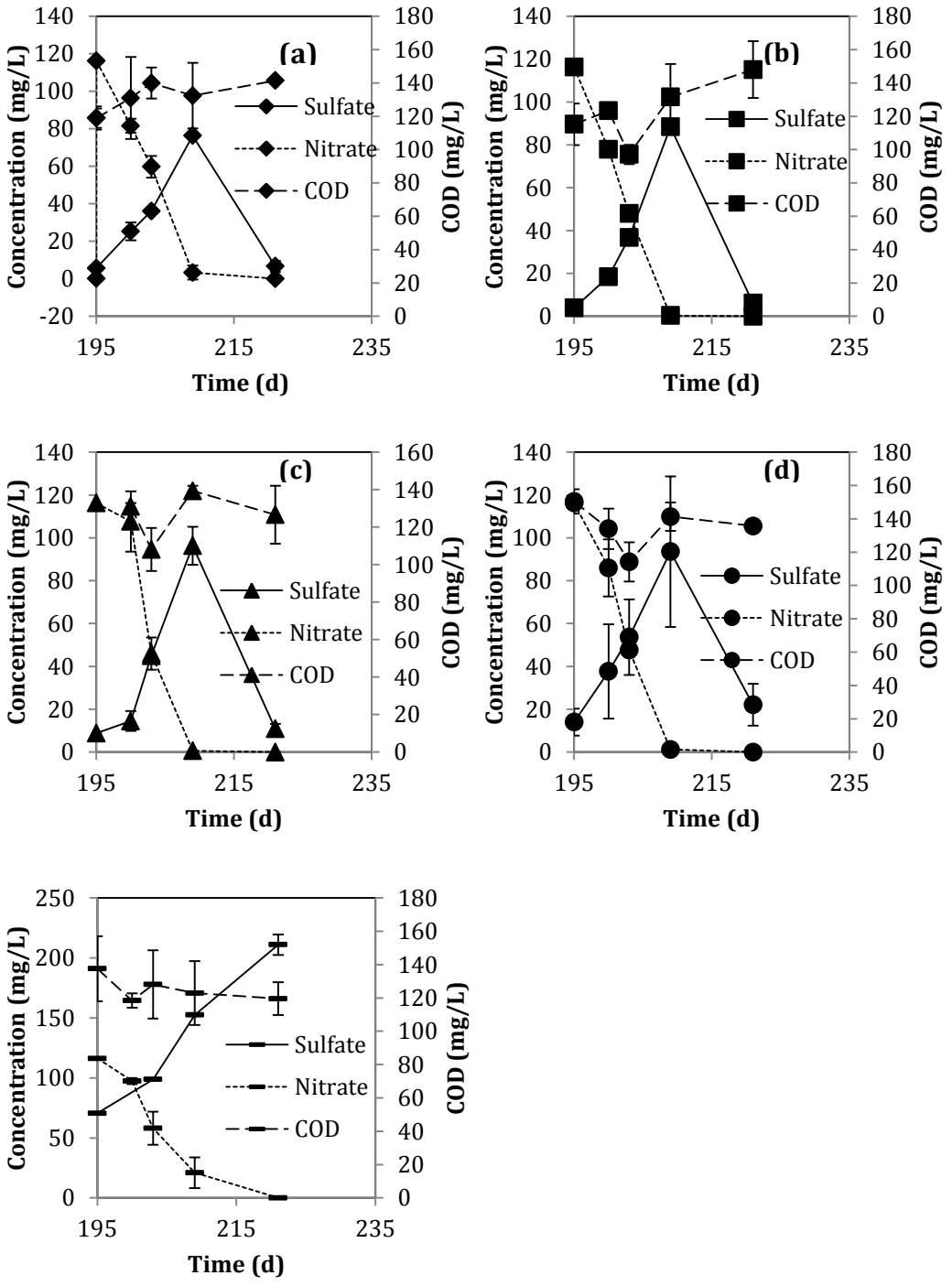

Figure 4-15. Nitrate and sulfate concentration profiles in the biodegradation tests of HiPOx-treated OSPW with MFT in the batch bioreactors with OSPW to MFT ratios of (a) 250 mL/L, (b) 500 mL/L, (c) 1000 mL/L, (d) 3000 mL/L, and (e) 7000 mL/L

Notably, when MFT was reduced to 250 mL, the sulfate concentration did not decrease after nitrate was depleted. The nitrate depletion is also much slower in this batch bioreactor indicating insufficient microbial activity.

Figure 4-16 shows that NAs concentration increased drastically during the experimental period suggesting MFT not only served as sources of microorganisms but also introduced more NAs to the system, which may be an issue during bioreactor operation. The mechanism of MFT leaching NAs into aqueous phase was not clear. It may be related to biodegradation and solubility equilibrium.

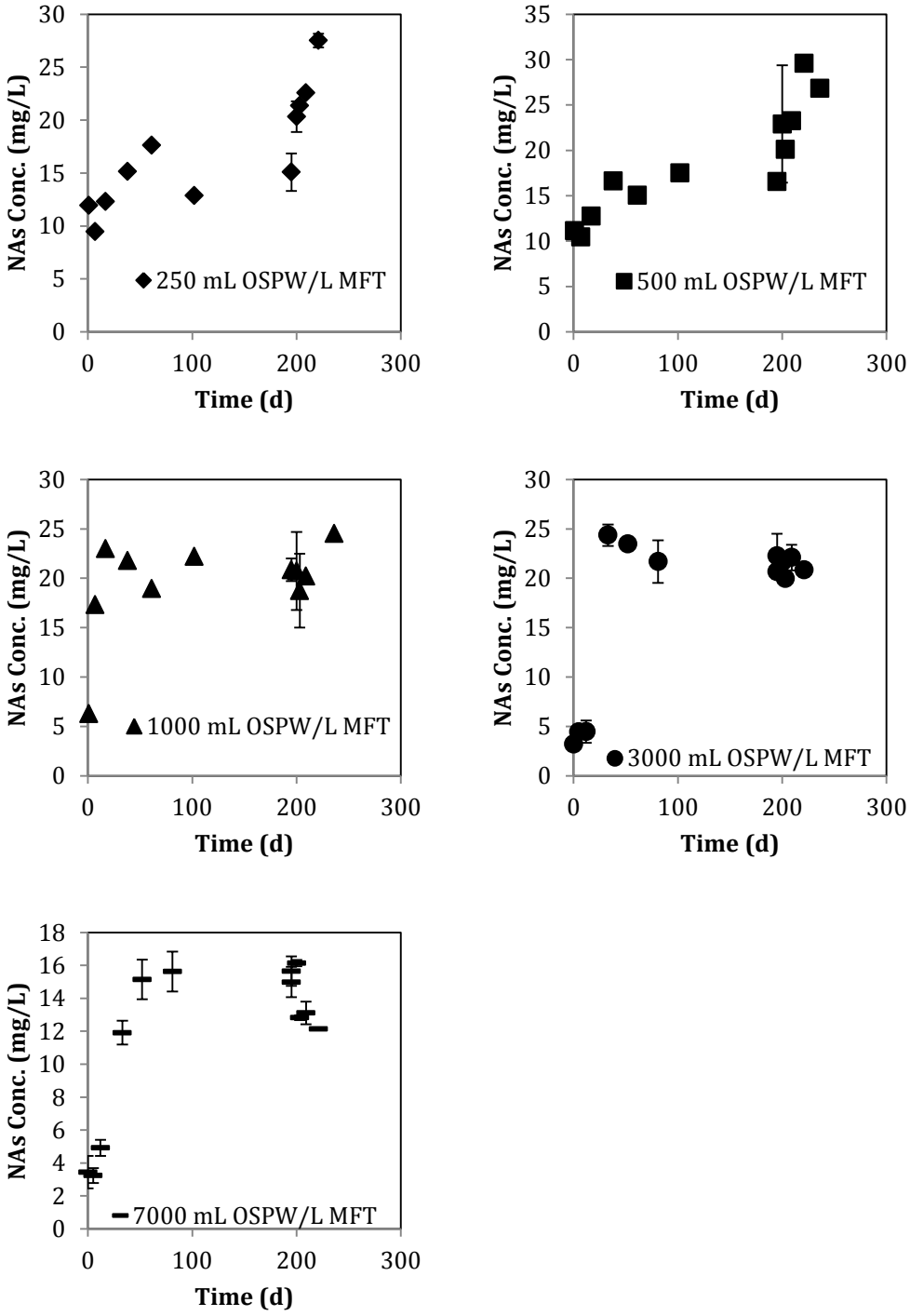

Figure 4-16 Biodegradation of NAs in HiPOx-treated OSPW in the batch reactors with OSPW to MFT ratios of 250 mL/L, 500 mL/L, 1000 mL/L, 3000 mL/L and 7000 mL/L

4.2.3 Anaerobic Biodegradation of HiPOx-treated OSPW with Biofilms Originated from MFT

Since the residue bitumen carried by MFT may cause fluctuation in tests results, biofilm originated from MFT was used as sole source of microorganisms to eliminate the interference of bitumen. In addition, batch bioreactors containing large amount of MFT would be very hard to operate in water treatment plant and developing a biofilm reactor to preserve the indigenous microbial communities while eliminating the MFT is necessary. In order to examine if there is viable biofilms recolonized on the surface of the growth media and the potential treatment efficiency it could achieve batch bioreactors using biofilms originated from MFT were set up.

Figure 4-17 shows the biodegradation of dissolved organic compounds in OSPW with biofilms originated from MFT at OSPW loadings of 8000 mL/m^2 of surface area and 16000 mL/m^2 of surface area. The COD could be reduced to less than150 mg/L when initial COD was 250 mg/L. In the batch reactor, COD was reduced from 125 mg/L to less than100 mg/L, indicating the oxidization products of the original organic matrix was biodegraded by microcosm biofilm.

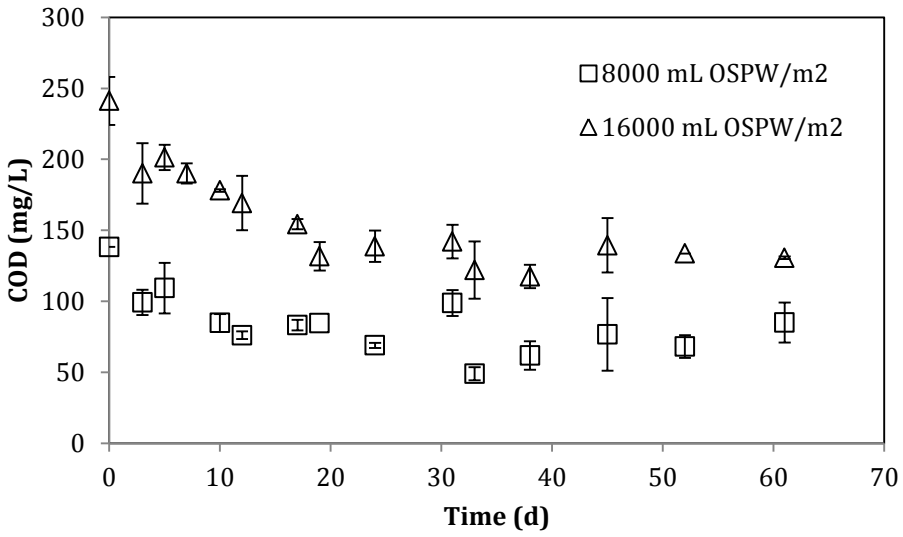

Figure 4-17. Biodegradation of dissolved organic compounds in OSPW with biofilms originated from MFT at OSPW loadings of 8000 mL OSPW/m^2 and 16000 mL OSPW/m^2

As it is shown in Figure 4-17, the overall removal rates for the biodegradation tests are around 50% for both batch bioreactors. Compared to the results yielded in the biodegradation tests conducted with MFT, the removal rates were slightly increased and the fluctuation of COD value in the end of biodegradation tests was also significantly reduced. With the results shown above, it is reasonable to conclude that using biofilm could effectively reduce the interference of residue bitumen and increase removal rate and process stability.

Figure 4-18 shows the sulfate utilization in the batch bioreactors treating HiPOx-treated OSPW with loadings of 8000 mL OSPW/m^2 of surface area and 16000 mL OSPW/m^2 of surface area.

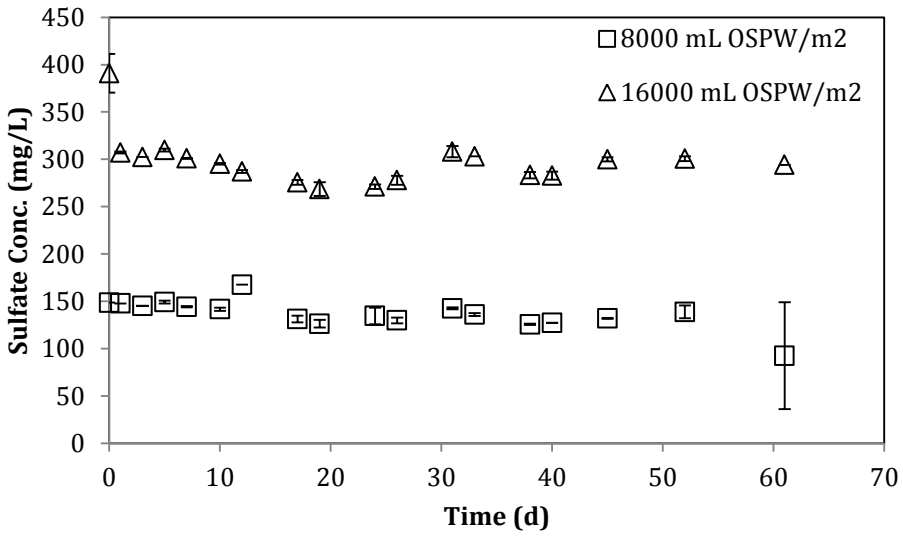

Figure 4-18. Utilization of sulfate in the biodegradation tests at OSPW loadings of 8000 mL/m^2 and 16000 mL/m^2

As it is shown in Figure 4-18, sulfate utilization rate is much slower compared to the tests using MFT where sulfate was depleted in 30 days. Sulfate depletion is not clear in these two batch reactors and could not be achieved in 8 weeks of incubation.

Figure 4-19 shows the interaction between nitrate and sulfate in the batch bioreactors after nitrate spiking.

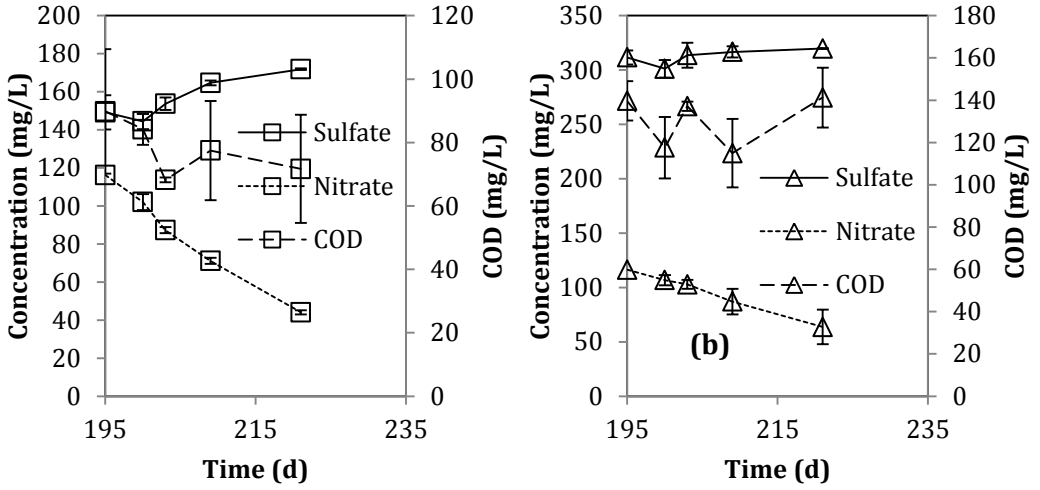

Figure 4-19. Nitrate and sulfate concentration profiles in the biodegradation tests of OSPW with biofilms originated from MFT at OSPW loadings of(a) 8000 mL/m^2 and (b) 16000 mL/m^2

As it is shown in the graph, nitrate was not depleted in the monitoring period. The increase on sulfate concentration suggests potential utilization of sulfide as inorganic electron donors.

Figure 4-20 shows that the NAs concentration changes in the batch bioreactors during the experimental period.

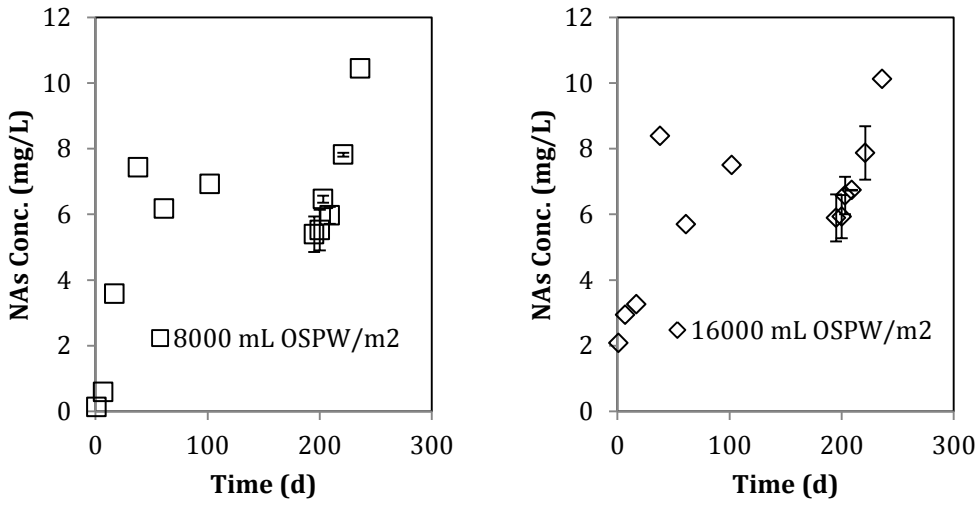

Figure 4-20. Biodegradation of NAs in HiPOx-treated OSPW with biofilms originated from MFT in the batch reactors with loadings of 8000 mL OSPW/m^2 of surface area and 16000 mL OSPW/m^2 of surface area

In both trials, the NAs concentration increased to 9 mg/L. The changes on NAs concentration were identical in both trials, indicating the NAs concentration was closely related to the biomass concentration in the system. Moreover, after MFT was eliminated from the system, 9 mg/L of NAs was yielded from the biomass suggesting that during the acclimation period the NAs could still be released to the aqueous phase. Further study on NAs leaching theory may be valuable for OSPW bioreactor operation.

4.3 Anaerobic Biodegradation of OSPW with Acclimatized Biofilms from a Bioreactor

4.3.1 Characterization of OSPW

Table 4-3 characterizes OSPW before anaerobic biodegradation tests with acclimatized biofilms. The OSPW is highly saline with alkalinity of 863.0 mg/L, indicating high total dissolved solids (TDS) concentration. Having been determined to be 341.2 after filtration to 0.45 µm, the COD value is quite similar to the municipal wastewater treatment plant influent, which is very suitable for

biological treatment. As two major contributors to toxicity, NAs concentration ranged from 40 mg/L to 80 mg/L and ammonium concentration is measured to be 40 mg/L using ion chromatography method. Nitrate in OSPW is under detected limit probably due to the active anaerobic biological process occurring in MFTs. Sulfate is determined to be 140 mg/L, indicating the presence of significant amount of electron acceptor. The other two major ions of concern, sodium and chloride concentration are measured to be 68.7 mg/L and 359.7 mg/L respectively in ion chromatography.

Table 4-3. Characterization of OSPW obtained for the anaerobic biodegradation tests with MFT

Parameter	Units	OSPW	River (2011[a])
pH	-	8.7 ± 0.1	6.5 - 9.0
Alkalinity	mg $CaCO_3$/L	863.0 ± 1.9	-
Sodium	mg/L	68.7	-
Ammonium	mg/L	43.5 ± 4.4	0.1
Chloride	mg/L	359.7	120
Sulfate	mg/L	140.9 ± 6.6	-
Nitrate	mg/L	ND	13
COD	mg/L	341.2 ± 20.4	-
NAs	mg/L	71.6	-

[a]Canadian aquatic water quality guidelines (CCME, 2011)

4.3.2 Anaerobic Biodegradation of OSPW with Acclimatized Biofilms From a Bioreactor

The bacteria that could biodegrade the toxic and recalcitrant organics need an acclimation time to induce and sustain the enzymes needed in biodegradation process. Through continuous adding target recalcitrant organics, the critical microorganicsms could be developed. However, specific environmental conditions may be necessary for the acclimation period. Acclimatized biofilms were taken from an experimental aerobic MBBR reactor operating under nitrification conditions in OSPW treatment for six months. With the presence of acetic acids as primary substrate, the experimental conditions are the same as anaerobic biodegradation of OSPW with MFT described previouse in Chapter 4.1 and the biofilms' ability of degradation NAs was tested in 10 weeks biodegradation trials in the laboratory.

Figure 4-21 indicates that the microbial community in acclimatized biofilms could biodegrade the organic compounds in OSPW. The organic compounds remaining after biodegradation was monitored to be 170 mg/L in COD, which is 50 mg/L less than that in the biodegradation with MFT and 25 mg/L less than that in the incubated with MFT-originated biofilms. When the COD in feed water was diluted to 300 mg/L, the COD could be reduced to 80 mg/L.

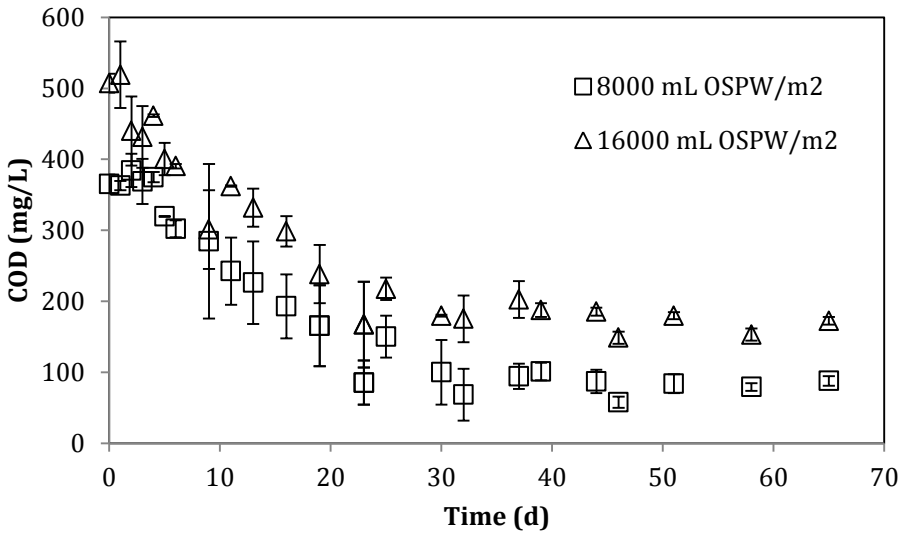

Figure 4-21. Biodegradation of dissolved organic compounds in OSPW with acclimatized biofilms at OSPW loadings of 8000 mL OSPW/m^2 of surface area and 16000 mL OSPW/m^2 of surface area.

Notably, after the biodegradable organic compounds were exhausted, the COD value did not increase indicating there is no dissolved organics leaching out from the acclimatized biofilms suggesting that after long-term acclimation, the MFT leaching effect could be eliminated. Since biofilms are consortia of microorganisms that did not contain fine particles or bitumen, it could eliminate the leaching effects and interferences. Without sands and clays that are present in large amount in MFT, biofilms reactor is also more manageable and requires less operation and maintenance work. It could largely reduce the operating cost.

Figure 4-25 shows the loss in NAs concentration during 10 weeks of anaerobic incubation. The biodegradation of NAs is evident in first 20 days of incubation. After the biodegradation was completed, NAs did not leach out from the acclimatized biofilms, which indicates a transformation in the structure of organic compounds, instead of bioadsorption. Cyclic Alkanes have considered as non-biodegradable under anaerobic conditions. However, the results show in the process of co-metabolism the recalcitrant organic compounds could be removed

by biological process. Furthermore, biofilms could eliminate the interference and reduce maintenance work, which is preferable in engineering applications.

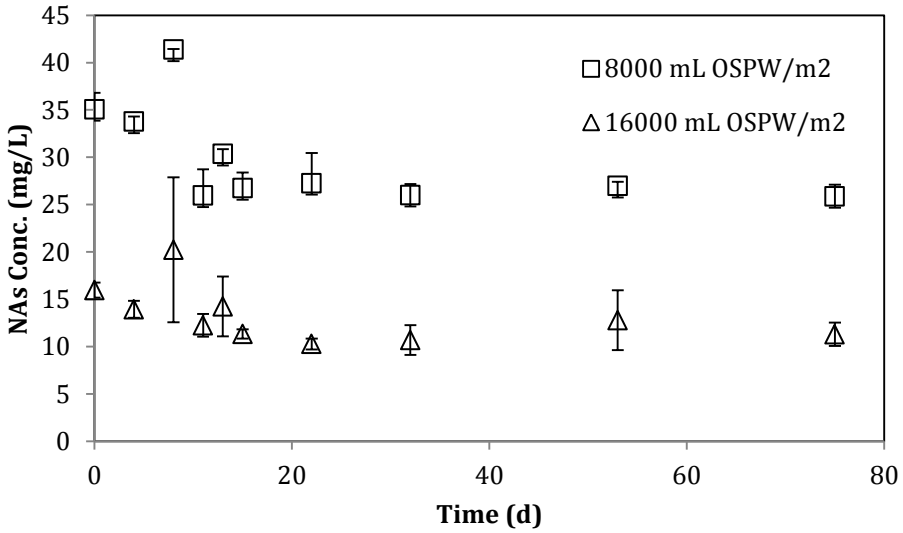

Figure 4-23 Biodegradation of NAs in OSPW with acclimatized biofilms at OSPW loadings of 8000 mL/m^2 of surface area and 16000 mL/m^2 of surface area

CHAPTER 5: CONCLUSIONS

This research has investigated the feasibility of selecting indigenous microorganisms to develop a biofilm reactor to treat OSPW. Through monitoring the COD concentration and biodegradation of organic compounds including naphthenic acids with MFT and biofilms, it was found that the indigenous microorganisms could be introduced to a biofilm reactor to biodegrade recalcitrant organic compounds. This study is important because it provides insight on anaerobic microbial activities existing in the MFT, promoting reclamation of oil sands tailings ponds and sustainable development in the Athabasca region.

5.1 Anaerobic Biodegradation of OSPW with MFT and Biofilms Originated from MFT

Both MFT and MFT-originated biofilms demonstrated the ability of utilizing dissolved organic compounds in OSPW to support different anaerobic microbial processes with the presence of acetic acids. During the batch biodegradation tests, COD was rapidly reduced from 600 mg/L to 200 mg/L in the first 10 days of incubation, indicating that the indigenous microbial community is very active and its activity could be promoted with supplemented readily biodegradable organic carbon source. Along with the rapid utilization of readily biodegradable substrates, sulfate was depleted in 16 days. Denitrification was observed immediately after nitrate spiking, suggesting that the indigenous microbial community could support multiple anaerobic biodegradation processes.

After biodegradation, the residue COD in acetic acids supplemented OSPW was reduced to 200 mg/L which is lower than the original COD in OSPW, indicating partial removal of OSPW-originated dissolved organic compounds by potentially co-metabolism.

Using MFT-originated biofilms could largely eliminate the interference caused by leaching of dissolved organic compounds from the MFT, leading to a more stabilized effluent water quality.

5.2 Anaerobic Biodegradation of HiPOx-treated OSPW with MFT and Biofilms Originated from MFT

HiPOx treatment breaks down recalcitrant and long-chain organic compounds into smaller molecules that are less resistant to biodegradation, promoting the biodegradation of dissolved organic compounds. After biodegradation test, the residue COD was further reduced to 150 mg/L.

Leaching of naphthenic acids from residue bitumen in the MFT is clear when MFT is present in the bioreactors. Using MFT-originated biofilms could achieve the same treatment effects while eliminating the leaching effects. Consequently, MFT-originated biofilms demonstrated clear advantages in removing dissolved organic compounds in the OSPW.

5.3 Anaerobic Biodegradation of OSPW with Acclimatized Biofilms from a Bioreactor

The biodegradation tests with acclimatized biofilms did not display a lag phase at the beginning of the incubation, suggesting that acclimatized biofilms carries complex microbial community that could sustain the biodegradation processes without acclimatization time.

The COD was reduced to 200 mg/L in 20 days, which is same as the biodegradation tests results with 1000 mL MFT. In the batch bioreactor, the COD removal mechanism is mainly biodegradation by the biomass present in the bioreactors. Since the treatment efficiency is very similar between batch bioreactors using acclimatized biofilms and 1000 mL of MFT, the biomass concentration is very similar in the two biodegradation trials suggesting that acclimatized biofilms demonstrated same treatment efficiency as MFT while eliminating the unnecessary interference. In addition, the acclimatize biofilms take about 250 mL, which was largely reduced compared to MFT volume leaving a great potential to further increase the biomass amount and the treatment efficiency.

NAs concentration was reduced to 25 mg/L after the biodegradation. The decrease on NAs concentration was detected by both HPLC-MS and fluorescence spectrometry, indicating that fluorescence spectrometry could be used to characterize NAs concentration change in daily bioreactors operations.

5.4 Recommendations

Future work should focus on development of bioreactor with continuous influent to encourage continuous growth of biofilms. Such experiments may include following:

Improvements of the laboratory bioreactors study technique. This can be tested using continuous biofilm reactors to observe whether biofilms, especially the key microorganisms indigenous to MFT, could continuously grow on the surface of biocarriers and sustain biodegradation processes.

Development of a convenient analytical method to monitor the biodegradation of naphthenic acids. Further research could be conducted with fluorescence spectrometry monitoring biodegraded naphthenic acids sample and comparing with other analytical methods (eg. HPLC-MS, FTIR, etc.).

The future work will add to the viable implementation of the biological process to the oil sands industry to remove dissolved organic compounds and detoxify OSPW. This will ultimately help oil sands industry to achieve oil sands tailings ponds reclamation and safe discharge of OSPW. Additional significance of the implementation of this technology includes prediction of naphthenic acids biodegradation pathway and evaluation of environmental impacts of biological reclamation process as well as improvements on recycling water quality. This research is a preliminary step towards creating sustainable development within the Athabasca oil sands region.

REFERENCES

Abbasnezhad, H., Foght, J.M. and Gray, M.R. (2011) Adhesion to the hydrocarbon phase increases phenanthrene degradation by Pseudomonas fluorescens LP6a. Biodegradation 22(3), 485-496.

AECOM (2012) Tailings water treatment technology evaluation study: final report.

Afzal, A., Drzewicz, P., Martin, J.W. and El-Din, M.G. (2012a) Decomposition of cyclohexanoic acid by the UV/H_2O_2 process under various conditions. Science of the Total Environment 426, 387-392.

Afzal, A., Drzewicz, P., Perez-Estrada, L.A., Chen, Y., Martin, J.W. and El-Din, M.G. (2012b) Effect of molecular structure on the relative reactivity of naphthenic acids in the UV/H_2O_2 advanced oxidation process. Environmental Science & Technology 46(19), 10727-10734.

Ahn, Y., Jung, H., Tatavarty, R., Choi, H., Yang, J.W. and Kim, I.S. (2005) Monitoring of petroleum hydrocarbon degradative potential of indigenous microorganisms in ozonated soil. Biodegradation 16(1), 45-56.

Allen, E.W. (2008a) Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. Journal of Environmental Engineering and Science 7(2), 123-138.

Allen, E.W. (2008b) Process water treatment in Canada's oil sands industry: II. A review of emerging technologies. Journal of Environmental Engineering and Science 7(5), 499-524.

Alostaz, M.d. (2008) Improved ultraviolet induced fluorescence (UVIF)-standard cone penetration testing (CPT) system to detect petroleum hydrocarbon contaminants. Ph.D. Thesis, University of Alberta (Canada).

Anderson, J., Wiseman, S.B., Moustafa, A., El-Din, M.G., Liber, K. and Giesy, J.P. (2012a) Effects of exposure to oil sands process-affected water from experimental reclamation ponds on Chironomus dilutus. Water Research 46(6), 1662-1672.

Anderson, J.C., Wiseman, S.B., Wang, N., Moustafa, A., Perez-Estrada, L., Gamal El-Din, M., Martin, J.W., Liber, K. and Giesy, J.P. (2012b) Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to Chironomus dilutus. Environmental Science & Technology 46(1), 486-493.

Anderson, R.T. and Lovley, D.R. (2000) Anaerobic bioremediation of benzene under sulfate-reducing conditions in a petroleum-contaminated aquifer. Environmental Science & Technology 34(11), 2261-2266.

Arbabi, M., Nasseri, S. and Chimezie, A. (2009) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in petroleum contaminated soils. Iranian Journal of Chemistry & Chemical Engineering-International English Edition 28(3), 53-59.

Arceivala, S. (1999) Design of Waste Water Irrigation Systems. Journal IWWA.

Bale, A.R., El-Din, M.G., Smith, D.W., Mazzei, A. and Overbeck, P. (2003) Ozone mass transfer analyses of two new ozone reactor designs, University of Alberta.

Barrow, M.P., Headley, J.V., Peru, K.M. and Derrick, P.J. (2009) Data Visualization for the characterization of naphthenic acids within petroleum samples. Energy & Fuels 23, 2592-2599.

Baskaran, V. and Nemati, M. (2006) Anaerobic reduction of sulfate in immobilized cell bioreactors, using a microbial culture originated from an oil reservoir. Biochemical Engineering Journal 31(2), 148-159.

Bataineh, M., Scott, A.C., Fedorak, P.M. and Martin, J.W. (2006) Capillary HPLC/QTOF-MS for characterizing complex naphthenic acid mixtures and their microbial transformation. Analytical Chemistry 78(24), 8354-8361.

Beltran, J.L., Ferrer, R. and Guiteras, J. (1998) Multivariate calibration of polycyclic aromatic hydrocarbon mixtures from excitation-emission fluorescence spectra. Analytica Chimica Acta 373(2-3), 311-319.

Bernal-Martinez, A., Patureau, D., Delgenes, J.P. and Carrere, H. (2009) Removal of polycyclic aromatic hydrocarbons (PAH) during anaerobic digestion with recirculation of ozonated digested sludge. Journal of Hazardous Materials 162(2-3), 1145-1150.

Borgund, A.E., Erstad, K. and Barth, T. (2007) Fractionation of crude oil acids by HPLC and characterization of their properties and effects on gas hydrate surfaces. Energy & Fuels 21(5), 2816-2826.

Brandal, O. and Sjoblom, J. (2005) Interfacial behavior of naphthenic acids and multivalent cations in systems with oil and water. II: Formation and stability of metal naphthenate films at oil-water interfaces. Journal of Dispersion Science and Technology 26(1), 53-58.

Burland, S.M. and Edwards, E.A. (1999) Anaerobic benzene biodegradation linked to nitrate reduction. Applied and Environmental Microbiology 65(2), 529-533.

Canadian Association of Petroleum Producers (CAPP). 2009. Water use in Canada's oil sands. 2009-0022. CAPP, Calgary, Alberta. Available at http://www.capp.ca/library/publications/crudeOilAndOilSands/pages/pubInfo.aspx?DocId=154986#qizoWOc35CWl (accessed November 29, 2010).

Chalatumyk, R.J., Scott, J.D., and Ozum, B. (2002) Natural agents and synthetic polymers for creating nonsegregating, thickened tailings. Proceedings of the Fifteenth International Conference on Soil Mechanics and Geotechnical Engineering, Vol 4.

Chalaturnyk, R.J., Don Scott, J. and Özüm, B. (2002) Management of oil sands tailings. Petroleum Science and Technology 20(9-10), 1025-1046.

Chen, Y.D., Barker, J.F. and Gui, L. (2008) A strategy for aromatic hydrocarbon bioremediation under anaerobic conditions and the impacts of ethanol: a microcosm study. Journal of Contaminant Hydrology 96(1-4), 17-31.

Chi Fru, E., Chen, M., Walshe, G., Penner, T. and Weisener, C. (2013) Bioreactor studies predict whole microbial population dynamics in oil sands tailings ponds. Applied Microbiology and Biotechnology 97(7), 3215-3224.

Clemente, J.S. and Fedorak, P.M. (2003) Development of a high performance liquid chromatography method to monitor the biodegradation of naphthenic acids. Journal of Environmental Engineering and Science 2(3), 177-186.

Clemente, J.S. and Fedorak, P.M. (2005) A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60(5), 585-600.

Clemente, J.S., MacKinnon, M.D. and Fedorak, P.M. (2004) Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science & Technology 38(4), 1009-1016.

Clemente, J.S., Prasad, N.G.N., MacKinnon, M.D. and Fedorak, P.M. (2003) A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. Chemosphere 50(10), 1265-1274.

Coates, J.D., Anderson, R.T. and Lovley, D.R. (1996a) Oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. Applied and Environmental Microbiology 62(3), 1099-1101.

Coates, J.D., Anderson, R.T., Woodward, J.C., Phillips, E.J.P. and Lovley, D.R. (1996b) Anaerobic hydrocarbon degradation in petroleum-contaminated harbor sediments under sulfate-reducing and artificially imposed iron-reducing conditions. Environmental Science & Technology 30(9), 2784-2789.

Couto, M.N., Monteiro, E. and Vasconcelos, M.T. (2010) Mesocosm trials of bioremediation of contaminated soil of a petroleum refinery: comparison of natural attenuation, biostimulation and bioaugmentation. Environmental Science and Pollution Research International 17(7), 1339-1346.

Da Silva, M.L.B., Ruiz-Aguilar, G.M.L. and Alvarez, P.J.J. (2005) Enhanced anaerobic biodegradation of BTEX-ethanol mixtures in aquifer columns amended with sulfate, chelated ferric iron or nitrate. Biodegradation 16(2), 105-114.

Del Rio, L.F., Hadwin, A.K., Pinto, L.J., MacKinnon, M.D. and Moore, M.M. (2006) Degradation of naphthenic acids by sediment micro-organisms. Journal of Applied Microbiology 101(5), 1049-1061.

Don Scott, J., Dusseault, M.B. and David Carrier Iii, W. (1985) Behaviour of the clay/bitumen/water sludge system from oil sands extraction plants. Applied Clay Science 1(1–2), 207-218.

Eckert, W.F., Masliyah, J.H., Gray, M.R. and Fedorak, P.M. (1996) Prediction of sedimentation and consolidation of fine tails. AlChE Journal 42(4), 960-972.

Edwards, E.A. and Grbicgalic, D. (1992) Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. Applied and Environmental Microbiology 58(8), 2663-2666.

EPCOR (2010) OSLI tailings water treatment pilot study: 2011 final report.

ERCB (2012) ST98-2012: Alberta's Energy Reserves 2011 and Supply/Demand Outlook 2012–2021.

Fan, T.P. (1991) Characterization of naphthenic acids in petroleum by fast-atom-bombardment mass-spectrometry. Energy & Fuels 5(3), 371-375.

Fedorak, P.M., Coy, D.L., Dudas, M.J., Simpson, M.J., Renneberg, A.J. and MacKinnon, M.D. (2003) Microbially-mediated fugitive gas production from oil sands tailings and increased tailings densification rates. Journal of Environmental Engineering and Science 2(3), 199-211.

Foght, J., Semple, K., Gauthier, C., Westlake, D.W.S., Blenkinsopp, S., Sergy, G., Wang, Z. and Fingas, M. (1999) Effect of nitrogen source on biodegradation of crude oil by a defined bacterial consortium incubated under cold, marine conditions. Environmental Technology 20(8), 839-849.

Gabryelski, W. and Froese, K.L. (2003) Characterization of naphthenic acids by electrospray ionization high-field asymmetric waveform ion mobility spectrometry mass spectrometry. Analytical Chemistry 75(17), 4612-4623.

Gamal El-Din, M., Fu, H., Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Drzewicz, P., Martin, J.W., Zubot, W. and Smith, D.W. (2011) Naphthenic acids speciation and removal during petroleum-coke adsorption and ozonation of oil sands process-affected water. Science of the Total Environment 409(23), 5119-5125.

Garcia-Garcia, E., Ge, J.Q., Oladiran, A., Montgomery, B., El-Din, M.G., Perez-Estrada, L.C., Stafford, J.L., Martin, J.W. and Belosevic, M. (2011a) Ozone treatment ameliorates oil sands process water toxicity to the mammalian immune system. Water Research 45(18), 5849-5857.

Garcia-Garcia, E., Pun, J., Perez-Estrada, L.A., Din, M.G., Smith, D.W., Martin, J.W. and Belosevic, M. (2011b) Commercial naphthenic acids and the organic fraction of oil sands process water downregulate pro-inflammatory gene expression and macrophage antimicrobial responses. Toxicology Letters 203(1), 62-73.

Gogate, P.R. and Pandit, A.B. (2004) A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions. Advances in Environmental Research 8(3-4), 501-551.

Golby, S., Ceri, H., Gieg, L.M., Chatterjee, I., Marques, L.L. and Turner, R.J. (2012) Evaluation of microbial biofilm communities from an Alberta oil sands tailings pond. FEMS Microbiology Ecology 79(1), 240-250.

Grewer, D.M., Young, R.F., Whittal, R.M. and Fedorak, P.M. (2010) Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? Sci Total Environ 408(23), 5997-6010.

Hagen, M.O., Garcia-Garcia, E., Oladiran, A., Karpman, M., Mitchell, S., El-Din, M.G., Martin, J.W. and Belosevic, M. (2012) The acute and sub-chronic exposures of goldfish to naphthenic acids induce different host defense responses. Aquatic Toxicology 109, 143-149.

Hall-Stoodley, L., Costerton, J.W. and Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews Microbiology 2(2), 95-108.

Han, X.M., Scott, A.C., Fedorak, P.M., Bataineh, M. and Martin, J.W. (2008) Influence of molecular structure on the biodegradability of naphthenic acids. Environmental Science & Technology 42(4), 1290-1295.

Harrison, J.J., Ceri, H. and Turner, R.J. (2007) Multimetal resistance and tolerance in microbial biofilms. Nature Reviews Microbiology 5(12), 928-938.

Herman, D.C., Fedorak, P.M., Mackinnon, M.D. and Costerton, J.W. (1994) Biodegradation of naphthenic acids by microbial-populations indigenous to oil sands tailings. Canadian Journal of Microbiology 40(6), 467-477.

Higashioka, Y., Kojima, H. and Fukui, M. (2011) Temperature-dependent differences in community structure of bacteria involved in degradation of petroleum hydrocarbons under sulfate-reducing conditions. Journal of Applied Microbiology 110(1), 314-322.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. (2002) Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research 36(11), 2843-2855.

Holowenko, F.M., MacKinnon, M.D. and Fedorak, P.M. (2000) Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. Canadian Journal of Microbiology 46(10), 927-937.

Holowenko, F.M., Mackinnon, M.D. and Fedorak, P.M. (2001) Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. Water Research 35(11), 2595-2606.

Huang, J., Nemati, M., Hill, G. and Headley, J. (2012) Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor. Journal of Hazardous Materials 201-202, 132-140.

Ince, N.H. and Apikyan, I.G. (2000) Combination of activated carbon adsorption with light-enhanced chemical oxidation via hydrogen peroxide. Water Research 34(17), 4169-4176.

Jones, D.M., Watson, J.S., Meredith, W., Chen, M. and Bennett, B. (2001) Determination of naphthenic acids in crude oils using nonaqueous ion exchange solid-phase extraction. Analytical Chemistry 73(3), 703-707.

Kannel, P.R. and Gan, T.Y. (2012) Naphthenic acids degradation and toxicity mitigation in tailings wastewater systems and aquatic environments: a review. Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering 47(1), 1-21.

Kasperski, K.L. (1992) A review of properties and treatment of oil sands tailings. Alberta Oil Sands Technology and Research Authority (AOSTRA) Journal of Research 8, 11-11.

Kiran, S.K., Ng, S. and Acosta, E.J. (2011) Impact of asphaltenes and naphthenic amphiphiles on the phase behavior of solvent−bitumen−water systems. Energy & Fuels 25(5), 2223-2231.

Ku, A.Y., Henderson, C.S., Petersen, M.A., Pernitsky, D.J. and Sun, A.Q. (2012) Aging of water from steam-assisted gravity drainage (SAGD) operations due to air exposure and effects on ceramic membrane filtration. Industrial & Engineering Chemistry Research 51(21), 7170-7176.

Lee, Y.C., Shin, H.J., Ahn, Y., Shin, M.C., Lee, M. and Yang, J.W. (2010) Biodegradation of diesel by mixed bacteria immobilized onto a hybrid support of

peat moss and additives: a batch experiment. Journal of Hazardous Materials 183(1-3), 940-944.

List, B.R. and Lord, E.R.F. (1997) Syncrude's tailings management practices from research to implementation. Cim Bulletin 90(1010), 39-44.

Lu, W., Ewanchuk, A., Perez-Estrada, L., Sego, D. and Ulrich, A. (2013) Limitation of fluorescence spectrophotometry in the measurement of naphthenic acids in oil sands process water. Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering 48(4), 429-436.

MacKinnon, M. and Sethi, A. (1993) A comparison of the physical and chemical properties of the tailings pond at the Syncrude and the Suncor oil sands plants, pp. 1-33, Alberta Oil Sands Technology and Research Authority (AOSTRA) Publications, Edmonton, AB.

Martin, J.W., Barri, T., Han, X.M., Fedorak, P.M., El-Din, M.G., Perez, L., Scott, A.C. and Jiang, J.T. (2010) Ozonation of oil sands process-affected water accelerates microbial bioremediation. Environmental Science & Technology 44(21), 8350-8356.

Merlin, M., Guigard, S.E. and Fedorak, P.M. (2007) Detecting naphthenic acids in waters by gas chromatography-mass spectrometry. Journal of Chromatography A 1140(1-2), 225-229.

Metcalf, E. (2002) Wastewater engineering: treatment and reuse. McGraw-Hill, New York.

Mohamed, M.H., Wilson, L.D., Headley, J.V. and Peru, K.M. (2008) Screening of oil sands naphthenic acids by UV-Vis absorption and fluorescence emission spectrophotometry. Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering 43(14), 1700-1705.

Mueller, J.G., Lantz, S.E., Blattmann, B.O. and Chapman, P.J. (1991) Bench-scale evaluation of alternative biological treatment processes for the remediation of pentachlorophenol- and creosote-contaminated materials: Slurry-phase bioremediation. Environmental Science & Technology 25 (6), 1055-1061.

Musat, F., Wilkes, H., Behrends, A., Woebken, D. and Widdel, F. (2010) Microbial nitrate-dependent cyclohexane degradation coupled with anaerobic ammonium oxidation. The ISME Journal 4(10), 1290-1301.

Nicolella, C., van Loosdrecht, M.C.M. and Heijnen, J.J. (2000) Wastewater treatment with particulate biofilm reactors. Journal of Biotechnology 80(1), 1-33.

Nopcharoenkul, W., Netsakulnee, P. and Pinyakong, O. (2013) Diesel oil removal by immobilized Pseudoxanthomonas sp. RN402. Biodegradation 24(3), 387-397.

Penner, T.J. and Foght, J.M. (2010) Mature fine tailings from oil sands processing harbour diverse methanogenic communities. Canadian Journal of Microbiology 56(6), 459-470.

Pollock, G.W., McRoberts, E.C., Livingstone, G., McKenna, G.T., Matthews, J.G., Csu, Csu and Csu (2000) Consolidation behaviour and modelling of oil sands composite tailings in the Syncrude CT prototype, A A Balkema Publishers, Leiden.

Purves, R.W. and Guevremont, R. (1999) Electrospray ionization high-field asymmetric waveform ion mobility spectrometry−mass spectrometry. Analytical Chemistry 71(13), 2346-2357.

Riser-Roberts, E. (1992) Bioremediation of petroleum contaminated sites, CK Smoley.

Scott, A.C., Whittal, R.M. and Fedorak, P.M. (2009) Coal is a potential source of naphthenic acids in groundwater. Science of the Total Environment 407(7), 2451-2459.

Scott, A.C., Zubot, W., MacKinnon, M.D., Smith, D.W. and Fedorak, P.M. (2008) Ozonation of oil sands process water removes naphthenic acids and toxicity. Chemosphere 71(1), 156-160.

Siddique, T., Gupta, R., Fedorak, P.M., MacKinnon, M.D. and Foght, J.M. (2008) A first approximation kinetic model to predict methane generation from an oil sands tailings settling basin. Chemosphere 72(10), 1573-1580.

Siddique, T., Penner, T., Semple, K. and Foght, J.M. (2011) Anaerobic biodegradation of longer-chain n-alkanes coupled to methane production in oil sands tailings. Environmental Science & Technology 45(13), 5892-5899.

Small, C.C. (2011) Activation of delayed and fluid petroleum coke for the adsorption and removal of naphthenic acids from oil sands tailings pond water. M.Sc. Thesis, University of Alberta (Canada).

St John, W.P., Rughani, J., Green, S.A. and McGinnis, G.D. (1998) Analysis and characterization of naphthenic acids by gas chromatography electron impact mass spectrometry of tert.-butyldimethylsilyl derivatives. Journal of Chromatography A 807(2), 241-251.

Staehelin, J. and Hoigne, J. (1982) Decomposition of ozone in water - rate of initiation by hydroxide ions and hydrogen-peroxide. Environmental Science & Technology 16(10), 676-681.

Stanford, B.D., Pisarenko, A.N., Holbrook, R.D. and Snyder, S.A. (2011) Preozonation effects on the reduction of reverse osmosis membrane fouling in water reuse. Ozone: Science & Engineering 33(5), 379-388.

Tomiyasu, H., Fukutomi, H. and Gordon, G. (1985) Kinetics and mechanism of ozone decomposition in basic aqueous-solution. Inorganic Chemistry 24(19), 2962-2966.

Tucker, S.A., Amszi, V.L. and Acree, W.E. (1992) Primary and secondary inner filtering - effect of $K_2Cr_2O_7$ on fluorescence emission intensities of quinine sulfate. Journal of Chemical Education 69(1), A8-A12.

Vlasopoulos, N., Memon, F.A., Butler, D. and Murphy, R. (2006) Life cycle assessment of wastewater treatment technologies treating petroleum process waters. Science of the Total Environment 367(1), 58-70.

Voordouw, G. (2013) Interaction of oil sands tailings particles with polymers and microbial cells: First steps toward reclamation to soil. Biopolymers 99(4), 257-262.

Wang, N. (2011) Ozonation and biodegradation of oil sands process water. M.Sc. Thesis, University of Alberta (Canada).

Wang, X. and Kasperski, K.L. (2010) Analysis of naphthenic acids in aqueous solution using HPLC-MS/MS. Analytical Methods 2(11), 1715.

Wu, L., Ge, G. and Wan, J. (2009) Biodegradation of oil wastewater by free and immobilized Yarrowia lipolytica W29. Journal of Environmental Sciences 21(2), 237-242.

Yen, T.-W., Marsh, W.P., MacKinnon, M.D. and Fedorak, P.M. (2004) Measuring naphthenic acids concentrations in aqueous environmental samples by liquid chromatography. Journal of Chromatography A 1033(1), 83-90.

Zhu, R., Liu, Q., Xu, Z., Masliyah, J.H. and Khan, A. (2011) Role of Dissolving Carbon Dioxide in Densification of Oil Sands Tailings. Energy & Fuels 25(5), 2049-2057.

CHAPTER 6: APPENDICES

APPENDIX A: Raw Data

Appendix A: Raw Data

Table A-1: Summary of operational data from batch bioreactor OSPW-MFT250

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	160.49	160.49	160.5	0	36.34	36.34	36.3	0	-	-	-	-	-	-	-	-
1	197.22	189.32	193.3	5.6	32.38	30.32	31.4	1.5	-	-	-	-	16.6	21.4	19.0	3.4
3	208.62	201.02	204.8	5.4	20.26	23.80	22.0	2.5	-	-	-	-	29.4	29.4	29.4	0.0
5	-	-	-	-	6.87	13.83	10.4	4.9	-	-	-	-	27.3	26.3	26.8	0.7
7	184.56	246.62	215.6	43.9	5.49	7.84	6.7	1.7	-	-	-	-	30.5	26.1	28.3	3.1
10	163.02	160.49	161.8	1.8	6.86	3.47	5.2	2.4	-	-	-	-	15.5	19.5	17.5	2.8
13	161.76	190.89	176.3	20.6	2.69	2.05	2.4	0.5	-	-	-	-	-	-	-	-
16	315.02	193.42	254.2	86.0	2.73	16.74	9.7	9.9	-	-	-	-	-	-	-	-
16	315.02	193.42	254.2	86.0	2.73	16.74	9.7	9.9	-	-	116.3	0.0	-	-	-	-
18	-	-	-	-	9.13	10.19	9.7	0.8	102.54	107.52	105.0	3.5	-	-	-	-
20	232.69	184.56	208.6	34.0	19.22	21.22	20.2	1.4	70.49	75.29	72.9	3.4	22.1	23.2	22.6	0.8
24	208.62	145.29	177.0	44.8	85.26	91.01	88.1	4.1	-	-	-	-	-	26.4	26.4	-
27	200.16	103.38	151.8	68.4	-	-	-	-	-	-	-	-	-	-	-	-
31	196.83	115.97	156.4	57.2	95.87	-	95.9	-	-	-	-	-	23.8	24.1	23.9	0.2
34	185.93	126.79	156.4	41.8	70.96	87.72	79.3	11.9	-	-	-	-	25.1	26.0	25.6	0.7
60	160.84	133.98	147.4	19.0	1.92	3.91	2.9	1.4	-	-	-	-	22.9	30.2	26.6	5.2

72	156.48	149.97	153.2	4.6	-	-	-	-	-	-	-	-	-	-	-	-

Table A-2: Summary of operational data from batch bioreactor OSPW-MFT500

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	299.82	299.82	299.8	-	63.18	63.18	63.2	-	-	-	-	-	-	-	-	-
1	277.02	261.82	269.4	10.7	57.18	58.28	57.7	0.8	-	-	-	-	26.0	20.9	23.4	3.6
3	263.09	263.09	263.1	0.0	35.88	32.75	34.3	2.2	-	-	-	-	27.9	28.9	28.4	0.8
5	-	-	-	-	22.90	19.68	21.3	2.3	-	-	-	-	27.1	32.7	29.9	4.0
7	169.36	175.69	172.5	4.5	20.88	14.31	17.6	4.6	-	-	-	-	32.3	31.0	31.7	0.9
10	189.62	183.29	186.5	4.5	9.15	6.96	8.1	1.6	-	-	-	-	19.5	19.9	19.7	0.3
13	190.89	182.02	186.5	6.3	3.86	3.20	3.5	0.5	-	-	-	-	-	-	-	-
16	235.22	222.56	228.9	9.0	4.31	3.84	4.1	0.3	-	-	-	-	-	-	-	-
16	235.22	222.56	228.9	9.0	4.31	3.84	4.1	0.3	-	-	116.3	0	-	-	-	-
18	-	-	-	-	10.63	11.02	10.8	0.3	110.19	107.68	108.9	1.8	-	-	-	-
20	208.62	163.02	185.8	32.2	21.00	20.71	20.9	0.2	79.45	75.41	77.4	2.9	21.9	23.5	22.7	1.1
24	223.82	217.49	220.7	4.5	111.94	102.12	107.0	6.9	-	-	-	-	26.6	16.9	21.7	6.8
27	201.73	187.63	194.7	10.0	-	-	-	-	-	-	-	-	-	-	-	-
31	225.94	184.08	205.0	29.6	109.77	-	109.8	-	-	-	-	-	23.2	23.4	23.3	0.1
34	212.62	173.53	193.1	27.6	88.26	82.69	85.5	3.9	-	-	-	-	25.2	25.3	25.3	0.1
60	207.97	163.08	185.5	31.7	2.61	2.45	2.5	0.1	-	-	-	-	28.3	25.6	26.9	1.9
72	184.63	160.52	172.6	17.0	-	-	-	-	-	-	-	-	-	-	-	-

Table A-3: Summary of operational data from batch bioreactor OSPW-MFT1000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	564.54	564.54	564.5	-	132.77	132.77	132.8	-	-	-	-	-	-	-	-	-
1	513.88	527.81	520.8	9.8	90.87	120.19	105.5	20.7	-	-	-	-	-	27.9	27.9	-
3	383.41	472.30	427.9	62.9	68.80	94.28	81.5	18.0	-	-	-	-	26.3	23.6	24.9	1.9
5	-	-	-	-	51.55	78.20	64.9	18.8	-	-	-	-	27.7	26.7	27.2	0.7
7	239.02	299.82	269.4	43.0	44.54	51.04	47.8	4.6	-	-	-	-	27.0	27.0	27.0	0.0
10	221.29	247.89	234.6	18.8	28.80	26.46	27.6	1.7	-	-	-	-	19.6	18.5	19.0	0.8
13	203.56	201.02	202.3	1.8	20.07	31.93	26.0	8.4	-	-	-	-	-	-	-	-
16	318.82	212.42	265.6	75.2	13.25	9.31	11.3	2.8	-	-	-	-	24.8	29.4		
16	318.82	212.42	265.6	75.2	13.25	9.31	11.3	2.8	-	-	116.3	-	-	-	-	-
18	-	-	-	-	22.55	14.39	18.5	5.8	95.62	118.52	107.1	16.2	-	-	-	-
20	211.16	213.69	212.4	1.8	33.27	32.71	33.0	0.4	65.61	76.35	71.0	7.6	24.0	25.3	24.6	0.9
24	221.29	239.02	230.2	12.5	122.47	121.92	122.2	0.4	0.08	-	0.1	0.1	24.2	18.6	21.4	4.0
27	205.98	217.64	211.8	8.2	-	-	-	-	-	-	-	-	-	-	-	-
31	214.95	225.74	220.3	7.6	108.89	-	108.9	-	-	-	-	-	28.3	28.6	28.4	0.2
34	204.52	216.86	210.7	8.7	94.68	142.82	118.8	34.0	-	-	-	-	29.0	28.3	28.7	0.5
60	206.84	247.24	227.0	28.6	-	3.20	3.2	2.3	-	-	-	-	29.7	26.5	28.1	2.3
72	192.63	186.37	189.5	4.4	-	-	-	-	-	-	-	-	-	-	-	-

Table A-4: Summary of operational data from batch bioreactor OSPW-MFT3000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	564.54	564.54	564.5	-	132.77	132.77	132.8	-	-	-	-	-	-	-	-	-
1	488.54	536.68	512.6	34.0	123.41	105.37	114.4	12.8	-	-	-	-	28.6	28.4	28.5	0.1
3	480.94	536.67	508.8	39.4	99.11	100.39	99.8	0.9	-	-	-	-	27.9	32.3	30.1	3.1
5	-	-	-	-	86.23	88.26	87.2	1.4	-	-	-	-	25.4	25.6	25.5	0.1
7	396.08	491.08	443.6	67.2	85.93	77.66	81.8	5.8	-	-	-	-	31.9	31.5	31.7	0.3
10	339.08	449.28	394.2	77.9	50.84	39.06	45.0	8.3	-	-	-	-	26.0	24.1	25.1	1.3
13	242.82	372.01	307.4	91.4	6.80	3.10	5.0	2.6	-	-	-	-	-	-	-	-
16	279.55	315.02	297.3	25.1	11.43	14.11	12.8	1.9	-	-	-	-	-	-	-	-
16	279.55	315.02	297.3	25.1	11.43	14.11	12.8	1.9	-	-	116.3	-	-	-	-	-
18	-	-	-	-	21.88	13.58	17.7	5.9	115.98	118.70	117.3	1.9	-	-	-	-
20	214.96	228.89	221.9	9.9	33.04	26.45	29.7	4.7	86.65	86.73	86.7	0.1	28.9	32.4	30.7	2.4
24	241.55	230.15	235.9	8.1	128.59	129.11	128.8	0.4	-	-	-	-	28.2	29.2	28.7	0.7
27	238.84	241.96	240.4	2.2	-	-	-	-	-	-	-	-	-	-	-	-
31	207.64	236.78	222.2	20.6	146.37	-	146.4	-	-	-	-	-	29.3	28.0	28.7	0.9
34	217.59	224.86	221.2	5.1	136.49	126.70	131.6	6.9	-	-	-	-	27.5	26.5	27.0	0.7
60	236.85	216.59	226.7	14.3	4.60	1.50	3.0	2.2	-	-	-	-	27.2	23.8	25.5	2.3

72	216.74	188.63	202.7	19.9	-	-	-	-	-	-	-	-	-	-	-	-

Table A-5: Summary of operational data from batch bioreactor OSPW-MFT7000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	564.54	564.54	564.5	-	132.77	132.77	132.8	-	-	-	-	-	-	-	-	-
1	536.68	521.44	529.1	10.8	127.99	126.94	127.5	0.7	-	-	-	-	28.4	32.9	30.7	3.2
3	536.67	539.20	537.9	1.8	118.96	115.91	117.4	2.2	-	-	-	-	29.3	29.7	29.5	0.3
5	-	-	-	-	113.72	108.12	110.9	4.0	-	-	-	-	29.6	29.1	29.3	0.4
7	491.08	464.48	477.8	18.8	115.23	107.19	111.2	5.7	-	-	-	-	31.1	32.0	31.6	0.7
10	449.28	432.81	441.0	11.6	82.44	71.89	77.2	7.5	-	-	-	-	23.3	23.3	23.3	0.0
13	372.01	327.68	349.8	31.3	45.30	30.86	38.1	10.2	-	-	-	-	-	-	-	-
16	315.02	263.09	289.1	36.7	17.22	9.55	13.4	5.4	-	-	-	-	-	-	-	-
16	315.02	263.09	289.1	36.7	17.22	9.55	13.4	5.4	-	-	116.3	-	-	-	-	-
18	-	-	-	-	7.21	8.88	8.0	1.2	97.46	102.23	99.8	3.4	-	-	-	-
20	228.89	220.02	224.5	6.3	21.41	16.59	19.0	3.4	39.66	75.81	57.7	25.6	35.6	36.3	35.9	0.5
24	230.15	212.42	221.3	12.5	72.74	97.07	84.9	17.2	5.36	15.97	10.7	7.5	32.6	31.3	31.9	0.9
27	241.96	213.85	227.9	19.9	-	-	-	-	-	-	-	-	-	-	-	-
31	236.78	224.75	230.8	8.5	96.61	-	96.6	-	-	-	-	-	29.5	28.2	28.8	0.9
34	224.86	196.37	210.6	20.1	90.35	124.80	107.6	24.4	-	-	-	-	28.4	29.0	28.7	0.4
60	216.59	185.62	201.1	21.9	34.39	29.47	31.9	3.5	-	-	-	-	27.9	24.5	26.2	2.5
72	188.63	153.28	171.0	25.0	-	-	-	-	-	-	-	-	-	-	-	-

Table A-6: Summary of operational data from batch bioreactor OSPW-biofilm8000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	299.82	299.82	299.8	-	63.18	63.18	63.2	-	-	-	-	-	-	-	-	-
1	264.36	260.56	262.5	2.7	63.70	63.45	63.6	0.2	-	-	-	-	18.1	17.8	17.9	0.2
3	289.69	290.95	290.3	0.9	66.73	61.80	64.3	3.5	-	-	-	-	21.4	21.3	21.3	0.1
5	-	-	-	-	59.11	59.77	59.4	0.5	-	-	-	-	22.0	21.6	21.8	0.3
7	306.15	290.95	298.6	10.7	65.19	62.02	63.6	2.2	-	-	-	-	21.8	22.9	22.4	0.8
10	293.48	268.15	280.8	17.9	55.05	54.53	54.8	0.4	-	-	-	-	15.8	16.0	15.9	0.1
13	260.55	255.49	258.0	3.6	43.72	44.77	44.2	0.7	-	-	-	-	-	-	-	-
16	235.22	227.62	231.4	5.4	36.21	38.43	37.3	1.6	-	-	-	-	-	-	-	-
16	235.22	227.62	231.4	5.4	36.21	38.43	37.3	1.6	-	-	116.3	0	-	-	-	-
18	-	-	-	-	33.00	39.81	36.4	4.8	26.69	22.80	24.7	2.8	-	-	-	-
20	136.43	144.03	140.2	5.4	35.58	39.54	37.6	2.8	0.29	-	0.3	0.2	31.2	35.8	33.5	3.3
24	156.69	189.62	173.2	23.3	47.43	43.73	45.6	2.6	-	-	-	-	22.3	24.6	23.5	1.6
27	157.75	196.42	177.1	27.3	-	-	-	-	-	-	-	-	-	-	-	-
31	185.63	157.26	171.4	20.1	56.62	-	56.6	-	-	-	-	-	18.6	19.8	19.2	0.9
34	166.83	169.39	168.1	1.8	53.85	40.40	47.1	9.5	-	-	-	-	19.2	22.1	20.7	2.1
60	184.74	137.27	161.0	33.6	51.07	26.61	38.8	17.3	-	-	-	-	20.9	19.1	20.0	1.2
72	210.42	118.48	164.5	65.0	-	-	-	-	-	-	-	-	-	-	-	-

Table A-7: Summary of operational data from batch bioreactor OSPW-biofilm16000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	564.54	564.54	564.5	0	132.77	132.77	132.8	0	-	-	-	-	-	-	-	-
1	534.14	577.21	555.7	30.5	133.57	134.87	134.2	0.9	-	-	-	-	32.1	33.6	32.8	1.1
3	560.74	572.14	566.4	8.1	130.44	130.33	130.4	0.1	-	-	-	-	34.2	33.7	33.9	0.3
5	-	-	-	-	127.56	127.68	127.6	0.1	-	-	-	-	33.9	34.1	34.0	0.2
7	544.28	554.40	549.3	7.2	137.01	135.75	136.4	0.9	-	-	-	-	32.2	32.9	32.5	0.5
10	535.41	524.01	529.7	8.1	123.17	123.22	123.2	0.0	-	-	-	-	23.0	23.8	23.4	0.6
13	501.21	531.61	516.4	21.5	106.34	108.04	107.2	1.2	-	-	-	-	-	-	-	-
16	479.67	508.81	494.2	20.6	92.78	95.29	94.0	1.8	-	-	-	-	-	-	-	-
16	479.67	508.81	494.2	20.6	92.78	95.29	94.0	1.8	-	-	116.3	-	-	-	-	-
18	-	-	-	-	75.12	88.44	81.8	9.4	80.02	47.13	63.6	23.3	-	-	-	-
20	479.67	508.81	494.2	20.6	53.48	76.27	64.9	16.1	-	-	-	-	35.5	38.5	37.0	2.1
24	280.82	273.22	277.0	5.4	35.69	39.39	37.5	2.6	-	-	-	-	35.2	36.5	35.8	0.9
27	268.36	253.75	261.1	10.3	-	-	-	-	-	-	-	-	-	-	-	-
31	257.95	238.96	248.5	13.4	43.98	-	44.0	-	-	-	-	-	39.2	36.9	38.1	1.6
34	246.76	266.57	256.7	14.0	69.94	71.98	71.0	1.4	-	-	-	-	34.8	34.7	34.8	0.1
60	216.86	213.63	215.2	2.3	51.03	25.14	38.1	18.3	-	-	-	-	21.5	29.9	25.7	5.9
72	207.53	196.73	202.1	7.6	-	-	-	-	-	-	-	-	-	-	-	-

Table A-8: Summary of operational data from batch bioreactor HiPOx-MFT250

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	139.58	139.58	139.6	0	79.0	74.9	76.9	2.9	-	-	-	-	-	-	-	-
1	-	-	-	-	64.5	64.5	64.5	0.0	-	-	-	-	12.8	11.0	11.9	1.3
3	124.44	113.09	118.8	8.0	62.4	60.7	61.6	1.2	-	-	-	-	-	-	-	-
5	118.13	110.56	114.3	5.4	57.4	51.8	54.6	4.0	-	-	-	-	-	-	-	-
7	143.37	144.63	144.0	0.9	44.3	42.5	43.4	1.2	-	-	-	-	9.9	9.0	9.5	0.6
10	158.51	166.08	162.3	5.4	37.7	33.9	35.8	2.6	-	-	-	-	-	-	-	-
12	134.89	109.03	122.0	18.3	26.2	17.9	22.1	5.9	-	-	-	-	-	-	-	-
17	138.77	141.36	140.1	1.8	23.4	10.1	16.8	9.4	-	-	-	-	11.9	12.7	12.3	0.5
19	93.52	98.69	96.1	3.7	5.1	6.2	5.6	0.8	-	-	-	-	-	-	-	-
24	160.75	137.48	149.1	16.5	13.6	25.5	19.5	8.4	-	-	-	-	-	-	-	-
26	119.38	79.3	99.3	28.3	5.9	8.9	7.4	2.1	-	-	-	-	-	-	-	-
31	147.82	134.89	141.4	9.1	11.5	6.5	9.0	3.5	-	-	-	-	-	-	-	-
33	134.89	150.41	142.7	11.0	5.5	5.4	5.4	0.1	-	-	-	-	-	-	-	-
38	180.14	181.44	180.8	0.9	4.6	19.4	12.0	10.5	-	-	-	-	15.9	14.4	15.1	1.1
40	-	-	-	-	6.4	5.4	5.9	0.7	-	-	-	-	-	-	-	-
45	145.23	155.58	150.4	7.3	9.3	6.8	8.0	1.8	-	-	-	-	-	-	-	-
52	132.3	129.72	131.0	1.8	6.5	12.8	9.6	4.5	-	-	-	-	-	-	-	-

61	116.27	100.01	108.1	11.5	7.9	n/a	7.9	5.6	-	-	-	-	17.9	17.3	17.6	0.4
69	141.28	137.53	139.4	2.7	-	-	-	-	-	-	-	-	-	-	-	-
81	137.53	211.32	174.4	52.2	-	-	-	-	-	-	-	-	-	-	-	-
102	117.52	105.01	111.3	8.8	-	-	-	-	-	-	-	-	12.6	13.1	12.9	0.3
195	122.96	114.84	118.9	5.7	-	-	-	-	0.2	-	0.2	-	-	-	-	-
195	122.96	114.84	118.9	5.7	5.7	-	5.7	-	-	-	116.3	-	13.4	16.7	15.1	2.3
200	126.07	135.86	131.0	6.9	28.6	21.9	25.2	4.8	84.2	79.0	81.6	3.7	21.6	19.1	20.3	1.8
203	157.35	122.59	140.0	24.6	35.4	36.8	36.1	1.0	63.8	55.6	59.7	5.8	20.4	22.4	21.4	1.4
209	138.94	125.85	132.4	9.3	75.9	76.8	76.3	0.6	5.9	0.6	3.3	3.8	22.8	22.4	22.6	0.3
221	127.43	155.37	141.4	19.8	8.7	4.6	6.6	2.9	-	-	-	-	27.5	-	27.5	-
236	123.01	105.01	114.0	12.7	-	-	-	-	-	-	-	-	26.5	25.6	26.1	0.6

Table A-9: Summary of operational data from batch bioreactor HiPOx-MFT500

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	167.34	154.72	161.0	8.9	148.6	148.6	148.6	0.0	-	-	-	-	-	-	-	-
1	-	-	-	-	142.5	128.7	135.6	9.7	-	-	-	-	11.8	10.5	11.2	1.0
3	135.80	139.58	137.7	2.7	137.1	123.2	130.1	9.9	-	-	-	-	-	-	-	-
5	124.44	126.97	125.7	1.8	104.1	98.3	101.2	4.1	-	-	-	-	-	-	-	-
7	147.15	157.24	152.2	7.1	87.7	88.7	88.2	0.7	-	-	-	-	12.6	8.4	10.5	3.0
10	158.51	166.08	162.3	5.4	76.2	71.5	73.9	3.3	-	-	-	-	-	-	-	-
12	137.48	146.53	142.0	6.4	56.7	54.2	55.4	1.7	-	-	-	-	-	-	-	-
17	158.16	140.06	149.1	12.8	59.2	41.8	50.5	12.3	-	-	-	-	13.1	12.4	12.7	0.5
19	129.72	99.98	114.9	21.0	15.8	17.8	16.8	1.4	-	-	-	-	-	-	-	-
24	145.23	137.48	141.4	5.5	13.6	25.5	19.5	8.4	-	-	-	-	-	-	-	-
26	-	-	-	-	8.9	8.6	8.7	0.2	-	-	-	-	-	-	-	-
31	162.04	143.94	153.0	12.8	9.7	18.0	13.9	5.8	-	-	-	-	-	-	-	-
33	147.82	155.58	151.7	5.5	9.3	9.9	9.6	0.4	-	-	-	-	-	-	-	-
38	165.92	121.96	143.9	31.1	6.9	8.2	7.6	0.9	-	-	-	-	15.3	17.9	16.6	1.9
40	-	-	-	-	10.9	17.9	14.4	4.9	-	-	-	-	-	-	-	-
45	195.66	158.16	176.9	26.5	4.8	8.7	6.7	2.8	-	-	-	-	-	-	-	-
52	140.06	147.82	143.9	5.5	8.2	9.3	8.7	0.8	-	-	-	-	-	-	-	-

61	101.26	90	95.6	8.0	12.4	6.7	9.5	4.0	-	-	-	-	14.3	15.8	15.1	1.1
69	132.53	148.79	140.7	11.5	-	-	-	-	-	-	-	-	-	-	-	-
81	178.8	160.04	169.4	13.3	-	-	-	-	-	-	-	-	-	-	-	-
102	121.27	126.27	123.8	3.5	-	-	-	-	-	-	-	-	18.1	17.0	17.5	0.8
195	106.43	124.06	115.2	12.5	3.9	-	3.9	-	-	-	116.3	-	16.0	17.1	16.6	0.8
200	119.75	126.87	123.3	5.0	19.9	17.0	18.5	2.0	75.6	80.2	77.9	3.3	22.4	23.4	22.9	0.7
203	101.26	93.06	97.2	5.8	36.7	-	36.7	-	48.0	-	48.0	-	24.7	15.5	20.1	6.5
209	117.58	145.63	131.6	19.8	88.1	88.8	88.4	0.5	0.3	-	0.3	0.2	23.5	23.0	23.3	0.3
221	135.96	160.04	148.0	17.0	5.3	6.9	6.1	1.2	-	-	-	-	29.6	-	29.6	-
236	137.06	122.39	129.7	10.4	-	-	-	-	-	-	-	-	26.2	27.5	26.8	0.9

Table A-10: Summary of operational data from batch bioreactor HiPOx-MFT1000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	248.80	240.51	244.7	5.9	376.5	405.3	390.9	20.3	-	-	-	-	-	-	-	-
1	-	-	-	-	287.5	295.1	291.3	5.4	-	-	-	-	5.4	7.1	6.3	1.2
3	212.76	203.93	208.3	6.2	270.9	274.5	272.7	2.6	-	-	-	-	-	-	-	-
5	182.48	196.83	189.7	10.1	244.7	241.8	243.3	2.0	-	-	-	-	-	-	-	-
7	186.26	187.52	186.9	0.9	223.6	212.5	218.0	7.9	-	-	-	-	18.2	16.4	17.3	1.3
10	190.05	186.26	188.2	2.7	205.0	190.8	197.9	10.0	-	-	-	-	-	-	-	-
12	158.16	95.07	126.6	44.6	170.1	140.0	155.0	21.3	-	-	-	-	-	-	-	-
17	199.54	165.92	182.7	23.8	165.8	139.0	152.4	18.9	-	-	-	-	25.9	20.0	23.0	4.2
19	163.33	163.33	163.3	0.0	79.5	84.7	82.1	3.7	-	-	-	-	-	-	-	-
24	172.38	172.38	172.4	0.0	63.4	121.7	92.6	41.3	-	-	-	-	-	-	-	-
26	-	-	-	-	27.9	37.2	32.6	6.5	-	-	-	-	-	-	-	-
31	160.75	184.02	172.4	16.5	18.1	30.3	24.2	8.6	-	-	-	-	-	-	-	-
33	190.49	203.41	197.0	9.1	13.8	18.3	16.0	3.2	-	-	-	-	-	-	-	-
38	193.07	186.61	189.8	4.6	19.3	16.6	17.9	1.9	-	-	-	-	22.9	20.6	21.8	1.6
	-	-	-	-	7.3	21.2	14.2	9.8	-	-	-	-	-	-	-	-
45	207.29	196.95	202.1	7.3	11.9	11.4	11.7	0.3	-	-	-	-	-	-	-	-
52	180.14	173.68	176.9	4.6	11.2	8.5	9.9	1.9	-	-	-	-	-	-	-	-

61	118.77	131.28	125.0	8.8	6.8	5.3	6.0	1.0	-	-	-	-	19.2	18.7	18.9	0.4
69	175.05	165.05	170.1	7.1	-	-	-	-	-	-	-	-	-	-	-	-
81	193.81	173.8	183.8	14.1	-	-	-	-	-	-	-	-	-	-	-	-
102	155.04	142.53	148.8	8.8	-	-	-	-	-	-	-	-	20.7	23.7	22.2	2.1
195	136.83	142.87	139.9	4.3	-	-	-	-	-	-	-	-	-	-	-	-
195	-	-	-	-	8.8	-	8.8	-	-	-	116.3	-	15.7	26.0	20.9	7.2
200	132.3	129.72	131.0	1.8	17.8	11.2	14.5	4.6	97.7	117.6	107.6	14.0	19.9	21.5	20.7	1.1
203	116.27	100.01	108.1	11.5	45.3	44.7	45.0	0.4	40.7	51.3	46.0	7.6	16.0	21.5	18.7	3.9
209	141.28	137.53	139.4	2.7	102.5	90.0	96.3	8.8	0.6	0.6	0.6	0.0	17.6	22.9	20.2	3.7
221	137.53	115.72	126.6	15.4	9.3	12.5	10.9	2.2	-	-	-	-	-	-	-	-
236	117.52	105.01	111.3	8.8	-	-	-	-	-	-	-	-	24.4	24.6	24.5	0.1

Table A-11: Summary of operational data from batch bioreactor HiPOx-MFT3000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	248.08	248.08	248.1	0	376.5	405.3	390.9	20.3	-	-	-	-	-	-	-	-
	-	-	-	-	311.6	302.5	307.1	6.4	-	-	-	-	3.5	3.0	3.2	0.4
3	216.54	237.99	227.3	15.2	301.4	294.8	298.1	4.7	-	-	-	-	-	-	-	-
5	230.42	234.21	232.3	2.7	288.1	281.7	284.9	4.5	-	-	-	-	-	-	-	-
7	212.76	234.21	223.5	15.2	265.4	268.8	267.1	2.5	-	-	-	-	4.7	4.2	4.4	0.3
10	187.52	177.43	182.5	7.1	264.9	263.7	264.3	0.8	-	-	-	-	-	-	-	-
12	169.80	150.41	160.1	13.7	221.7	246.4	234.0	17.5	-	-	-	-	-	-	-	-
17	150.41	156.87	153.6	4.6	196.4	220.9	208.7	17.3	-	-	-	-	5.3	3.7	4.5	1.1
19	146.53	168.51	157.5	15.5	167.9	185.8	176.8	12.7	-	-	-	-	-	-	-	-
24	167.21	169.8	168.5	1.8	163.1	185.4	174.2	15.8	-	-	-	-	-	-	-	-
26	-	-	-	-	127.7	152.7	140.2	17.7	-	-	-	-	-	-	-	-
31	160.75	184.02	172.4	16.5	130.2	156.0	143.1	18.2	-	-	-	-	-	-	-	-
33	203.41	194.36	198.9	6.4	70.9	103.2	87.0	22.8	-	-	-	-	-	-	-	-
38	171.09	171.09	171.1	0.0	51.2	74.8	63.0	16.7	-	-	-	-	25.1	23.6	24.4	1.1
	-	-	-	-	14.5	47.8	31.1	23.5	-	-	-	-	-	-	-	-
45	198.24	199.54	198.9	0.9	13.8	14.1	13.9	0.2	-	-	-	-	-	-	-	-
52	167.21	195.66	181.4	20.1	8.7	7.3	8.0	0.9	-	-	-	-	-	-	-	-

61	143.78	178.8	161.3	24.8	13.6	45.9	29.7	22.8	-	-	-	-	23.9	23.0	23.4	0.6
69	187.56	201.32	194.4	9.7	-	-	-	-	-	-	-	-	-	-	-	-
81	181.31	187.56	184.4	4.4	-	-	-	-	-	-	-	-	-	-	-	-
102	147.53	143.78	145.7	2.7	-	-	-	-	-	-	-	-	23.2	20.2	21.7	2.2
195	-	-	-	-	-	-	-	-	-	0.2	0.2	0.2	-	-	-	-
195	145.23	155.58	150.4	7.3	9.5	18.5	14.0	6.3	-	-	116.3	-	23.8	20.6	22.2	2.3
200	125.47	142.56	134.0	12.1	22.1	53.3	37.7	22.0	95.4	76.6	86.0	13.3	21.1	20.3	20.7	0.5
203	105.82	122.48	114.2	11.8	41.2	66.1	53.7	17.6	50.1	45.2	47.6	3.5	22.0	21.2	21.6	0.5
209	147.34	135.25	141.3	8.5	68.7	118.4	93.6	35.1	1.2	-	1.2	0.9	20.3	19.7	20.0	0.4
221	137.53	133.87	135.7	2.6	15.2	29.0	22.1	9.8	-	-	-	-	21.2	23.0	22.1	1.3
236	121.86	103.12	112.5	13.3	-	-	-	-	-	-	-	-	21.2	20.5	20.9	0.5

Table A-12: Summary of operational data from batch bioreactor HiPOx-MFT7000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	271.63	260.7	266.2	7.7	376.55	405.25	390.9	20.3	-	-	-	-	-	-	-	-
1	-	-	-	-	308.59	311.79	310.2	2.3	-	-	-	-	4.1	2.7	3.4	1.0
3	243.04	251.87	247.5	6.2	311.67	315.52	313.6	2.7	-	-	-	-	-	-	-	-
5	239.25	249.35	244.3	7.1	301.36	303.89	302.6	1.8	-	-	-	-	-	-	-	-
7	244.30	225.37	234.8	13.4	287.58	295.78	291.7	5.8	-	-	-	-	3.6	2.9	3.2	0.5
10	249.35	216.54	232.9	23.2	287.37	291.68	289.5	3.1	-	-	-	-	-	-	-	-
12	171.12	187.52	179.3	11.6	271.64	272.12	271.9	0.3	-	-	-	-	-	-	-	-
17	180.14	171.09	175.6	6.4	261.23	264.92	263.1	2.6	-	-	-	-	4.6	5.3	4.9	0.5
19	163.33	164.63	164.0	0.9	249.97	250.71	250.3	0.5	-	-	-	-	-	-	-	-
24	151.7	155.58	153.6	2.7	249.69	256.89	253.3	5.1	-	-	-	-	-	-	-	-
26	-	-	-	-	234.72	227.72	231.2	5.0	-	-	-	-	-	-	-	-
31	141.36	187.9	164.6	32.9	246.81	250.37	248.6	2.5	-	-	-	-	-	-	-	-
33	141.36	172.38	156.9	21.9	219.85	217.68	218.8	1.5	-	-	-	-	-	-	-	-
38	140.06	152.99	146.5	9.1	203.05	188.11	195.6	10.6	-	-	-	-	11.4	12.4	11.9	0.7
40	-	-	-	-	165.47	159.41	162.4	4.3	-	-	-	-	-	-	-	-
45	150.41	151.7	151.1	0.9	125.59	129.37	127.5	2.7	-	-	-	-	-	-	-	-
52	186.61	155.58	171.1	21.9	73.31	81.22	77.3	5.6	-	-	-	-	-	-	-	-

61	167.21	159.46	163.3	5.5	-	-	-	-	-	-	-	-	14.3	16.0	15.1	1.2
69	190.06	178.8	184.4	8.0	-	-	-	-	-	-	-	-	-	-	-	-
81	207.57	208.82	208.2	0.9	-	-	-	-	-	-	-	-	-	-	-	-
102	208.82	192.56	200.7	11.5	-	-	-	-	-	-	-	-	14.8	16.5	15.6	1.2
195	123.77	151.29	137.5	19.5	70.2	70.9	70.5	0.5	-	-	116.3	-	14.3	15.6	15.0	0.9
	121.53	115.34	118.4	4.4	-	-	-	-	95.6	99.2	97.4	2.5	15.0	16.3	15.7	0.9
203	113.64	142.56	128.1	20.4	99.6	98.0	98.8	1.1	67.8	48.2	58.0	13.8	16.0	16.3	16.1	0.2
209	109.35	136.47	122.9	19.2	146.4	158.5	152.5	8.6	30.1	12.0	21.0	12.8	12.7	12.9	12.8	0.1
221	112.56	126.47	119.5	9.8	224.6	197.5	211.1	19.2	-	-	-	-	13.6	12.6	13.1	0.7
236	131.57	145.37	138.5	9.8	-	-	-	-	-	-	-	-	12.2	12.1	12.1	0.1

Table A-13: Summary of operational data from batch bioreactor HiPOx-biofilm8000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	138.32	138.32	138.3	0	148.6	148.6	148.6	0.0	-	-	-	-	-	-	-	-
1	-	-	-	-	147.7	147.6	147.7	0.0	-	-	-	-	0.1	0.1	0.1	0.0
3	105.52	92.90	99.2	8.9	145.0	145.3	145.1	0.2	-	-	-	-	-	-	-	-
5	121.92	96.69	109.3	17.8	150.4	148.2	149.3	1.6	-	-	-	-	-	-	-	-
7	-	-	-	-	143.7	144.7	144.2	0.7	-	-	-	-	1.3	-	0.6	1.0
10	80.28	89.12	84.7	6.3	140.4	142.7	141.6	1.6	-	-	-	-	-	-	-	-
12	74.12	78.00	76.1	2.7	167.5	-	167.5	-	-	-	-	-	-	-	-	-
17	85.76	80.59	83.2	3.7	133.7	128.8	131.3	3.4	-	-	-	-	4.1	3.1	3.6	0.7
19	85.76	83.17	84.5		129.2	123.4	126.3	4.1	-	-	-	-	-	-	-	-
24	67.66	70.25	69.0	1.8	128.3	140.7	134.5	8.8	-	-	-	-	-	-	-	-
26	-	-	-	-	131.9	127.7	129.8	3.0	-	-	-	-	-	-	-	-
31	105.15	92.22	98.7	9.1	143.3	141.8	142.6	1.1	-	-	-	-	-	-	-	-
33	52.15	45.68	48.9	4.6	137.1	134.9	136.0	1.5	-	-	-	-	-	-	-	-
38	54.73	68.95	61.8	10.1	125.1	126.2	125.7	0.8	-	-	-	-	7.3	7.6	7.4	0.2
40	-	-	-	-	127.2	127.4	127.3	0.2	-	-	-	-	-	-	-	-
45	94.81	58.61	76.7	25.6	132.2	131.5	131.9	0.5	-	-	-	-	-	-	-	-
52	73.83	62.49	68.2	8.0	143.5	134.1	138.8	6.7	-	-	-	-	-	-	-	-

61	95	74.99	85.0	14.1	52.5	132.5	92.5	56.6	-	-	-	-	5.3	7.0	6.2	1.2
69	97.5	-	97.5	-	-	-	-	-	-	-	-	-	-	-	-	-
81	79.99	77.49	78.7	1.8	-	-	-	-	-	-	-	-	-	-	-	-
102	49.98	98.76	74.4	34.5	-	-	-	-	-	-	-	-	6.1	7.8	6.9	1.2
195	75.97	103.68	89.8	19.6	142.8	155.6	149.2	9.0	-	-	116.3	-	5.3	5.5	5.4	0.1
200	80.74	87.62	84.2	4.9	141.6	147.2	144.4	4.0	105.0	99.1	102.1	4.2	5.9	5.1	5.5	0.5
203	67.68	68.75	68.2	0.8	151.3	156.0	153.7	3.3	86.1	88.4	87.3	1.7	6.9	6.0	6.5	0.6
209	66.38	88.54	77.5	15.7	163.7	165.6	164.7	1.3	72.3	70.0	71.2	1.6	5.9	6.0	6.0	0.1
221	59.63	83.75	71.7	17.1	172.2	171.6	171.9	0.4	44.9	43.4	44.1	1.1	8.0	7.6	7.8	0.3
236	73.54	54.68	64.1	13.3	-	-	-	-	-	-	-	-	10.4	10.5	10.4	0.1

Table A-14: Summary of operational data from batch bioreactor HiPOx-biofilm16000

	COD (mg/L)				Sulfate (mg/L)				Nitrate (mg/L)				NAs (mg/L)			
Time (d)	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD	Rep1	Rep2	Mean	STD
0	253.13	229.16	241.1	16.9	376.5	405.3	390.9	20.3	-	-	-	-	-	-	-	-
1	-	-	-	-	306.4	307.8	307.1	1.0	-	-	-	-	1.8	2.3	2.1	0.3
3	174.91	205.19	190.1	21.4	302.4	302.4	302.4	0.0	-	-	-	-	-	-	-	-
5	195.09	207.71	201.4	8.9	310.8	308.6	309.7	1.6	-	-	-	-	-	-	-	-
7	185.00	195.09	190.0	7.1	300.5	301.3	300.9	0.6	-	-	-	-	2.6	3.3	2.9	0.5
10	177.43	178.69	178.1	0.9	294.5	295.7	295.1	0.9	-	-	-	-	-	-	-	-
12	155.58	182.73	169.2	19.2	286.1	288.2	287.1	1.5	-	-	-	-	-	-	-	-
17	151.7	156.87	154.3	3.7	273.8	277.4	275.6	2.5	-	-	-	-	3.5	3.0	3.3	0.3
19	138.77	124.55	131.7	10.1	263.4	273.6	268.5	7.3	-	-	-	-	-	-	-	-
24	146.53	131.01	138.8	11.0	272.8	269.5	271.1	2.3	-	-	-	-	-	-	-	-
26	-	-	-	-	274.5	281.3	277.9	4.8	-	-	-	-	-	-	-	-
31	150.41	133.6	142.0	11.9	304.0	312.2	308.1	5.8	-	-	-	-	-	-	-	-
33	136.18	107.74	122.0	20.1	303.0	303.2	303.1	0.1	-	-	-	-	-	-	-	-
38	123.25	111.62	117.4	8.2	280.8	285.7	283.3	3.4	-	-	-	-	8.7	8.1	8.4	0.4
40	-	-	-	-	279.6	285.6	282.6	4.3	-	-	-	-	-	-	-	-
45	152.99	125.84	139.4	19.2	301.4	298.3	299.8	2.2	-	-	-	-	-	-	-	-
52	133.6	133.6	133.6	0.0	302.4	299.0	300.7	2.4	-	-	-	-	-	-	-	-

61	131.28	130.02	130.7	0.9	-	294.1	294.1	-	-	-	-	-	6.7	4.7	5.7	1.4
69	142.53	157.54	150.0	10.6	-	-	-	-	-	-	-	-	-	-	-	-
81	126.27	170.05	148.2	31.0	-	-	-	-	-	-	-	-	-	-	-	-
102	126.27	126.27	126.3	0.0	-	-	-	-	-	-	-	-	7.9	7.1	7.5	0.6
195	133.1	146.29	139.7	9.3	316.1	307.0	311.6	6.4	-	-	116.3	-	6.7	5.1	5.9	1.1
200	127.86	107.29	117.6	14.5	295.5	306.7	301.1	8.0	103.46	110.07	106.8	4.7	6.4	5.4	5.9	0.7
203	138.73	135.65	137.2	2.2	305.6	321.7	313.6	11.4	99.96	105.68	102.8	4.0	7.0	6.1	6.6	0.7
209	103.52	126.38	115.0	16.2	313.0	320.2	316.6	5.1	78.70	95.31	87.0	11.7	7.1	6.3	6.7	0.6
221	131.28	151.25	141.3	14.1	319.6	319.9	319.7	0.2	52.51	74.92	63.72	15.845	7.9	7.9	7.9	0.0
236	124.38	122.58	123.5	1.3	-	-	-	-	-	-	-	-	10.7	9.5	10.1	0.8