Application of Anoxic-Aerobic Membrane Bioreactors (MBRs) for Oil Sands Process-Affected Water (OSPW) Treatment

by

Jinkai Xue

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

Doctor of Philosophy

in
Environmental Engineering

Department of Civil and Environmental Engineering
University of Alberta

Abstract

The enormous volumes of oil sands process-affected water (OSPW) produced during oil sands bitumen extraction have been a public concern due to the toxicity and persistence of the organic contaminants contained in the water. Among all the contaminants in OSPW, naphthenic acids (NAs) are regarded as the most problematic due to their bio-persistency and acute toxicity to aquatic life. It has been suggested that biodegradation is the most cost-effective approach for OSPW treatment. However, the *in situ* biodegradation half-lives of NAs in tailings ponds could be as long as 13.6 years, making it necessary to accelerate the biodegradation process through the application of engineered bioreactors. To address this need, two identical anoxic-aerobic membrane bioreactors (MBRs) with a submerged ceramic membrane were continuously operated for 742 days to treat raw and mildly ozonated (utilized ozone dose 30 O_3 mg/L) process-affected water.

Efforts were made to firstly evaluate their feasibility for OSPW NA degradation and then optimize their treatment performance. A > 300-day system startup/sludge acclimatization phase was performed to gradually acclimatize microorganisms in the MBRs to 100% OSPW environment, revealing the feasibility of the MBR configuration for OSPW treatment. To better understand the contribution of anoxic and nitrifying aerobic compartments to the degradation of OSPW classical NAs and oxidized NAs (oxy-NAs), a batch study was performed on biodegradation of raw and ozonated OSPWs under decoupled anoxic and nitrifying aerobic conditions. The batch study suggested that both anoxic and aerobic conditions could contribute to biodegradation of OSPW NAs though the latter demonstrated much better NA degradation.

Once the MBRs were stabilized on around Day 300, a 442-day continuous operating condition optimization phase was conducted. The MBR's performance on OSPW NA degradation was

successfully improved through changing the supplemented inorganic nitrogen composition and hydraulic retention time (HRT). To assess the systems' classical and oxidized NA degradation performance, ultra performance liquid chromatography coupled with high resolution mass spectrometry (UPLC/HRMS) analysis was performed. Among all the six examined operating conditions, the condition with an NH_4-N concentration of 25 mg/L and HRT of 12 h demonstrated the best removal rates of total classical NAs (37.6%) and total oxy-NAs (23.9%) from raw OSPW. Under this particular operating condition, the MBR removed 49.7% of total classical NAs and 35.4% of total oxy-NAs from the ozonated OSPW. The hybridization of low-dose ozone pretreatment and MBR process (HRT = 12 h) degraded 94.0% of classical NAs and 43.9% of total oxy-NAs from the raw OSPW.

Impacts of molecular structures of NAs on their biodegradation efficiency were discussed; and possible correlations between certain microorganisms and biodegradation of NAs with particular molecular features were found. Meanwhile, the fouling behaviors of the two MBRs for raw and ozonated OSPW treatment were closely monitored and studied over the whole operation period. By performing mathematical model fitting, it is suggested that cake layer filtration was the dominating mechanism during the long-term slow TMP growth phase. In addition, HRT might be the factor that determined the dominating fouling mechanism during the sharp TMP jump phase. Moreover, the membrane fouling cake layers were examined using techniques including scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDX) and confocal laser scanning microscopy (CLSM).

Effects of ozone pretreatment on NA biodegradation, microbial community structure, and membrane fouling development were evaluated. NAs with more alkyl branches, longer carbon chains and higher cyclicity are undoubtedly the most bio-refractory fractions in OSPW. Through

preferably degrading those recalcitrant NAs, the mild ozonation pretreatment remarkably enhanced subsequent biological treatment in terms of higher NA degradation rates and lower post-biodegradation residual NAs concentrations. In addition, the ozone pretreatment substantially affected the microbial community structure in the subsequent biological systems by altering the relative abundances of the dominant microbial species and encouraging existence of numerically minor species. Moreover, a prolonged long-term slow TMP growth phase and reduced frequency of TMP jump were observed in the MBR treating ozonated OSPW.

To our best knowledge, this is the very first study that combines low-dose ozone pretreatment and MBR process for OSPW treatment. In addition, this project is one of the few studies that extensively investigate the biodegradation of oxy-NAs under various operating conditions throughout the whole system operation. Out of the limited research on MBR fouling mitigation using ozone pretreatment of feed water, this study provides insightful information on how mild ozonation pretreatment impacts the MBR's feed water quality, microbial community structure, and fouling behavior. The results obtained in this study would be illuminating for future studies on improving biological systems treating OSPW and other industrial wastewaters containing bio-persistent organic contaminants. Industries in Canada, including oil sands exploitation, refinery plants, petrochemical industry, foundry industry, pulp and paper mills, produce large quantities of wastewater containing high recalcitrant organic contents. The knowledge gained in this study could be applied to these industrial scenarios to help improve their effluent water quality, and thus minimizing the impacts on receiving water bodies including rivers, lakes and groundwaters. It is, therefore, anticipated that this study may contribute in the future to the better protection of both environment and public health in Canada.

Preface

All of the research covered in this thesis was proposed, designed, and planned by myself and supervised by my principal supervisor, Professor Mohamed Gamal El-Din, and my co-supervisor, Professor Yang Liu, at the University of Alberta. As a team member of this project, Dr. Yanyan Zhang has contributed significantly after her joining the research group, in terms of system operation, parameter measurements, and manuscript reviewing. Most of the experiments performed in this thesis were accomplished in collaboration with Dr. Yanyan Zhang. All the raw data used in this thesis were mined, processed and analyzed solely by myself unless otherwise specified in the following context. All the manuscripts published, submitted and to be submitted were compiled by myself with the following contributions from collaborators and co-authors.

Chapter 2: Dr. Yanyan Zhang and Dr. Kerry N. McPhedran contributed to the manuscript edits. Miss Nian Sun, Shimiao Dong and Mr. Yuan Chen contributed by performing the UPLC/HRMS and Ion Mobility Spectrometry (IMS) analyses, as well as preliminarily processing the raw data.

Chapter 3: Dr. Yanyan Zhang and Dr. Kerry N. McPhedran contributed to the manuscript edits. Miss Nithya Loganathan contributed to routine water chemistry measurements when she was working in the research groups as a summer student. Miss Nian Sun, Shimiao Dong and Mr. Yuan Chen contributed by performing the UPLC/HRMS analyses and preliminarily processing the raw data.

Chapter 4, 5, and 6: Dr. Yanyan Zhang contributed to the manuscript edits. Dr. Rongfu Huang performed UPLC/HRMS analyses.

I dedicate my thesis work,

firstly, to my very strong parents and heroic brothers,

secondly, to my beloved wife and my little hero Yanlai!

Acknowledgements

I would like to show my acknowledgement and thanks to my supervisors, Dr. Yang Liu and Dr. Mohamed Gamal El-Din, for their enlightening guidance, constructive advice, and extraordinary patience throughout my course work and research. It is their continuous supervision and comments that enabled me to enrich my knowledge in the realm of environmental engineering. It was my utmost honor and pleasure to work under their supervision. In addition, my sincere acknowledgement and gratitude go to Dr. Yanyan Zhang, who has been a trustworthy, reliable, supportive and illuminating collaborator during my research. It was my absolute fortune to have an opportunity to work with her.

I would also acknowledge and thank the professors in the Department of Civil and Environmental Engineering, including Dr. Ania Ulrich, Dr. Tong Yu, and Dr. Leonidas Perez-Estrada for their informative and enlightening courses. I feel grateful to the technicians in the department, including Yupeng Zhao, Chen Liang and Elena Dlusskaya. My gratitude also goes to the former and current colleagues in Dr. Mohamed Gamal El-Din's research group, including Maria Demeter, Pamela Chelme-Ayala, Nian Sun, Rongfu Huang, Yuan Chen, Shimiao Dong, Kerry N. McPhedran, and Chao Li. The former and current members from Dr. Yang Liu's research group should also be appreciated for their help, including Jeongdong Choi, Yijing Shi, and Zhiya Sheng. In addition, Nithya Loganathan is thanked for her assistance during the batch study. It is the valuable support and suggestions from all those aforementioned people that ensured the smoothness of my research.

Moreover, I would like to express my appreciation to the sponsors of this research for their generosity. They are Meidensha Corporation, the Helmholtz - Alberta Initiative, Natural Sciences and Engineering Research Council of Canada (NSERC), Syncrude Canada Ltd., Suncor

Energy Inc., Shell Canada, Canadian Natural Resources Ltd., Total E&P Canada Ltd., EPCOR Water Services, IOWC Technologies Inc., Alberta Innovates - Energy and Environment Solution, and Alberta Environment and Parks. Besides, I appreciate the Alberta Innovates - Technology Futures Graduate Student Scholarship and Dr. Donald R. Stanley Graduate Scholarship.

Last but not least, I am grateful to all my amazing friends. Without the support and friendship from them, I would not have come this far.

TABLE OF CONTENTS

List of Tables

List of Figures

List of abbreviations

AEF	acid extractable fractions
BOD	biochemical oxygen demand
BOD_5	5-day biochemical oxygen demand
CLSM	confocal laser scanning microscopy
COD	chemical oxygen demand
DCM	dichloromethane
DNA	deoxyribonucleic acids
DO	dissolved oxygen
DOC	dissolved organic carbon
EDX	energy dispersive x-ray spectroscopy
EPS	extracellular polymeric substances
FA	free ammonia
F/M	food to microorganism ratio
FT-IR	Fourier transform infrared spectrometry
GAC	granular activated carbon
h	hour
HRT	hydraulic retention time
IMS	ion mobility spectrometry
MBR	membrane bioreactor
min	minute
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
NAs	naphthenic acids
OLR	organic loading rate
oxy-NAs	oxidized naphthenic acids
OSPW	oil sands process-affected water

OTU	operational taxonomic unit
PAH	polycyclic aromatic hydrocarbon
PCR	polymerase chain reaction
PSD	particle size distribution
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	real-time quantitative polymerase chain reaction
RNA	ribonucleic acids
SEM	scanning electron microscopy
SRT	solid retention time
SMP	soluble microbial products
TDS	total dissolved solids
TMP	transmembrane pressure
TSS	total suspended solids
UPLC/HRMS	ultra performance liquid chromatography coupled with high resolution mass spectrometry

1 Introduction and objectives

1.1 General introduction and research motivation

Canada has the world's third largest oil reserves (~173 billion barrels), of which ~167 billion barrels are reserved as oil sands (CAPP 2014). Due to the huge water demand (2 – 4.5 barrels of water per barrel of bitumen produced) in the modified Clark caustic hot water extraction process, the total area of existing tailings ponds storing oil sands process-affected water (OSPW) had reached 182 km^2 (including associated structures) by the year 2013 (CAPP 2014, Gamal El-Din et al. 2011). Wide public concerns are arising on the impacts of OSPW on the regional environment, including soil, water bodies, and wild life. The recently announced *Tailings Management Framework* further strengthens environmental protections in the oil sands region by restricting the accumulated amount of tailings water as well as pushing oil sands producers to invest in technology development to treat tailings water (The Government of Alberta 2015).

As a complex mixture of suspended solids, salts and organic compounds, this problematic water features high salinity, high alkalinity (pH ranging from 8.0 – 8.4) and acute toxicity to aquatic organisms (Allen 2008a). The organic fraction in OSPW consists of naphthenic acids (NAs), humic and fulvic acids, benzene, toluene, ethyl benzene and xylene (BTEX), phenols, and polycyclic aromatic hydrocarbons (PAHs), etc. (Klamerth et al. 2015, Wang et al. 2013b). Among all the organic contaminants contained in OSPW, NAs (typically ranging from 20 to 50 mg/L) in particular have been reported as the major contributors to OSPW toxicity and persistency, and regarded as the target pollutants (Choi and Liu 2014, Hwang et al. 2013, MacKinnon et al. 1993, Martin et al. 2010, Wang et al. 2013b).

NAs are naturally occurring, aliphatic or alicyclic carboxylic acids found in petroleum. The structure of classical NAs could be generalized using a formula $C_nH_{2n+Z}O_x$, where n denotes the carbon number; Z is zero or a negative, even integer specifying the hydrogen deficiency resulting from ring formation; and x refers to the number of oxygen atoms. Species with $x = 2$ are referred as classical NAs, while those with $x \geq 3$ are termed as oxidized NAs (oxy-NAs) (Islam et al. 2014). Sample classical NA structures are illustrated in Figure 1-1 (Clemente and Fedorak 2005). The growing demand of recycling OSPW and the stringent potential discharge policy to the receiving environments impel the industry to seek efficient and economical technologies to reclaim OSPW (Allen 2008a, Peng et al. 2004). The detoxification of OSPW is a challenge due to the persistence of NAs as well as the large volumes of OSPW produced.

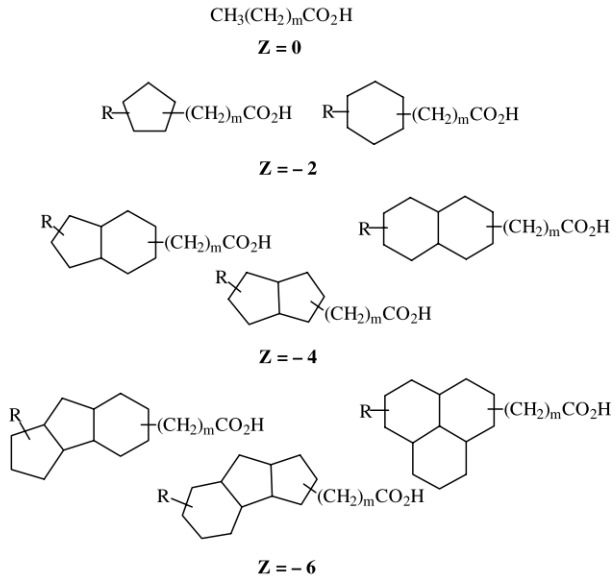


Figure 1-1. Sample NA structures (R is an alkyl chain, and m is the number of CH_2 units).

Various treatment methods have been investigated for their efficacy in removing NAs from OSPWs, such as adsorption, membrane filtration, and advanced oxidation processes (Allen 2008b, Gamal El-Din et al. 2011, Hwang et al. 2012b, Kim et al. 2011a, Martin et al. 2010,

Moustafa et al. 2014). According to Allen (2008b)'s review, most physical methods are deficient in mitigating OSPW organic substances. High-dose ozonation has proven its capability of substantially removing OSPW NAs including those highly branched and cyclic species (Gamal El-Din et al. 2011, Scott et al. 2008, Wang et al. 2013b). However, some fractions existed even if a high ozone dose was used (e.g., 80 mg/L) (Wang et al., 2013). Moreover, the high energy consumption of high-dose ozonation prevents it from being an economically feasible way to treat OSPW on a large scale.

Among all the investigated treatment processes, biodegradation is generally considered the most cost-effective and practical way to reduce NAs and detoxify OSPW through selectively removing NAs with low molecular weights and cyclicity (Han et al. 2008, Kannel and Gan 2012, Scott et al. 2005b). However, it is estimated that the *in situ* biodegradation half-life of NAs is within a range of 12.8 – 13.6 years (Han et al. 2009). To accelerate the biodegradation of OSPW NAs, it is crucial to develop engineered biological processes that are efficient in mitigating the OSPW organic compounds.

In fact, the biodegradation of commercial NAs and OSPW indigenous NAs has been extensively studied using various bench-scale suspended and biofilm systems (Choi et al. 2014, Clemente et al. 2004, Gunawan et al. 2014, Han et al. 2008, Martin et al. 2010, Misiti et al. 2013c, Scott et al. 2005b). As illustration, Han et al. (2008) observed that 48% of OSPW NAs were removed in their aerobic batch suspension reactor after 98 days of operation. Choi and Liu (2014) performed a study on the treatment of OSPW by using two sequencing batch reactors (SBR) and achieved acid extractable fraction (AEF) removal rates of 8.7% and 16.6% in the activated sludge seeded and the mature fine tailings (MFT) seeded SBRs, respectively. Hwang et al. (2013) reported that 18.5% of the OSPW parent NAs was eliminated by OSPW indigenous microorganisms in a

biofilm reactor with a hydraulic retention time (HRT) of 19 h. Recently, Zhang et al. (2015b) reported that aromatic NAs (more toxic than classical NAs) were degraded by *Pseudomonas fluorescens* and *Pseudomonas putida*. However, it should be noticed that most of the previously studied biological systems only achieved a low removal rate of NAs, making it necessary to put more effort to further improve the efficiency of biological treatment of OSPW.

As a relatively novel technology, membrane bioreactors (MBRs) have been widely applied for municipal and industrial wastewater treatment in recent decades (Cicek 2003, Lew et al. 2009, Liao et al. 2006, Lin 2012, Skouteris et al. 2012, Visvanathan et al. 2010, Zhou and Smith 2002). An MBR system is typically composed of a membrane filtration component for liquid/solid separation and a biological reactor system for biological degradation of contaminants in wastewater (Allen 2008b, Lin 2012, Wen et al. 2010). MBR processes have several advantages including outstanding effluent quality, great disinfection performance, smaller footprint, low sludge production, and excellent nitrification/denitrification performance. The application of membrane filtration module makes it realistic to decouple sludge retention time (SRT) from HRT. Additionally, the enrichment of microorganisms in the reactor helps improve the overall efficiency of the system (Allen 2008b, Lew et al. 2009, Lin 2012, Wen et al. 2010).

However, the wider application of MBRs is constrained by its major disadvantage: membrane fouling. Membrane fouling is a collective concept of a variety of phenomena that are caused by the accumulation of rejected constituents (e.g., substrate components, cells, cell debris and microbial metabolites) in the retentate on the membrane surface or inside the membrane pores, and lead to a reduced permeate flux at a given trans-membrane pressure (TMP), or conversely an increased TMP at a constant permeate flux (Chang et al. 2002, Judd and Judd 2011). Membrane fouling results in deteriorated system performance, declined permeate flux, high energy

consumption, more frequent membrane cleaning and replacement, and consequently higher cost (Chang et al. 2002, Gander et al. 2000, Meng et al. 2009). Factors that affect membrane fouling could be categorized into four groups, including membrane properties (surface charges, hydrophobicity, morphology, etc.), biomass properties (particle size, sludge viscosity, extracellular polymeric substances (EPS) production, etc.), feed water quality, and operating conditions (SRT, HRT, permeate flux, food to microorganism ratio (F/M), etc.) (Le-Clech et al. 2006). Some commonly employed fouling control strategies are aeration/gas sparging, subcritical flux operation (Robles et al. 2012), SRT control, relaxation, backwashing (Liao et al. 2013, Lin 2012, Zsirai et al. 2012), and physical and chemical cleaning (Le-Clech et al. 2006).

Previous studies have disclosed that MBRs are effective in treating petrochemical wastewaters, which are usually considered toxic and refractory (Lin 2012, Wen et al. 2010). However, to our best knowledge, no researchers have conducted investigations on MBR applications for OSPW treatment, resulting in a significant need to narrow this knowledge gap.

Furthermore, all previous studies revealed a bio-recalcitrant fraction of OSPW NAs remaining after biological treatment (Choi et al. 2014, Han et al. 2008, Hwang et al. 2013, Scott et al. 2005b), making it clear that a certain pre-treatment of OSPW to reduce this fraction prior to biological processes would be necessary to improve the removal efficiency. The most extensively studied pretreatment method for the biodegradation of OSPW is ozonation, which has been reported to effectively degrade NAs, and increase the biodegradability of OSPW (Anderson et al. 2012, Gamal El-Din et al. 2011, Garcia-Garcia et al. 2011, He et al. 2012, Hwang et al. 2013, Martin et al. 2010, Pereira et al. 2013, Scott et al. 2008, Wang et al. 2013b). Martin et al. (2010) found that ozonation selectively breaks down the most recalcitrant fractions (higher ring numbers and more alkyl branches) of the NAs in OSPW. It was also concluded that

lower ozone doses (e.g., 30 mg O_3/L) effectively accelerated subsequent detoxification and biodegradation of OSPW NAs (Martin et al. 2010, Scott et al. 2008). Overall, there have been numerous recent studies indicating that a combination of ozonation and biological treatment processes may be helpful for the reclamation of OSPW. As a powerful oxidizing agent, ozone is capable of oxidizing a wide spectrum of recalcitrant organic compounds without inducing foreign chemicals. Some researchers reported that ozone effectively reduced MBR fouling through modifying sludge (Meng et al. 2009). Some others evidenced that ozone pretreatment of feed water prior to membrane filtration resulted in a significant decrease in fouling (Van Geluwe et al. 2011). However, to our best knowledge, there is limited published research on the effect of ozone pretreatment of feed water on membrane fouling of MBRs.

Therefore, in this research, an anoxic-aerobic MBR was investigated for its OSPW treatment performance. In addition, to investigate the effect of mild-dose ozone pretreatment on the MBR's performance, an identical MBR system was operated in parallel for mildly ozonated (utilized ozone dose of 30 mg O_3/L) OSPW treatment.

1.2 Research objectives

The overall objective of this project was to investigate the feasibility of the anoxic-aerobic MBR to treat OSPW. This objective was further divided into three phases: Phase I) system set-up and start-up/sludge acclimatization of the anoxic-aerobic MBRs for raw and ozonated OSPW treatment; Phase II) optimization of operating conditions of the anoxic-aerobic MBRs treating raw and ozonated OSPWs; and Phase III) characterization and comparison of membrane fouling behaviors of anoxic-aerobic MBRs for raw and ozonated OSPW treatment.

Phase I: Setup and start-up of the anoxic-aerobic MBRs for raw and ozonated OSPW treatment. Through the reactor start-up/sludge acclimatization phase, the potential of the proposed MBR system for OSPW treatment was investigated by evaluating the system's performance in NA degradation. In addition, adjustments of operating conditions during the start-up stages were made to determine the operating factors required for further optimization.

Phase II: Optimization of operating conditions of the anoxic-aerobic MBRs treating raw and ozonated OSPWs.

A. Once the systems for the two feed water types (i.e., raw and ozonated OSPWs) were successfully acclimatized, the optimal operating parameters were investigated. Supplemented NH_4-N concentrations of 75, 50, and 25 mg/L at an HRT of 48 h, and HRTs of 72, 48, 24, and 12 h with a supplemented NH_4-N concentration of 25 mg/L have been examined for the MBRs' OSPW classical NA and oxy-NA degradation efficiency. The optimal operating condition was determined through comparing the removal rates of classical and oxidized NAs achieved at different stages.
B. Through measuring their concentrations before and after the MBR processes, the biodegradabilities of NAs with different molecular features (i.e., carbon number, cyclicity and oxygen atom number) were studied under different operating conditions.
C. To investigate the effects of denitrification and nitrification processes on the OSPW NA degradation efficiency in the anoxic-aerobic MBRs, a batch study on biodegradation of raw and ozonated OSPWs under decoupled anoxic and aerobic conditions was conducted. The results of the batch study were taken into account to determine a favorable composition of supplemented inorganic nitrogen for the MBRs.

D. Quantitative real-time polymerase chain reaction (qPCR) and next generation sequencing techniques (454 Pyrosequencing and Illumina MiSeq sequencing analyses) were conducted to characterize the microbial communities in the reactors. Dominating microorganisms were identified at different operation conditions. Correlations between microbial communities of the systems at different operating conditions were investigated. Statistical calculations were performed for correlations between abundances of different microorganisms and removal efficiencies of NAs with different structures.
E. Effects of the mild-dose ozone pretreatment on the biodegradation of OSPW classical and oxidized NAs and the structure of microbial community were evaluated. Underlying mechanisms of ozone pretreatment's enhancement effect on the subsequent biodegradation of OSPW NAs are explored.

Phase III: Characterization and comparison of membrane fouling behaviors of the anoxic-aerobic MBRs for raw and ozonated OSPW treatment under different operating conditions.

A. To explore the effect of ozone pretreatment on membrane fouling behavior of the MBR. This was achieved through comparing fouling mechanisms, characteristics of fouling layers and compositions of microbial communities of the two MBRs under different operating conditions.
B. To characterize membrane fouling in the anoxic-aerobic MBRs treating raw and ozonated OSPWs under different HRTs. Effect of HRT on membrane fouling behavior was studied.
C. To study the characteristics of microbial communities inhabiting the membrane fouling cake layers. Dominating microbes on membrane fouling layers and correlations

between membrane cake layer microbial communities and MBR suspended microbial communities were investigated.

1.3 Significance of the study

To our best knowledge, this is the very first study that combines low-dose ozone pretreatment and MBR process for OSPW treatment. In addition, this project is one of the few studies that extensively investigate the biodegradation of oxy-NAs under various operating conditions. Out of the limited research on MBR fouling mitigation using ozone pretreatment of feed water, this study provides insightful information on how mild ozone pretreatment impacts feed water quality and the microbial community structure of the subsequent MBR, and eventually alleviates MBR fouling. Microbial community characterization results under different operating conditions statistically reveal potential correlations between certain microorganisms and degradation of NAs with particular molecular features, which may be a good starting point for future studies on identification of degraders of different NAs. Overall, the study provided future researchers with in-depth information on anoxic-aerobic MBR's efficiency, bacterial community characteristics of MBR (both suspended growth and membrane fouling layer growth) and their potential functions in OSPW treatment.

This study would be illuminating for future studies on improving biological systems treating OSPW and other industrial wastewaters containing refractory organic contaminants. Industries in Canada, including oil sands exploitation, refinery plants, petrochemical industry, foundry industry, pulp and paper mills, produce large quantities of wastewater containing high recalcitrant organic contents. The knowledge obtained through this study could be applied to these industrial scenarios to improve their effluent water quality, and thus minimizing the

impacts on receiving water bodies including rivers, lakes and groundwaters. It is therefore anticipated that this study may contribute in the future to the better protection of both environment and public health in Canada.

1.4 Thesis organization

This thesis is divided into seven chapters. Chapter 1 consists of general introduction and research motivation, research objectives, and significance of the study.

Chapter 2 contains preliminary evaluation (Day 0 – 426) of the feasibility of the anoxic-aerobic MBR for raw OSPW treatment. The whole system start-up/sludge acclimatization phase and the beginning of the stabilized phase are covered. The MBR's performance in COD and inorganic nitrogen removing was assessed. In addition, the variations of biomass concentration and F/M ratio over time were monitored. To evaluate the MBR's NA degradation efficiency, UPLC/HRMS analysis was performed for NAs concentrations measurement. Moreover, quantitative real-time polymerase chain reaction (qPCR) and 454 Pyrosequencing techniques were used for characterization of the microbial communities at different stages of the system startup/sludge acclimatization phase. The reactor startup/sludge acclimatization phase of the anoxic-aerobic MBR for ozonated OSPW treatment is not covered in this thesis, as it will be published under the first co-authorship of the project's other investigator Dr. Yanyan Zhang.

In order to explore the contribution of anoxic tank and aerobic tanks in the MBRs, Chapter 3 includes the batch study on the biodegradation of raw and ozonation OSPWs under decoupled denitrifying (anoxic) and nitrifying (aerobic) conditions. The removal efficiencies of COD, NH_4-N, NO_3-N, classical NAs, and oxy-NAs under different conditions were evaluated. The influence of mild ozonation pretreatment on subsequent biological processes is evaluated in terms of

classical and oxidized NA removal, and microbial community structures. In addition, the effect of NAs molecular structure (i.e., carbon number, hydrogen deficiency, and oxygen atom number) on their biodegradation is discussed.

Chapter 4 focuses on the optimization of operating conditions of the anoxic-aerobic MBR for raw OSPW treatment. The MBR's performance is evaluated for the degradation of classical NAs and oxy-NAs under different operating conditions. The effects of supplemented NH_4-N concentration and HRT are explored. The condition that resulted in the best overall removal of classical NAs and oxy-NAs is selected as the optimal among all the examined operating conditions. In addition, effort has been made to analyze and compare the microbial communities under different operating conditions.

With the aim of enhancing the treatment efficiency, ozone pretreatment was applied prior to the MBR process, Chapter 5 compares NA (classical and oxidized) degradation efficiencies of the two MBRs treating raw and ozonated OSPWs. Illumina MiSeq technique was used to characterize the microbial communities under different operating conditions. Dominating microorganisms were identified for different operating conditions. Through this chapter, the enhancement of low-dose ozone pretreatment on the MBR's biodegradation performance is revealed.

Chapter 6 is on membrane fouling behaviors of the two MBRs. The fouling patterns of the two MBRs under different operating conditions were elucidated and compared. The effects of mild-dose ozone pretreatment and HRT on membrane fouling are evaluated. Mathematical fouling models were used to describe the membrane fouling behaviors. SEM-EDX and CLSM were

performed to analyze fouling layers on membrane specimens. In addition, the microbial communities on fouled membrane surfaces were characterized using Illumina MiSeq sequencing.

Finally, Chapter 7 presents general conclusions and recommendations for future work.

References

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.

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., 2012. Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to Chironomus dilutus. Environmental science & technology 46(1), 486-493.

CAPP, 2014. The facts on: oil sands, Canadian Association of Petroleum Producers, oilsandstoday.ca.

Chang, I.-s., Clech, P.L., Jefferson, B. and Judd, S., 2002. Membrane Fouling in Membrane Bioreactors for Wastewater Treatment. JOURNAL OF ENVIRONMENTAL ENGINEERING (November), 1018-1029.

Choi, J., Hwang, G., Gamal El-Din, M. and Liu, Y., 2014. Effect of reactor configuration and microbial characteristics on biofilm reactors for oil sands process-affected water treatment. International Biodeterioration & Biodegradation 89(2014), 74-81.

Choi, J. and Liu, Y., 2014. Biodegradation of oil sands process affected water in sequencing batch reactors and microbial community analysis by high-throughput pyrosequencing. International Biodeterioration & Biodegradation 92, 79-85.

Cicek, N., 2003. A review of membrane bioreactors and their potential application in the treatment of agricultural wastewater. CANADIAN BIOSYSTEMS ENGINEERING 45, 37-49.

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.

Gamal El-Din, M., Fu, H., Wang, N., Chelme-Ayala, P., Pérez-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. The Science of the total environment 409(23), 5119-5125.

Gander, M., Jefferson, B. and Judd, S., 2000. Aerobic MBRs for domestic wastewater treatment: a review with cost considerations. Separation and purification Technology 18(2), 119-130.

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., 2011. Ozone treatment ameliorates oil

sands process water toxicity to the mammalian immune system. Water research 45(18), 5849-5857.
Gunawan, Y., Nemati, M. and Dalai, A., 2014. Biodegradation of a surrogate naphthenic acid under denitrifying conditions. Water research 51, 11-24.
Han, X., MacKinnon, M.D. and Martin, J.W., 2009. Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS. Chemosphere 76(1), 63-70.
Han, X., 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.
He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., El-Din, M.G., Giesy, J.P. and Wiseman, S.B., 2012. Toxicity of untreated and ozone-treated oil sands process-affected water (OSPW) to early life stages of the fathead minnow (Pimephales promelas). Water research 46(19), 6359-6368.
Hwang, G., Dong, T., Islam, M.S., Sheng, Z., Pérez-Estrada, L.a., Liu, Y. and Gamal El-Din, M., 2013. The impacts of ozonation on oil sands process-affected water biodegradability and biofilm formation characteristics in bioreactors. Bioresource technology 130C, 269-277.
Hwang, G., Kang, S., El-Din, M.G. and Liu, Y., 2012. Impact of conditioning films on the initial adhesion of Burkholderia cepacia. Colloids and surfaces. B, Biointerfaces 91, 181-188.
Islam, M.S., Moreira, J., Chelme-Ayala, P. and El-Din, M.G., 2014. Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Science of the total environment 493, 282-290.
Judd, S. and Judd, C., 2011. The MBR Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, Elsevier, Oxford.
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.
Kim, E.-S., Liu, Y. and Gamal El-Din, M., 2011. The effects of pretreatment on nanofiltration and reverse osmosis membrane filtration for desalination of oil sands process-affected water. Separation and purification Technology 81(3), 418-428.
Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and El-Din, M.G., 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. Science of the total environment 506, 66-75.
Le-Clech, P., Chen, V. and Fane, T.a.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. Journal of Membrane Science 284(1-2), 17-53.
Lew, B., Tarre, S., Beliavski, M., Dosoretz, C. and Green, M., 2009. Anaerobic membrane bioreactor (AnMBR) for domestic wastewater treatment. Desalination 243(1-3), 251-257.
Liao, A.B.Q., Bagley, D.M., Kraemer, H.E., Leppard, G.G. and Liss, S.N., 2013. A Review of Biofouling and its Control in Membrane Separation Bioreactors. Water Environment Research 76(5), 425-436.
Liao, B.-Q., Kraemer, J.T. and Bagley, D.M., 2006. Anaerobic Membrane Bioreactors: Applications and Research Directions.
Lin, H., 2012. Membrane Bioreactors for Industrial Wastewater Treatment : A Critical Review. Critical Reviews in Environmental Science and Technology 42(7), 677-740.

MacKinnon, M., Meer, T.V. and Verbeek, A., 1993. Assessment of biological impact from a wet landscape option for the reclamation of fine tails from oil sands. Baddaloo, E.G., Ramamoorthy, S. and Moore, J.W. (eds), Alberta Environment, Environmental Protection, Edmonton.

Martin, J.W., Barri, T., Han, X., 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.

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S. and Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water research 43(6), 1489-1512.

Misiti, T.M., Tezel, U., Tandukar, M. and Pavlostathis, S.G., 2013. Aerobic biotransformation potential of a commercial mixture of naphthenic acids. Water research 47(15), 5520-5534.

Moustafa, A.M., Kim, E.-S., Alpatova, A., Sun, N., Smith, S., Kang, S. and El-Din, M.G., 2014. Impact of polymeric membrane filtration of oil sands process water on organic compounds quantification. Water Science & Technology 70(5), 771-779.

Peng, H., Volchek, K., MacKinnon, M., Wong, W.P. and Brown, C.E., 2004. Application of nanofiltration to water management options for oil sands operation. Desalination 170(2), 137-150.

Pereira, A.S., Islam, S., El-din, M.G. and Martin, J.W., 2013. Ozonation degrades all detectable organic compound classes in oil sands process-affected water ; an application of high-performance liquid chromatography / obitrap mass spectrometry. Rapid communications in mass spectrometry : RCM (27), 1-10.

Robles, a., Ruano, M.V., Ribes, J. and Ferrer, J., 2012. Sub-critical long-term operation of industrial scale hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system. Separation and purification Technology 100, 88-96.

Scott, A.C., Mackinnon, M.D. and Fedorak, P.M., 2005. Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. Environmental science & technology 39(21), 8388-8394.

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.

Skouteris, G., Hermosilla, D., López, P., Negro, C. and Blanco, Á., 2012. Anaerobic membrane bioreactors for wastewater treatment: A review. Chemical Engineering Journal 198-199, 138-148.

The Government of Alberta, 2015. Lower Athabasca Region-Tailings Management Framework for the Mineable Athabasca Oil Sands. The Government of Alberta (ed).

Van Geluwe, S., Braeken, L. and Van der Bruggen, B., 2011. Ozone oxidation for the alleviation of membrane fouling by natural organic matter: A review. Water research 45(12), 3551-3570.

Visvanathan, C., Aim, R.B. and Parameshwaran, K., 2010. Membrane Separation Bioreactors for Wastewater Treatment. Critical Reviews in Environmental Science and Technology 30(1), 1-48.

Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Garcia-Garcia, E., Pun, J., Martin, J.W., Belosevic, M. and Gamal El-Din, M., 2013. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. Environmental science & technology 47(12), 6518-6526.

Wen, G., Ma, J., Zhang, L. and Yu, G., 2010. Integrated Membrane Operations in Various Industrial Sectors: Case Studies, pp. 195-209, Elsevier B.V.

Zhang, Y., McPhedran, K.N. and El-Din, M.G., 2015. Pseudomonads biodegradation of aromatic compounds in oil sands process-affected water. Science of the total environment 521, 59-67.

Zhou, H. and Smith, D.W., 2002. Advanced technologies in water and wastewater treatment. Journal of Environmental Engineering and Science (1), 247-264.

Zsirai, T., Buzatu, P., Aerts, P. and Judd, S., 2012. Efficacy of relaxation, backflushing, chemical cleaning and clogging removal for an immersed hollow fibre membrane bioreactor. Water research 46(14), 4499-4507.

2 Treatment of oil sands process-affected water (OSPW) using an anoxic-aerobic membrane bioreactor (MBR) with a submerged flat-sheet ceramic microfiltration membrane[1]

2.1 Introduction

The rapidly developing oil sands industry in the Athabasca region of Alberta, Canada, has caused severe environmental impact in the area due to the generation of large volumes of toxic oil sands process-affected water (OSPW) during bitumen extraction. This OSPW is toxic to aquatic life mainly because of the naphthenic acids (NAs) present in the water. The structures of classical NAs are represented by $C_nH_{2n+Z}O_x$, where n denotes the carbon number, Z is zero or a negative, even integer specifying the hydrogen deficiency resulting from ring formation (Holowenko et al. 2001), and x refers to the number of oxygen atoms. Species with $x = 2$ are referred as classical NAs, while those with $x \geq 3$ are termed oxidized NAs (oxy-NAs) (Islam et al. 2014). The growing demand for recycling OSPW in bitumen production and the ZERO discharge policy of the Alberta Government impel the industry to seek efficient and economical technologies to reclaim OSPW. Various treatment methods, such as adsorption, membrane filtration and advanced oxidation have been investigated for their efficiency in removing NAs from OSPW (Allen 2008b). However, a technology is yet to be found to treat OSPW sufficiently and economically to allow its recycling or safe discharge into the environment.

Indigenous microorganisms in oil sands tailings ponds (discarded bodies of OSPW containing sand and clay) are capable of degrading NAs (Han et al. 2009). However, the estimated *in situ*

[1] This chapter has been partially published: Jinkai Xue, Yanyan Zhang, Yang Liu, Mohamed Gamal El-Din, 2016. Treatment of oil sands process-affected water (OSPW) using a membrane bioreactor with a submerged flat-sheet ceramic microfiltration membrane. Water Research 88, 1-11.

biodegradation half-life of NAs in tailings ponds is as long as 13 years (Han et al. 2009). Many researchers have published their studies on biodegradation of OSPW NAs, most of which were focused on batch reactors (Clemente et al. 2004, Han et al. 2008, Holowenko et al. 2001). Thus, engineered biological systems with enriched microbial cultures need to be developed in order to improve biodegradation efficiency.

Recently, bench-scale engineered biofilm systems have been employed for the biodegradation of OSPW (Choi et al. 2014, Hwang et al. 2013, Islam et al. 2013). Hwang et al. (2013) achieved a NA removal rate of around 18.5% using continuous biofilm reactors inoculated with indigenous microorganisms in OSPW, suggesting that biological treatment has a great potential for OSPW treatment if reactors are properly configured. Islam et al. (2013) observed that 56% of the acid extractable fraction (AEF) and 96% of classic NAs were removed from OSPW under a combined granular activated carbon (GAC) adsorption and biodegradation process in a fluidized bed biofilm reactor (FBBR). To further explore the potential of biological treatment of OSPW, more efforts should be done on either optimizing the previously studied systems or testing other uninvestigated biological systems, such as membrane bioreactors (MBRs).

A membrane bioreactor (MBR) combines a membrane module for liquid/solid separation with suspended biomass (activated sludge) to remove contaminants in wastewater (Judd and Judd 2011, Lin 2012). MBR's advantages include outstanding effluent quality, a smaller environmental footprint, low sludge production, and excellent nitrification/denitrification performance (Allen 2008b, Lin 2012). MBR also enables the accumulation of slow growing bacteria, which might contribute more than fast growing bacteria to the removal of recalcitrant pollutants. A disadvantage associated with membrane bioreactors is membrane fouling, which lowers system capacity and raises operation costs. MBRs have shown the effectiveness in

treating toxic and refractory petrochemical wastewaters (Lin 2012); however, to our knowledge MBRs have not yet been applied to OSPW treatment.

In order to enhance the performance of MBR in NA removal, the reactor was designed to allow the occurrence of both nitrification and denitrification as illustrated by De Gusseme et al. (2009), Deni and Penninckx (1999), and Gunawan et al. (2014). Ammonia oxidizing bacteria (AOB) were believed to be tolerant to persistent substances such as endocrine disrupting compounds and refractory petroleum hydrocarbons, and be capable of enhancing biodegradation of these chemicals by generating ammonium monooxygenase (*amo*) that has an ability to degrade a wide spectrum of substrates (De Gusseme et al. 2009, Roh et al. 2009). Deni and Penninckx (1999) found that *in vitro* AOB are capable of partially oxidizing hydrocarbons. However, it is still unclear whether denitrification conditions are beneficial to OSPW NA degradation or not. Gunawan et al. (2014) investigated anoxic degradation of a surrogate NA trans-4-methyl-1-cyclohexane carboxylic acid in continuous stirred-tank and biofilm reactors. They found that NA removal coupled with denitrification was two times faster than that under aerobic conditions. In contrast, Misiti et al. (2013a) observed no commercial NA degradation under nitrate reducing conditions regardless of the presence of a degradable carbon source. To date, no studies have been performed to evaluate OSPW indigenous NA biodegradation under denitrification or nitrification condition.

In this research, an MBR with a modified Ludzack-Ettinger (MLE) configuration with internal recirculation from the aerobic tank to the anoxic tank was used to remove recalcitrant organics such as NAs in OSPW under nitrification and denitrification conditions. A submerged ceramic membrane was applied to reduce membrane fouling. The objectives of this study include 1) to

investigate the feasibility of the MLE-MBR system for OSPW treatment; and 2) to characterize the microbial community structure in the system.

2.2 Materials and Methods

2.2.1 Source water

Table 2-1. Characteristics of OSPW I and OSPW II.

Parameter	OSPW I	OSPW II
pH	8.9	8.9
COD (mg/L)	186	209
NO_3^--N (mg/L)	0.8	2.4
NO_2^--N (mg/L)	6.4	6.2
NH_3-N (mg/L)	5.1	5.2
Total Phosphate (mg P/L)	-	-
Sulfate (mg/L)	362.5	525.5
AEF by FTIR (mg/L)	77.2	86.2
NAs (mg/L)	22.2	25.2

OSPW water was obtained from the same tailings pond in Northern Alberta in two shipments that differed slightly (Table 2-1) in terms of water quality characteristics. OSPW I (first shipment) was used for the system start-up/acclimatization stage. OSPW II (second shipment) was treated after biomass in the two systems was successfully acclimatized. To investigate the feasibility of MLE-MBR in treating OSPW with a higher NA concentration, the system was then switched to OSPW II on Day 228 when 100% OSPW I had been treated for around one month. Sodium acetate was added as an external carbon source to ensure the growth of microorganisms in the MBR system. Supplementary nitrate, ammonium, and phosphate were added to ensure that nitrogen and phosphorus were not limiting factors in bacterial growth. A modified Bushnell-Haas medium (BHM) was utilized to supply necessary trace metals to the microorganisms (Clemente et al. 2004). Gold Bar Wastewater Treatment Plant (GBWWTP) mixed sludge and

mature fine tailings (MFT) sediments (Fort McMurray, Alberta) were used as reactor inocula. To gradually acclimatize the microorganisms in the MBR, the volumetric ratio of OSPW in the feed water was increased stepwise from 10% to 100%. In addition, a commercial NA mixture (Sigma-Aldrich, ON, CA) was added to accelerate the acclimatization of bacteria in the first stage of operation (Day 0 – Day 75).

2.2.2 MBR system

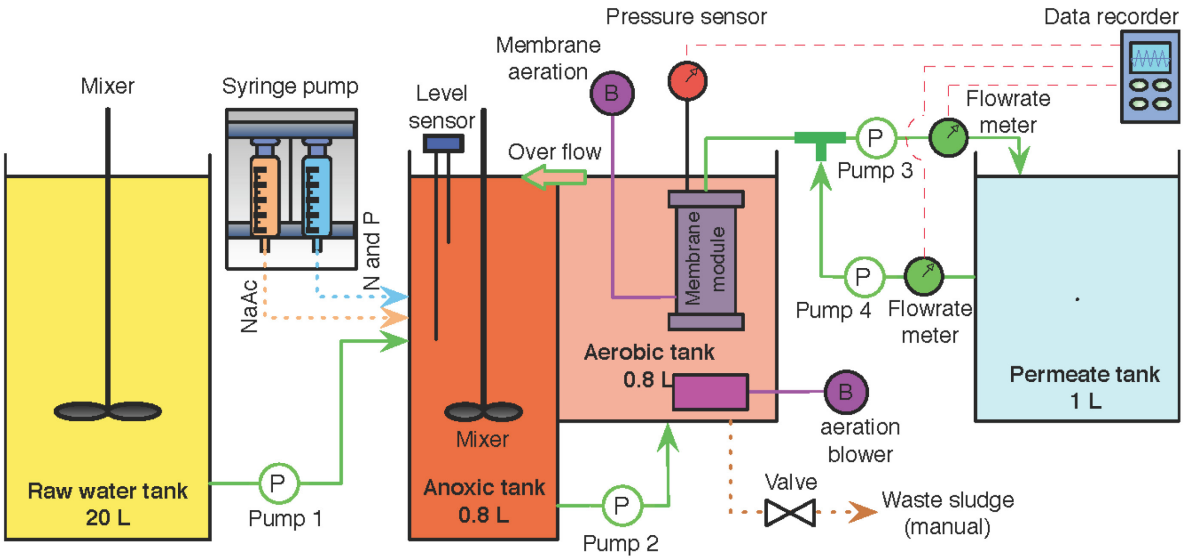


Figure 2-1. Schematic diagram of the MBR system.

As shown in Figure 2-1, the MLE-MBR system was composed of a feed water tank, an anoxic tank, an aerobic tank with an immersed membrane module, and a permeate tank. The feed OSPW tank was equipped with a stirrer to ensure homogeneity of the feed. The anoxic tank was stirred constantly to maintain the suspension of anoxic sludge. A flat sheet ceramic microfiltration membrane (Meidensha Corporation, Japan) with a pore size of 0.1 µm and an effective area of 128 cm^2 was immersed in the aerobic tank. Dissolved oxygen (DO) concentrations of < 0.3 mg/L and > 4.0 mg/L were maintained in the anoxic tank and the aerobic

tank, respectively. The retentate in the aerobic tank was recycled to the anoxic tank at a recycling ratio of 2 through overflow. Permeate water was pumped back to the membrane module for backwashing regularly.

The MBR was operated with a hydraulic retention time (HRT) of 48 h. Inflow and outflow rates were 0.8 L/day. A 30-second backwashing was conducted after 9.5 minutes of membrane filtration. Chemical cleaning (soaking the membrane module in 0.1% HClO solution for 1 h) was applied when the transmembrane pressure (TMP) reached -35 kPa, as suggested by the manufacturer.

2.2.3 Analyses of water quality

MBR influent and effluent were collected every week for measurements of pH, chemical oxygen demand (COD), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N). For conventional water quality analysis, disposable syringes were used to directly draw samples from the feed tank, the anoxic tank and the aerobic tank; while permeate samples were collected through leaving the permeate tubing into a clean glass bottle until a sufficient volume was obtained. DO concentration was measured directly by inserting a DO probe into the reactor tanks. Ultra performance liquid chromatography coupled with high resolution mass spectrometry (UPLC/HRMS) (Choi et al. 2014) followed by Ion Mobility Spectrometry (IMS) (Sun et al. 2014) was conducted to measure NA concentration profiles. A volume of 10 mL was collected from totally 2.4 L of permeate water produced over three consecutive days for UPLC/HMRS analysis. The second of the three days was used as the nominal sampling date. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in anoxic and aerobic tanks were measured according to standard methods to evaluate the biomass growth in the reactors.

2.2.4 Characterization of microbial communities

A PowerSoil® DNA Isolation Kit from Mo-Bio Laboratories, Inc. (CA, USA) was used to extract DNA from sludge samples according to the manufacturer's instructions. Extracted DNA was eluted to a final volume of 100 μL and stored at -20 °C. Molecular microbiological techniques including real-time polymerase chain reaction (qPCR) and 454 Pyrosequencing were performed to characterize microbial communities.

Table 2-2. Target genes and primers for qPCR analyses.

Function	Target gene	Primer	Sequence	References
Total bacteria	16s rDNA	341F	CCTACGGGAGGCAGCAG	(Higashioka et al. 2009)
		907R	CCGTCAATTCMTTTGAGTTT	
Nitrification	AOB *amoA*	amoA-1F	GGGGTTTCTACTGGTGGT	(Mincer et al. 2007)
		amoA-2R	CCCCTCKGSAAAGCCTTCTTC	
	Nitrospira spp. 16S rDNA	NSR 1113f	CCTGCTTTCAGTTGCTACCG	(Kim et al. 2011c)
		NSR 1264r	GTTTGCAGCGCTTTGTACCG	
	Nitrobacter spp. 16S rDNA	Nitro 1198f	ACCCCTAGCAAATCTCAAAAAACCG	
		Nitro 1423r	CTTCACCCCAGTCGCTGACC	
Denitrification	*narG* gene	narG 1960 m2f	TAYGTSGGGCAGGARAAACTG	
		narG 2050 m2r	CGTAGAAGAAGCTGGTGCTGTT	
	nirS gene	nirS 1f	TACCACCCSGARCCGCGCGT	
		nirS 3r	GCCGCCGTCRTGVAGGAA	
	nirK gene	nirK 876	ATYGGCGGVCAYGGCGA	
		nirK 1040	GCCTCGATCAGRTTRTGGTT	
	nosZ gene	nosZ 2f	CGCRACGGCAASAAGGTSMSSGT	
		nosZ 2r	CAKRTGCAKSGCRTGGCAGAA	

Three qPCR assays were used to detect nitrifying bacteria, including the *amoA* gene of ammonia oxidizing bacteria (AOB) (Mincer et al. 2007) and the 16S rDNA genes of two nitrite oxidizing bacteria (*Nitrospira* spp. and *Nitrobacter* spp.) (Kim et al. 2011c). Four qPCR assays were used for quantification of denitrifying bacteria, targeting the nitrate reductase gene (*narG*), nitrite reductase genes (*nirS* and *nirK*), and the nitrous oxide reductase gene (*nosZ*) (Kim et al. 2011c). A qPCR assay for total bacteria (16S rDNA of domain bacteria) was also conducted (Higashioka et al. 2009). Primers for the qPCR assays are listed in Table 2-2. A Bio-Rad CFX96 real-time PCR system with a C1000 Thermal Cycler (Bio-Rad Laboratories, Inc.) was used for qPCR

reactions. Each 20 μL reaction was composed of 10 μL of 2×SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Inc.), 6 μL of sterile water, 1 μL of each primer with a concentration of 10 μM, and 2 μL of diluted sample DNA. The qPCR amplification program for each individual gene is summarized in Table 2-3. All PCR runs used plasmid standards for quantification; and reactions without the DNA template were used as negative controls. Standard plasmids containing target genes were constructed using a TOPO TA Cloning kit (Invitrogen Corporation, Carlsbad, CA).

Table 2-3. QPCR amplification programs.

Target gene	Initial denaturation	Cycles				Final extension (72 °C)	Reference
		Cycles	Denaturation	Annealing	Extension (72 °C)		
16s rDNA	95 °C, 5 min	30	95 °C, 1 min	55 °C, 1 min	2 min	15 min	(Higashioka et al. 2009)
AOB *amoA*	95 °C, 15 min	45	95 °C, 1 min	54 °C, 1 min	1 min	10 min	(Mincer et al. 2007)
Nitrospira spp. 16S rDNA	50 °C, 2 min; 95 °C, 10 min	50	95 °C, 30 s	60 °C, 60 s	-	-	(Kim et al. 2011c)
Nitrobacter spp. 16S rDNA	50 °C, 2 min; 95 °C, 10 min	50	94 °C, 20 s	58 °C, 60 s	40 s	-	
narG gene	95 °C, 30 s	35	95 °C, 15 s	58 °C, 30 s	31 s	-	
nirS gene	95 °C, 30 s	30	95 °C, 15 s	60 °C, 20 s	31 s	-	
nirK gene	95 °C, 30 s	30	95 °C, 15 s	58 °C, 30 s	31 s	-	
nosZ gene	95 °C, 30 s	30	95 °C, 15 s	60 °C, 30 s	31 s	-	

To better understand the microbial community structures in the MBR, source waters, and inocula, the V1–V3 region of the 16S rDNA was amplified using the forward primer 28F (GAGTTTGATCNTGGCTCAG) and the reverse primer 519R (GTNTTACNGCGGCKGCTG) for bacteria. The amplified DNA was sequenced using a 454/Roche GS-FLX instrument at the Research and Testing Laboratory in Lubbock, Texas. Raw sequence data were processed with the Quantitative Insights Into Microbial Ecology (QIIME, http://qiime.org) pipeline with default settings. The process consists of quality checking, assigning samples to multiplexed reads, picking operational taxonomic units (OTUs) through making an OTU table, summarizing

communities by taxonomic composition, and diversity analysis. Sequences from all samples were clustered into OTUs at a similarity of 97% with the UCLUST algorithm. Representative OTUs were selected based on the most abundant sequences, and the taxonomic assignment was performed with the UCLUST consensus taxonomy classifier. OTU sequences were thereafter aligned with the Python Nearest Alignment Space Termination (PyNAST) tool. Both α diversity (diversity within a sample) and β diversity (diversity among a group of samples) were conducted based on the OTU table. To measure α diversity, Chao1, Shannon, observed species, and phylogenetic diversity whole tree (PD whole tree) were generated. β diversity was measured through building weighted and unweighted UniFrac distance matrices on the basis of a phylogenetic tree created by a FastTree algorithm to interpret relationships among microbial communities. Principal coordinate analysis (PCoA) was performed to examine the correlation among microbial communities from different samples. PCoA is commonly applied to extract and visualize highly informative components of variation from complex, multidimensional data.

2.3 Results and discussion

2.3.1 Treatment efficiency of the MBR

2.3.1.1 COD and nitrogen species removal

Figure 2-2 A shows COD concentrations over time in feed water and permeate water of the MBR. COD removals of 83%, 82%, 77%, 70%, 60%, 58%, and 51% were observed at volumetric ratios of OSPW of 10%, 20%, 30%, 40%, 60%, 80%, and 100%, respectively. It should be noticed that both OSPW organics and supplemented sodium acetate contributed to the COD in feed water. No substantial difference was observed between the residual COD in permeate water and the original COD in OSPW throughout the MBR operation. Therefore, in

spite of the significant COD reduction (Figure 2-2 A) in the MBR, it is difficult to conclude that OSPW organics were mitigated on the basis of COD results.

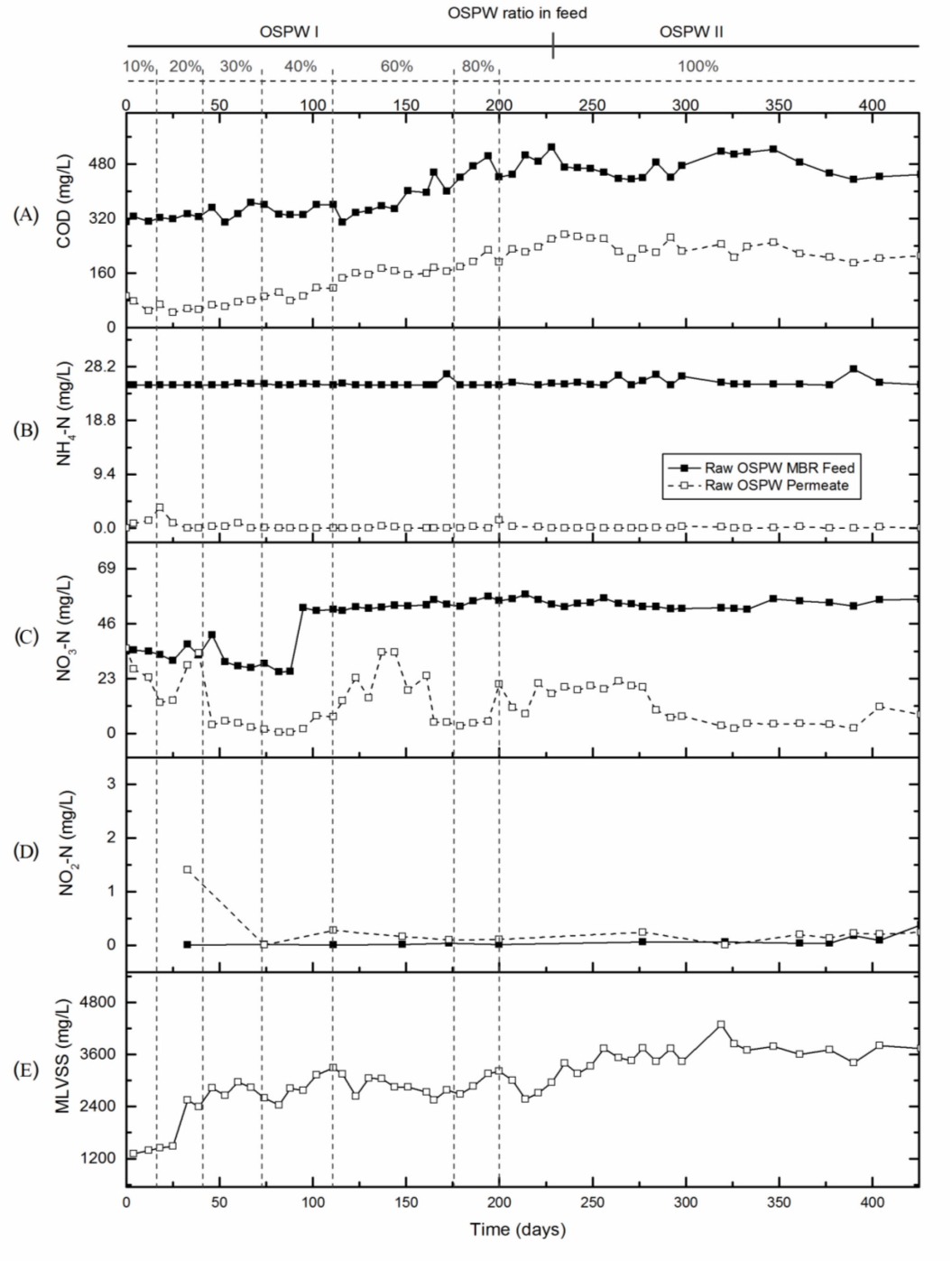


Figure 2-2. Water chemistry parameters: (A) COD; (B) NH_4-N; (C) NO_3-N; (D) NO_2-N; and (E) MLVSS.

MBR nitrogen removal efficiency was high (nearly 100%) (Figure 2-2 B, C and D) though there were minor fluctuations. NH_4-N removal efficiency has been constantly near 100% despite that OSPW ratio in the feed water increased from 10% to 100%. It is surprising to see efficient NH_4-N removal under an increasing OSPW NA load, because nitrifiers are believed to be very sensitive to toxic contaminants. The excellent ammonium oxidation performance of the system indicates that the nitrifiers in the MBR were tolerant to OSPW toxicity.

Figure 2-2 C shows that the concentration of NO_3-N in the feed water of the MBR was maintained at 25 mg N/L from 0% to 40% OSPW. At 40% OSPW, the feed water NO_3-N concentration was increased to 50 mg N/L to enhance the denitrification in the reactor and study its effect on NA removal. Initially, the permeate NO_3-N concentration was not significantly affected by the increased nitrate concentration in the influent, suggesting an excellent denitrification performance in this MBR system. However, the permeate nitrate concentration became higher after the OSPW ratio in the feed was increased from 40% to 60%, indicating that denitrifier activities were inhibited by the increased OSPW toxicity in the feed water. At the 60% OSPW stage, it took around 2 months for the MBR to recover its denitrification efficiency to a level of 90%. A similar deterioration of denitrification performance was observed after the feed OSPW percentage was further increased from 80% to 100%, suggesting that denitrifiers were sensitive toward OSPW concentration changes. About 80 days after the OSPW ratio in the feed water was increased to 100%, the denitrification rate eventually came to a higher level. A nitrate removal rate of > 80% was achieved in the MBR from day 284 to the end of MBR operation. Fluctuations in denitrification efficiency caused by increased OSPW ratio in the feed water suggest that OSPW organics may have impeded denitrifier activities, though denitrifying microorganisms eventually acclimatized to the water with higher OSPW ratios. Previous studies

showed that nitrate reduction rates in bioreactors were not affected by commercial NAs concentrations (0, 20, 80, 200, and 400 mg NA/L) (Misiti et al. 2013a), suggesting that commercial NAs and OSPW NAs may have different impacts on denitrifiers though OSPW has substances other than NAs.

Figure 2-2 D depicts NO_2-N concentration changes over time in the MBR. At the beginning of the study, the permeate had a nitrite concentration of 1.5 mg/L at 20% OSPW. After the OSPW was increased to 30%, negligible nitrite production (< 0.5 mg NO_2-N/L) was observed. The low nitrite concentration in the MBR permeate over the time of reactor operation suggests that nitrite oxidizers adapted to the toxic environment posed by OSPW.

2.3.1.2 Biomass concentrations and F/M ratio

The mixed liquor volatile suspended solids (MLVSS) concentration (Figure 2-2 E) was used as an indicator of biomass levels in the MBR system over the operation period. Because of the internal recirculation between the anoxic tank and the aerobic tank, the average value of the MLVSS concentrations in the two tanks was used to describe the biomass level in the system. To accelerate the start-up of the MBR process, activated sludge was re-inoculated in the MBR on Day 25 in the middle of the 20% OSPW step. There was a noticeable increasing trend in MLVSS concentration after this re-inoculation. No further re-inoculation was conducted. In spite of the fluctuations in MLVSS over the period from 30% – 80% OSPW (from Day 34 to Day 200), the biomass concentration showed a plateau trend as the OSPW ratio increased in the feed water. The MLVSS concentration began to increase on Day 214, the middle of the 100% OSPW I step, indicating that microorganisms in the MBR became gradually acclimatized to the high OSPW environment. OSPW II (100%) was used as the MBR influent after Day 228 (after the 100% OSPW I step) to further challenge the microorganisms since OSPW II had a slightly higher NA

concentration (Table 2-1). The MLVSS concentration in the MBR had stabilized since around Day 235 with an MLVSS and MLSS concentration (Table 2-4) of around 3730 mg/L and 5282 mg/L, respectively. The food to microorganism (F/M) ratio at the stabilized stage was 0.065 kg COD/(kg MLVSS·d) (Table 2-4), which is much lower than MBRs treating other industrial wastewater (Qin et al. 2007). Under this low F/M, more complete oxidation of recalcitrant substrate is expected.

Table 2-4. Selected parameters of the MBR at stabilized status (Days 298 – 426).

Parameters	Unit	Value
Organic loading rate (OLR)	mg COD/(L·d)	240.4±16.1
NA loading rate	mg NAs/(L·d)	12.7±0.1
NA degradation rate	mg NAs/(L·d)	3.2
Specific NA removal rate	mg NAs/(mg MLVSS·d)	8.5×10^{-4}
NA removal efficiency	%	24.7 (Day 361)
HRT	h	48
Calculated SRT	days	~280
Flux	LMH*	10.4
MLSS	mg/L	5257±360
MLVSS	mg/L	3730±233
F/M ratio	kg COD/(kg MLVSS·d)	0.065

*LMH: $L/m^2/h$.

2.3.1.3 Classical NA removal

Figure 2-3 A shows the NA removal achieved in the MBR at increasing percentages of OSPW in the feed water. NA removal at 40%, 60%, 80%, 100% OSPW I, and 100% OSPW II (Day 361) was 22.8%, 4.8%, 12.6%, 23.1%, and 24.7%, respectively. In comparison, Hwang et al. (2013) reported that a continuous biofilm reactor removed 18.5% of parent NAs in OSPW. The bio-persistence of NAs in OSPW has been demonstrated in a batch study, suggesting that the half-life of OSPW NAs is around 44 – 240 days (Han et al. 2008). It was also estimated that NAs have undergone slow biodegradation with a half-life in the range of 12.8 – 13.6 years for residual recalcitrant fraction of OSPW in tailing ponds (Han et al. 2009).

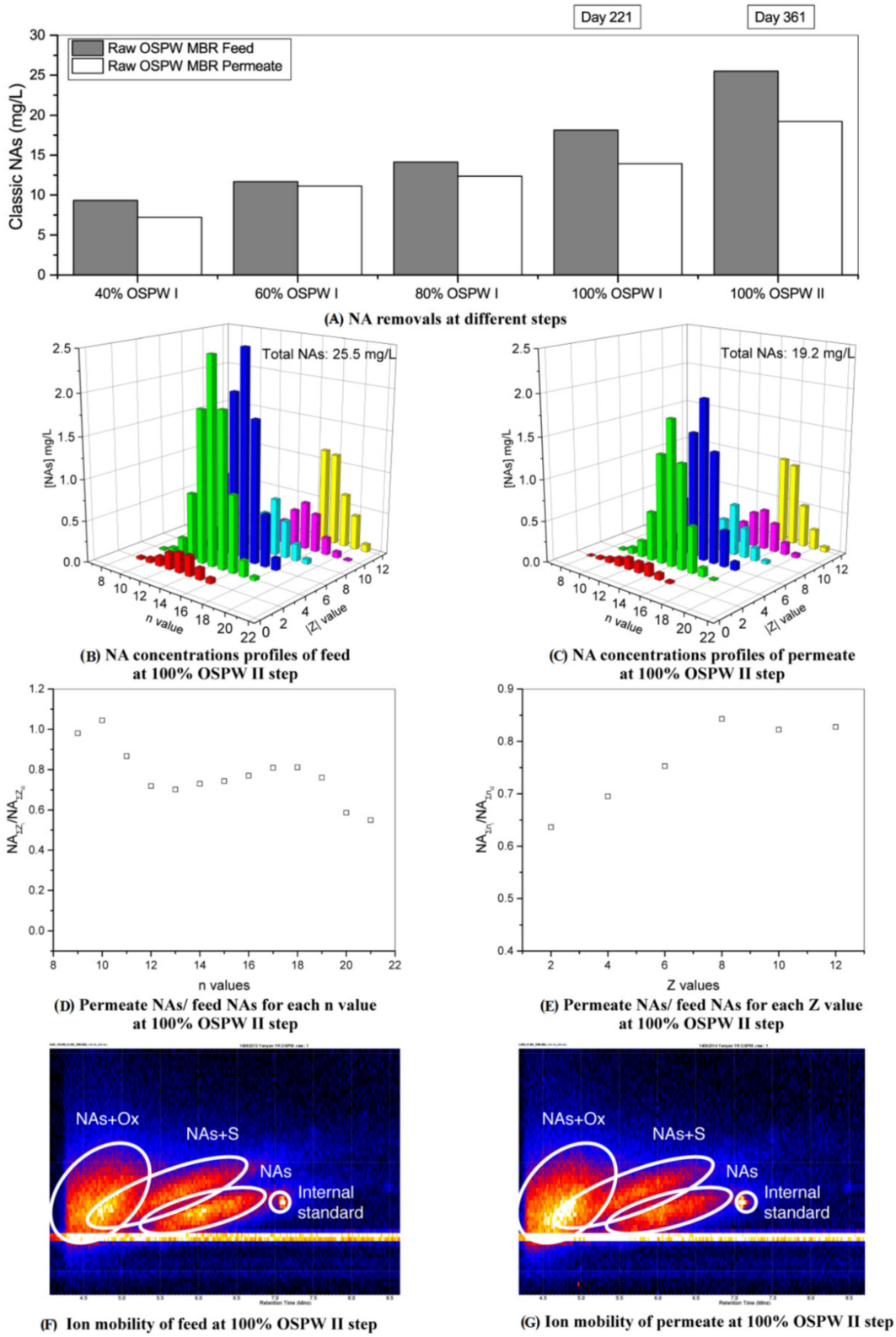


Figure 2-3. UPLC/HRMS and IMS results.

The rapid and high NA removal in this study indicates that the MBR is a competitive candidate for OSPW treatment. Although NA degradation efficiency achieved by this MBR was relatively lower than ozonation especially when high ozone dosage is applied (Wang et al. 2013b), ozonation is not economically feasible for treatment of OSPW due to a heavy demand of energy and chemicals, especially when considering the huge amount of tailing water stored in Athabasca region (976 million m^3) (The Government of Alberta 2015). Moreover, by-products of ozonation are still being evaluated for their associated toxicity issues. Increased toxicity has been observed during ozonation of some types of organic compounds (Germirli Babuna et al. 2009, Ledakowicz et al. 2006, Wang et al. 2003).

The classical NAs concentrations profiles of samples collected on Day 361 (100% OSPW II) are presented in Figure 2-3 B and C. After biodegradation, the concentrations of NAs with all *n* values detected decreased, except those with $n = 10$. NAs with $n = 12 – 16$ were removed by around 30%, whereas NAs with $n = 17 – 19$ were removed by around 22%. The MBR removed 40% of NAs with $n = 20$ and $n = 21$. It was reported that the biodegradation rate of OSPW NAs was inversely proportional to the carbon numbers (especially $n > 17$) (Clemente et al. 2004, Han et al. 2009). In this study, the higher levels of NAs with $n = 9 – 11$ in permeate might be attributed to the bioconversion of NAs with higher *n* values into NAs with lower *n* values (see Figure 2-3 D). In addition, the higher permeate abundance of NAs with lower molecular weight implies that derivative NAs caused by bioconversion are more bio-persistent, and a longer HRT may be needed to achieve complete NA removal.

Figure 2-3 E clearly indicates that the NA removal became less effective as the $|Z|$ value (proportional to the ring number) increased. Our observation that higher cyclicity led to less biodegradation of NAs is consistent with conclusions drawn by Han et al. (2008) and Wang et al.

(2013b). Indeed, the removal of NAs with |*Z*| values of 2 – 8 was negatively and linearly correlated with the value of |*Z*|; whereas, NAs with |*Z*| values higher than 8 were subject to similar degradation efficiency, regardless of the |*Z*| value. Han et al. (2008) found that the biodegradation rate of NAs decreased with increasing cyclicity (higher |*Z*|) in both of commercial NAs and OSPW NAs. Wang et al. (2013b) found that biodegradation substantially decreased NAs with $|Z| < 6$ in untreated OSPW, leaving concentrations of NAs with $|Z| > 8$ relatively unchanged.

Ion mobility spectrometry (IMS) was used to further characterize MBR samples in 100% OSPW II (Figure 2-3 F and G). A 2-D separation image was generated for each sample using IMS after UPLC/HRMR analysis. Three characteristic regions could be identified in the OSPW sample, including a classical NAs (NAs) cluster, an oxy-NAs (NAs+O_x) cluster, and a heteroatom NAs (e.g., NAs+S) cluster (Sun et al. 2014). The signal strength in an IMS spectrum qualitatively estimates the concentration of the corresponding NAs. In Figure 2-3 F, the region associated with oxy-NAs shows the most intensified signal, indicating that oxy-NAs were the most abundant species among all three NA related groups in the untreated OSPW II sample, contradicting with previously reported results (Sun et al. 2014). The region representing classical NAs also showed an intense signal whereas the cluster for heteroatom NAs has the least brightness, implying that the abundance of heteroatom NAs in the untreated OSPW II sample was relatively low compared with classical and oxidized NA species. By comparing Figure 2-3 F and Figure 2-3 G, it can be seen that the classical NAs cluster signal in the permeate sample image is substantially less intensive than that in the feed sample image, indicating that the MBR greatly reduced the concentration of classical NAs in the feed water. IMS analysis also shows that the MBR process removed some sulfur containing NAs. However, the oxy-NAs signal is more intensified in the

MBR permeate compared with the feed water IMS image, indicating that oxy-NAs were produced from classical NAs during the MBR biological treatment of OSPW. Our results are consistent with Han et al. (2009)'s study, which showed that classical NAs were converted to oxy-NAs during biodegradation.

2.3.1.4 Oxy-NA removal[2]

Oxy-NAs were also measured for the 100% OSPW II stage as is shown in Figure 2-4. O3 and O6 oxy-NAs were increased by 15.9% and 1.28%, respectively. In contrast, removal rates of 12.9% and 9.1% were achieved for O4 and O5 oxy-NAs, respectively. Regarding the overall degradation of total oxy-NAs (O3 – 6), the MBR's efficiency was marginal (0.1%). O3 and O4 oxy-NAs species are possibly intermediates during biodegradation of classical NAs according to some researchers (Grewer et al. 2010, Han et al. 2009, Martin et al. 2010). To be specific, O3 species are likely hydroxylated NAs (Han et al. 2008) and O4 are probably dicarboxylic acids (Headley et al. 2009). The recalcitrance of O3 oxy-NAs has been reported. Han et al. (2008) and Brown et al. (2013) performed a 98-day and 84-day incubation, respectively, without observing any appreciable changes of O3 or O4 oxy-NAs. Headley et al. (2009) did detect removal of O4 and O5 oxy-NAs in their study, which is in accordance to the results in this study. Therefore, it is suggested that the anoxic-aerobic MBR process is effective in removing certain oxy-NAs (i.e., O4 and O5 species in this study). In addition, the increase in O3 species migth be a result of bio-conversion of other NAs species, such as classical NAs.

Our COD results, and UPLC/HRMS analysis suggest that biotransformation other than mineralization was the major degradation pathway of NAs in the MBR, which is consistent with what has been concluded in previous studies (Choi et al. 2014, Misiti et al. 2013c).

[2] This paragraph is not included in the published manuscript.

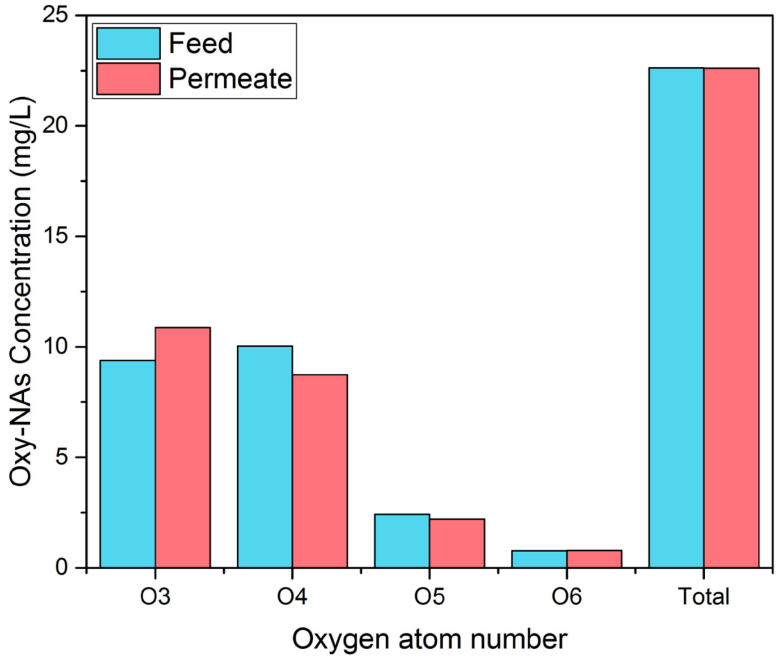
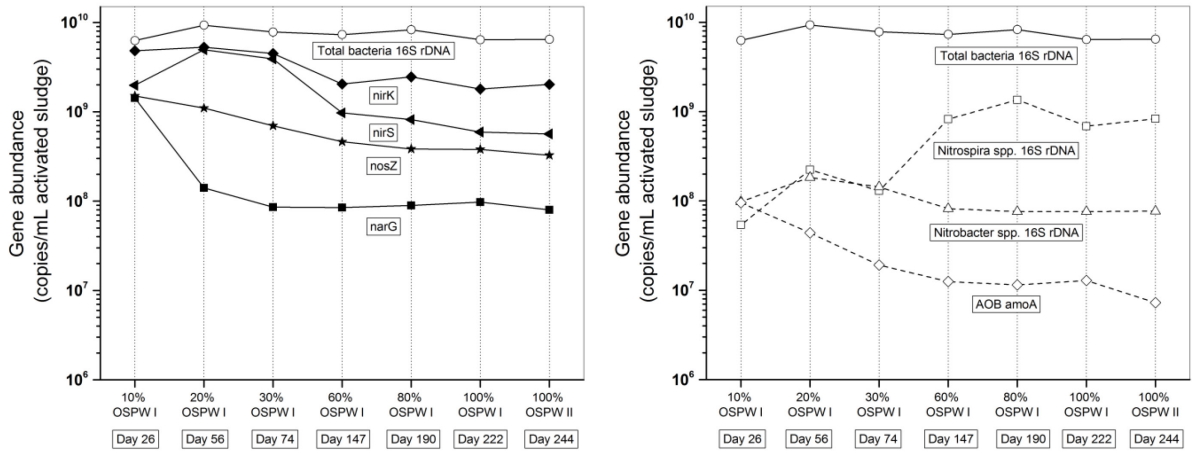


Figure 2-4. Oxy-NAs concentrations of the feed and permeate at the 100% OSPW II stage[3].

2.3.2 Characterization of microbial community structures

2.3.2.1 Real-time Quantitative Polymerase Chain Reaction (qPCR)



(A) Abundances of denitrifying genes

(B) Abundances of nitrifying genes

Figure 2-5. Abundances of selected genes at different OSPW percentages.

[3] This figure is not included in the published manuscript.

Figure 2-5 A and B illustrate the qPCR results for denitrifiers and nitrifiers, respectively, in the MBR at different OSPW percentages. The abundance of each gene was calculated by averaging the copy numbers of that gene in the anoxic and aerobic tanks. Over the MBR operating period, the total bacteria 16S rDNA gene showed a relatively stable abundance of around 7.6×10^9 copies/mL of sludge sample. Nitrite reductase genes *nirS* and *nirK* were the dominant denitrifying genes over the MBR operating period; the latter showed a higher abundance than the former. The *narG* gene abundance decreased from 1.42×10^9 copies/mL of sludge at 10% OSPW to 1.41×10^8 copies/mL of sludge at 20% OSPW, which is consistent with the fluctuation of nitrate concentration in the effluent (Figure 2-2 C). The *narG* abundance eventually stabilized at around 9.00×10^7 copies/mL of sludge at 100% OSPW II (Day 244). The abundances of *nirS* and *nirK* were higher than those of the *narG* and *nosZ* genes throughout the whole operation period of the MBR, which was in accordance with previous results (Kim et al. 2011c). The substantially decreased abundance of *nirS* and *nirK* at 60% OSPW stage might be related to the deterioration of denitrification performance in that stage (Figure 2-2 C).

Figure 2-5 B is a plot of the abundance of total bacteria, ammonia oxidizing bacteria (AOB), *Nitrospira* spp. (NOB), and *Nitrobacter* spp. (NOB), based on 16S rDNA gene analysis. Nitrifying bacterial genes *Nitrobacter* spp. 16S rDNA gene (NOB) and *amoA* (AOB) demonstrated a decreasing trend over the MBR operation. The abundance of *amoA* gradually decreased from 9.54×10^7 copies/mL of sludge at 10% OSPW to 6.24×10^6 copies/mL of sludge at 100% OSPW II step. NOBs are usually considered more sensitive to detrimental environmental conditions than AOBs. However, there are always exceptions. For instance, AOBs are more sensitive to silver nano-particles (AgNPs) than NOBs (Yang et al. 2014a); and AOBs are also more sensitive than NOB to the exposure of chromium (Zhang et al. 2015a). Thus, NAs or other

organic or inorganic components in OSPW might have impacted nitrifying bacteria in a different way. Take into consideration the ammonium concentration of ~25 mg N/L in the feed water to the MBR, the population of AOB reached in this study was in agreement with previous studies (Li et al. 2006, Wells et al. 2009), implying that the AOB population in the MBR was sufficient for the NH_4-N concentration used in the feed. According to Philippot and Hallin (2005), the existence of functional genes of target bacteria in the activated sludge samples does not necessarily guarantee that the corresponding microorganisms present in the system demonstrate the expected activities. Moreover, anammox might also contribute to the nearly complete ammonia removal in the reactor. The anaerobic zone in the anoxic tank might provide favorable environment for the growth of anammox.

The abundance of *Nitrobacter* spp. 16S rDNA gene was only 7.72×10^7 copies/mL of sludge at the 100% OSPW II step (Day 244). Contrarily, the abundance of the *Nitrospira* spp. 16S rDNA gene increased from 5.39×10^7 copies/mL of sludge at 10% OSPW to 1.35×10^9 copies/mL of sludge at 80% OSPW (Day 190), and then slightly declined to a relatively stable level of around 7.00×10^8 copies/mL of sludge at 100% OSPW steps (Day 222 and Day 244). When the OSPW ratio increased to 30%, the abundance of *Nitrospira* spp. increased considerably while the permeate nitrite became non-detectable. *Nitrospira* spp. turned out to be the dominating species among nitrifiers in the MBR at the stabilized stage. As a known K-strategist, *Nitrospira* spp. tends to dominate the NOB community when a nitrite-weak environment (i.e., a K_m of 0.01 mM nitrite) is present (Schramm et al. 1999); whereas *Nitrobacter* spp. (r-strategist) prefers high nitrite concentrations (> 0.5 mM nitrite) (Bock and Koops 1992) which would inhibit *Nitrospira* spp. (Cebron and Garnier 2005). Figure 2-2 D shows that the nitrite concentration in the MBR was mostly below 0.025 mg N/L (0.018 mM nitrite), which justifies the dominance of *Nitrospira*

spp. in the MBR suspension system. It has been reported that rather than small organics including acetate, butyrate, and propionate, *Nitrospira* spp. only utilizes inorganic carbon and pyruvate under aerobic conditions; whereas many species of *Nitrobacter* spp. were found to be capable of taking up all the above organic substrates mixotrophically. Moreover, *Nitrobacter* spp. is able to use pyruvate under anoxic condition (Kim and Kim 2006, Prosser 1989). Thus, *Nitrobacter* spp. might still be playing an indispensable role in the MBR system in the current study. No literature was found to support *Nitrospira* spp.'s degradation of OSPW NAs or other recalcitrant organic compounds. The increase in *Nitrospira* spp. abundance along with the increase of OSPW NAs concentrations observed in this study implies that *Nitrospira* spp. was not inhibited by increasing OSPW NAs, and may play a remarkable role in the pathway of OSPW NA biodegradation in the MBR system. Further investigation to examine the effects of OSPW NAs on the growth of *Nitrospira* spp. and *Nitrobacter* spp. are necessary to illuminate their activities in OSPW.

2.3.2.2 454 Pyrosequencing

The microbial compositions of the MFT, GBWWTP mixed sludge, raw OSPW I, raw OSPW II, and MBR mixed liquors at 60% OSPW I, 100% OSPW I, and 100% OSPW II steps were characterized using the 454 Pyrosequencing technique. Figure 2-6 A shows that the most dominating phylum in all samples was *Proteobacteria*, which accounted for 48 – 74% of the bacterial kingdom. *Proteobacteria* are believed to play an essential role in organic substance degradation, nutrient removal and activated sludge floc structural stability, which is critical to the biodegradation efficiency of the system (Yang et al. 2014b). Members affiliated with the genra *Thauera* and *Azoarcus* within the β-class of *Proteobacteria* have been reported to simultaneously

denitrify and mineralize a variety of hydrocarbons (Etchebehere and Tiedje 2005, Heider et al. 1999).

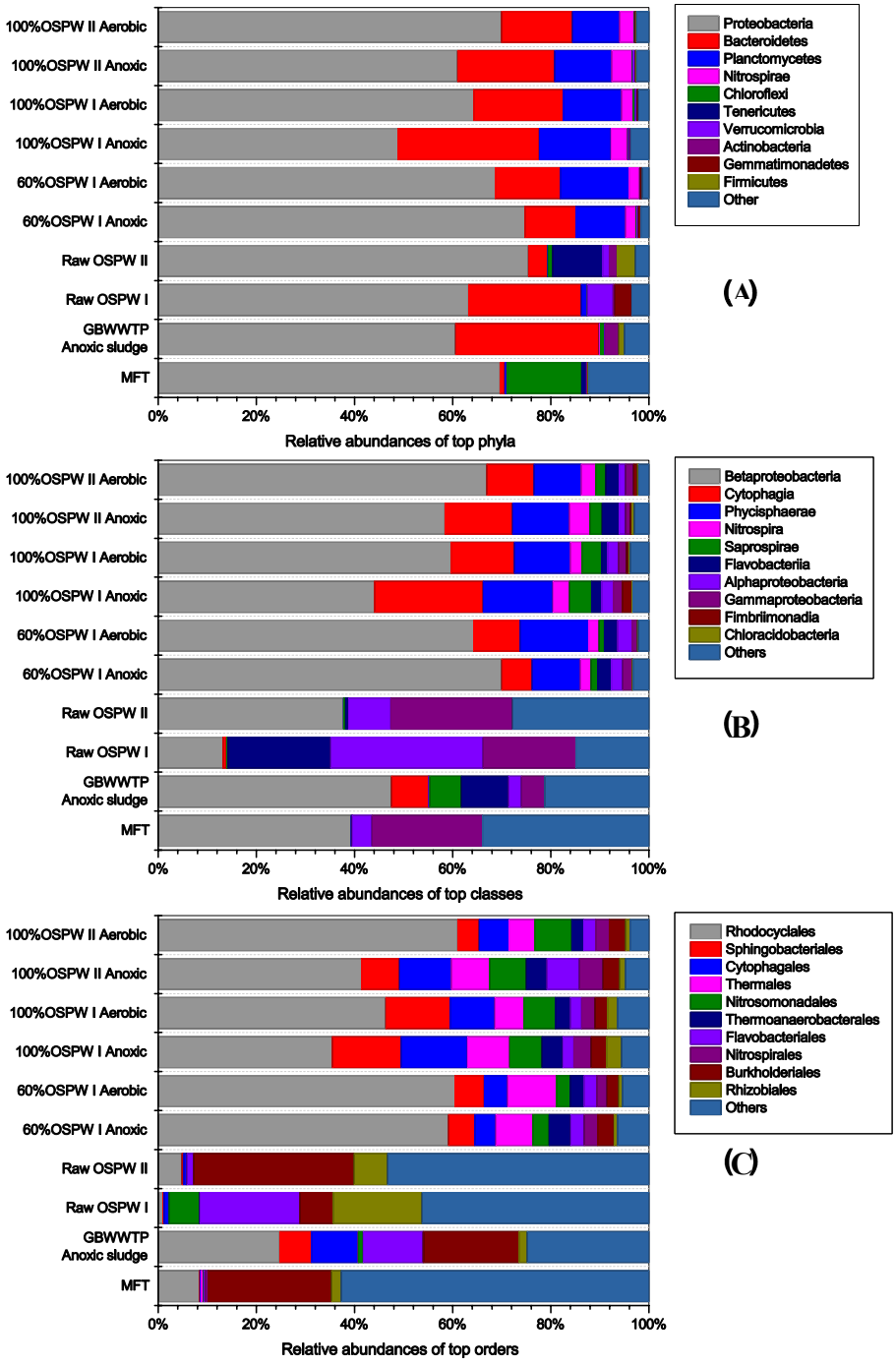


Figure 2-6. Relative abundance of (A) phyla; (B) classes; and (C) orders.

Bacteroidetes, the second most abundant phylum in the MBR, are considered to specialize in degrading complex organic matter, including those substances in the forms of polysaccharides and proteins (Kim and Kwon 2010, Thomas et al. 2011). Members within *Bacteroidetes* are thus assumed to play a role in degradation of polysaccharides and proteins produced through bacterial secretion and cell lysis, which might contribute to the limited TMP increase in this study. *Yeosuana aromativorans* under *Bacteroidetes* have been shown to degrade hydrocarbons (Kim and Kwon 2010). It is believed the bacteria that perform anaerobic ammonium oxidation (anammox) belong to the phylum *Planctomycetes* (Schmidt et al. 2003). In this study, substantial abundance of *Planctomycetes* was observed only in the MBR (not in OSPW or inocula); this might be attributed to the addition of nitrogen and low DO in the anoxic tank. The possible existence of anammox bacteria within the phyla of *Planctomycetes* help explain the nearly 100% removal of inorganic nitrogen in the MBR. *Nitrospirae* is one of the four phyla that include all known NOB (Siripong and Rittmann 2007). The high abundance of *Nitrospirae* in the MBR tanks at all percentages of OSPW explains the excellent nitrification performance of the system (Figure 2-6 A). The predominance of *Proteobacteria*, *Bacteroidetes*, and *Planctomycetes* in the MBR is consistent with previous MBR studies (Gao et al. 2011a) and is similar with a previous study on sequencing batch reactor for OSPW treatment (Choi and Liu 2014).

Figure 2-6 B shows the relative abundance of dominant classes in different samples. *β-Proteobacteria* and *γ-Proteobacteria* were dominant in the OSPW and MFT samples. After inoculating OSPW and MFT into the reactor, the proportion of *γ-Proteobacteria* declined while *β-Proteobacteria*, *Cytophagia* and *Phycisphaerae* became the only predominant class in the MBR. Dominating *β-Proteobacteria* have been found in oil sands tailings ponds and Athabasca river sediments as degraders of naphthenic acids and aromatic hydrocarbons (Yergeau et al.

2012) while *Cytophagia* have been linked to degradation of hydrocarbons (Röling et al. 2002). Closer scrutiny of the bacterial orders in different samples (Figure 2-6 C) showed that *Rhodocyclales* is the most dominating bacterial orders in the MBR tanks with an abundance of 35% – 60%. *Sphingobacteriales*, *Cytophagales*, and *Nitrosomonadales* also showed a relative high abundance. Among those dominating groups, *Rhodocyclales* are responsible for denitrification and reduction of aromatic hydrocarbons (Loy et al. 2005) while *Sphingobacteriales* and *Cytophagales* for degradation of a vast variety of bio-recalcitrant organic compounds (Drury et al. 2013, Kertesz and Kawasaki 2010, Röling et al. 2002). A study of Arctic soil with petroleum contamination indicated that *Nitrosomonadales* is also closely related to the degradation of hydrocarbons (Bell et al. 2013b).

Considering the origins of the major bacterial groups in MBR, it was found that the most dominating bacteria *Rhodocyclales* might be from OSPW, MFT, or GBWWTP mixed sludge. However, other dominant bacteria such as *Sphingobacteriales*, *Cytophagales* and *Nitrosomonadales* are more likely to come from the anoxic activated sludge. Although the abundance of *Thermales*, *Thermoanaerobacterales* and *Nitrospirales* was negligible in all the inocula and OSPW, they became dominant during the operation of the reactor for OSPW treatment. The comparisons between microbial community compositions of the MBR samples, OSPW samples, and inocula samples reveal the contributions of MFT, GBWWTP mixed sludge, raw OSPW I and raw OSPW II to the MBR microbial community development.

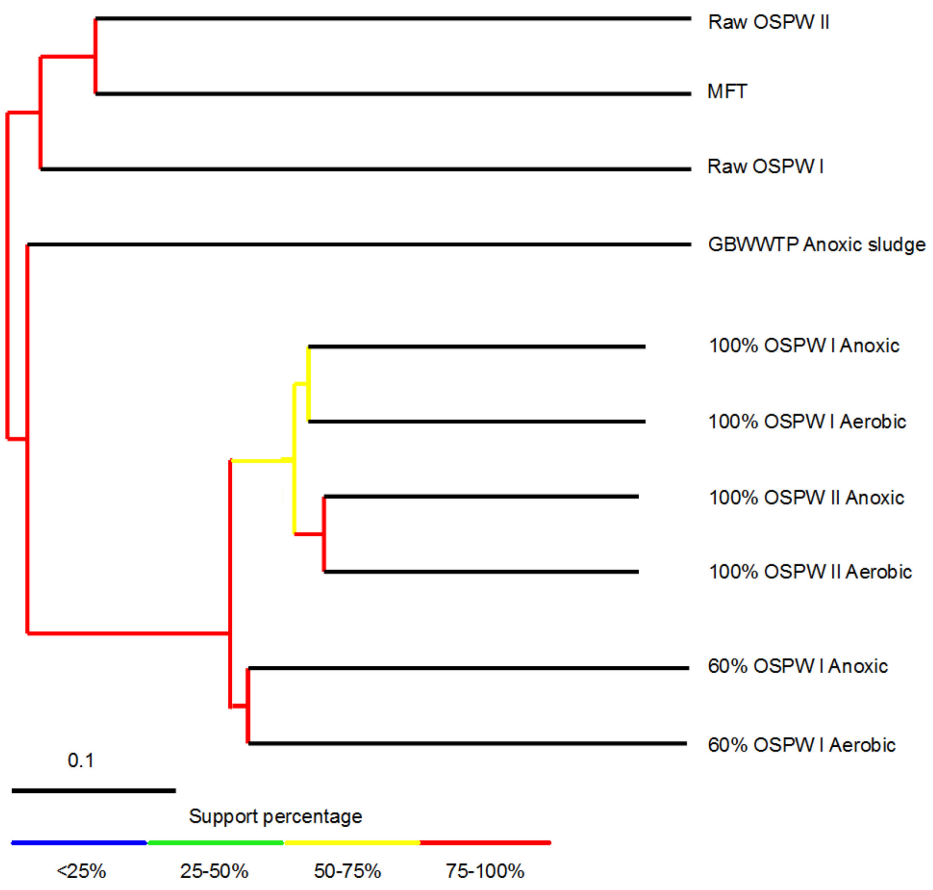


Figure 2-7. Bootstrapped tree.

The phylogenetic distance cladogram for different samples was built based on UniFrac distance (Figure 2-7). The bacterial communities in the MBR were clustered together while bacteria in MFT and OSPW were closely grouped. PCoA based on UniFrac distances is another good way to visually disclose the distance between microbial communities (Figure 2-8) (Liu et al. 2014). Bacterial groups in the anoxic sludge from the wastewater treatment plant show a clear separation from those in OSPW I and II and MFT, indicating that only bacteria acclimatized to OSPW could survive in the reactor.

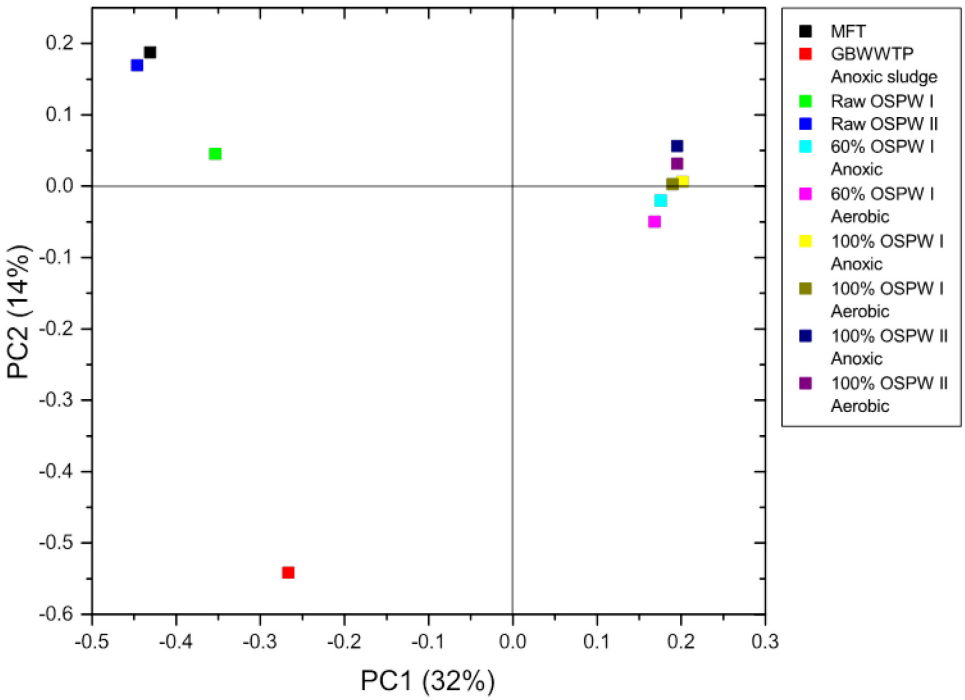


Figure 2-8. PCoA analysis.

Table 2-5 shows the bacterial diversity indices of MFT, GBWWTP mixed sludge, OSPW I and II, and sludge samples in the MBR. The Chao1 indices indicate that the richness of samples in the MBR is higher than that in OSPW and MFT samples (Table 2-5), suggesting the enrichment of bacteria in the MBR. This decreased Shannon diversity in the reactor may be attributed to the chronic toxicity of NAs accumulated in the MBR, which allows the survival of only bacteria with high tolerance. Similar numbers of bacterial species were observed in most samples except for the samples of anoxic sludge from the GBWWTP. The high abundances of bacteria involved in removing nitrogen, aromatic compounds, and hydrocarbons in the MBR system accounted for the MBR system's good nitrification and denitrification performance and effective removal of OSPW NAs. However, more effort is necessary to further expand the understanding on how different members of the microbial community affect and contribute to degradation of OSPW organic compounds in the system.

Table 2-5. Alpha diversity analyses.

Sample	Parameters (N=1525 sequences/sample)			
	Chao1[a]	Shannon[b]	Observed species[c]	PD whole tree[d]
MFT	280	5.42	204	17.5
GBWWTP mixed sludge	508	7.13	362	22.6
Raw OSPW I	342	6.20	224	13.4
Raw OSPW II	326	6.34	234	14.9
MBR anoxic at 60% OSPW I	375	5.42	209	13.3
MBR aerobic at 60% OSPW I	420	5.44	230	13.0
MBR anoxic at 100% OSPW I	461	5.47	231	14.4
MBR aerobic at 100% OSPW I	375	5.56	215	14.3
MBR anoxic at 100% OSPW II	342	5.45	206	12.6
MBR aerobic at 100% OSPW II	413	5.30	209	13.8

a. Chao1: The species richness estimator, the total number of OTUs estimated in the community.
b. Shannon: An index characterizing the species diversity in a community. A higher value indicates higher diversity.
c. Observed species: The count of the unique OTUs in the sample.
d. PD whole tree: Faith's Phylogenetic Diversity based on the phylogenetic tree. It adds up all the branch lengths as a measure of diversity.

2.3.3 Transmembrane pressure

Compared to other filtration membranes, the ceramic membrane provided a great advantage in wastewater treatment in regard to fouling resistance (Alpatova et al. 2014). Figure 2-9 shows the TMP values recorded over the operating period of the MBR. The manufacturer of the membrane suggested that a critical TMP of -35 kPa indicates that the membrane is severely fouled and should be chemically cleaned. However, throughout > 300 days of continuous operation, the membrane used in the aerobic MBR tank was chemically cleaned only once due to sensor failure which caused rapid increase of TMP (shown in Figure 2-9). The TMP value never exceeded -20 kPa under normal operating conditions, indicating the excellent fouling resistance of the ceramic membrane used in this study. Compared with conventional polymeric membrane used in MBR system, ceramic membrane has been demonstrated to have the less irreversible fouling (Hofs et al. 2011) and could be operated with longer period without cleaning (Tewari et al. 2012). In addition, taking into account its outstanding physical/chemical stability and resistance to

biodegradation, ceramic membranes have a good potential to be used in MBR system for treating the OSPW or other wastewater.

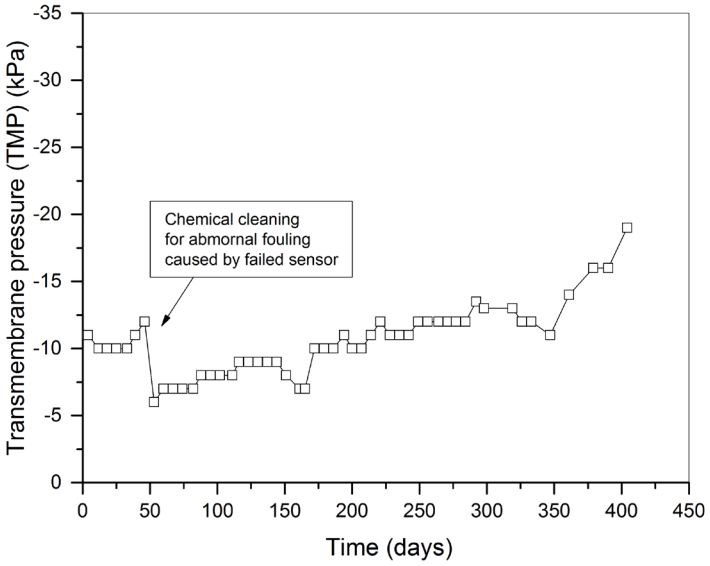


Figure 2-9. TMP over time.

2.4 Conclusions

The anoxic-aerobic MBR employed in this study has shown its effectiveness in OSPW treatment with a stabilized NA removal of 24.7% within 48 h, indicating that the anoxic-aerobic MBR is a promising technology for OSPW remediation. It has been demonstrated that biotransformation instead of complete mineralization was the dominant biodegradation pathway of OSPW parent NAs in the MBR. While parent NAs with complex molecular structures were broken down into molecules with fewer carbons, more degradation was observed for NAs with fewer degrees of cyclization. The presence of carbon degraders (such as *Rhodocyclales* and *Sphingobacteriales*) in the MBR indicated the biodegradation of organic constituents in OSPW. The dominating *Bacteroidetes* in the MBR might play an important role in excellent antifouling performance of ceramic membrane. To further improve the performance of this anoxic-aerobic MBR for OSPW

treatment, efforts could be done to optimize the operating conditions of the MBR as well as to combine the anoxic-aerobic MBR system with other treatment processes, such as ozonation pre-treatment/post-treatment and biofilm carriers.

References

Allen, E.W., 2008. 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.

Alpatova, A., Kim, E.-S., Dong, S., Sun, N., Chelme-Ayala, P. and El-Din, M.G., 2014. Treatment of Oil Sands Process-Affected Water with Ceramic Ultrafiltration Membrane: Effects of Operating Conditions on Membrane Performance. Separation and purification Technology 122, 170-182.

Bell, T.H., Yergeau, E., Maynard, C., Juck, D., Whyte, L.G. and Greer, C.W., 2013. Predictable Bacterial Composition and Hydrocarbon Degradation in Arctic Soils Following Diesel and Nutrient Disturbance. ISME J 7(6), 1200-1210.

Bock, E. and Koops, H., 1992. The Genus Nitrobacter and Related Genera. Balows A, Tr€ uper HG, Dworkin M, Harder W, Schleifer KH, editors. The prokaryotes. New York: Springer, 2302-2309.

Brown, L.D., Pérez-Estrada, L., Wang, N., El-Din, M.G., Martin, J.W., Fedorak, P.M. and Ulrich, A.C., 2013. Indigenous Microbes Survive in Situ Ozonation Improving Biodegradation of Dissolved Organic Matter in Aged Oil Sands Process-Affected Waters. Chemosphere.

Cebron, A. and Garnier, J., 2005. Nitrobacter and Nitrospira Genera as Representatives of Nitrite-Oxidizing Bacteria: Detection, Quantification and Growth Along the Lower Seine River (France). Water research 39(20), 4979-4992.

Choi, J., Hwang, G., Gamal El-Din, M. and Liu, Y., 2014. Effect of Reactor Configuration and Microbial Characteristics on Biofilm Reactors for Oil Sands Process-Affected Water Treatment. International Biodeterioration & Biodegradation 89(2014), 74-81.

Choi, J. and Liu, Y., 2014. Biodegradation of Oil Sands Process Affected Water in Sequencing Batch Reactors and Microbial Community Analysis by High-Throughput Pyrosequencing. International Biodeterioration & Biodegradation 92, 79-85.

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.

De Gusseme, B., Pycke, B., Hennebel, T., Marcoen, A., Vlaeminck, S.E., Noppe, H., Boon, N. and Verstraete, W., 2009. Biological Removal of 17 Alpha-Ethinylestradiol by a Nitrifier Enrichment Culture in a Membrane Bioreactor. Water research 43(9), 2493-2503.

Deni, J. and Penninckx, M.J., 1999. Nitrification and Autotrophic Nitrifying Bacteria in a Hydrocarbon-Polluted Soil. Applied and environmental microbiology 65(9), 4008-4008.

Drury, B., Rosi-Marshall, E. and Kelly, J.J., 2013. Wastewater Treatment Effluent Reduces the Abundance and Diversity of Benthic Bacterial Communities in Urban and Suburban Rivers. Applied and environmental microbiology 79(6), 1897-1905.

Etchebehere, C. and Tiedje, J., 2005. Presence of Two Different Active Nirs Nitrite Reductase Genes in a Denitrifying Thauera Sp. From a High-Nitrate-Removal-Rate Reactor. Applied and environmental microbiology 71(9), 5642-5645.

Gao, D.-w., Fu, Y., Tao, Y., Li, X.-x., Xing, M., Gao, X.-h. and Ren, N.-q., 2011. Linking Microbial Community Structure to Membrane Biofouling Associated with Varying Dissolved Oxygen Concentrations. Bioresource technology 102(10), 5626-5633.

Germirli Babuna, F., Camur, S., Alaton, I.A., Okay, O. and Iskender, G., 2009. The Application of Ozonation for the Detoxification and Biodegradability Improvement of a Textile Auxiliary: Naphtalene Sulphonic Acid. Desalination 249(2), 682-686.

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? The Science of the total environment 408(23), 5997-6010.

Gunawan, Y., Nemati, M. and Dalai, A., 2014. Biodegradation of a Surrogate Naphthenic Acid under Denitrifying Conditions. Water research 51, 11-24.

Han, X., MacKinnon, M.D. and Martin, J.W., 2009. Estimating the in Situ Biodegradation of Naphthenic Acids in Oil Sands Process Waters by Hplc/Hrms. Chemosphere 76(1), 63-70.

Han, X., 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.

Headley, J.V., Peru, K.M., Armstrong, S.A., Han, X., Martin, J.W., Mapolelo, M.M., Smith, D.F., Rogers, R.P. and Marshall, A.G., 2009. Aquatic Plant‐Derived Changes in Oil Sands Naphthenic Acid Signatures Determined by Low‐, High‐and Ultra High‐Resolution Mass Spectrometry. Rapid Communications in Mass Spectrometry 23(4), 515-522.

Heider, J., Spormann, A.M., Beller, H.R. and Widdel, F., 1999. Anaerobic Bacterial Metabolism of Hydrocarbons. FEMS Microbiology Reviews 22, 459-473.

Higashioka, Y., Kojima, H., Sato, S. and Fukui, M., 2009. Microbial Community Analysis at Crude Oil-Contaminated Soils Targeting the 16s Ribosomal Rna, Xylm, C23o, and Bcr Genes. Journal of applied microbiology 107(1), 126-135.

Hofs, B., Ogier, J., Vries, D., Beerendonk, E.F. and Cornelissen, E.R., 2011. Comparison of Ceramic and Polymeric Membrane Permeability and Fouling Using Surface Water. Separation and purification Technology 79(3), 365-374.

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.

Hwang, G., Dong, T., Islam, M.S., Sheng, Z., Pérez-Estrada, L.a., Liu, Y. and Gamal El-Din, M., 2013. The Impacts of Ozonation on Oil Sands Process-Affected Water Biodegradability and Biofilm Formation Characteristics in Bioreactors. Bioresource technology 130C, 269-277.

Islam, M.S., Dong, T., Sheng, Z., Zhang, Y., Liu, Y., Gamal El-Din, M. and El-Din, M.G., 2013. Microbial Community Structure and Operational Performance of a Fluidized Bed Biofilm Reactor Treating Oil Sands Process-Affected Water. International Biodeterioration & Biodegradation 91, 111-118.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and El-Din, M.G., 2014. Prediction of Naphthenic Acid Species Degradation by Kinetic and Surrogate Models During the Ozonation of Oil Sands Process-Affected Water. Science of The Total Environment 493, 282-290.

Judd, S. and Judd, C., 2011. The Mbr Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, Elsevier, Oxford.

Kertesz, M. and Kawasaki, A., 2010. Handbook of Hydrocarbon and Lipid Microbiology, pp. 1693-1705, Springer.
Kim, D.-J. and Kim, S.-H., 2006. Effect of Nitrite Concentration on the Distribution and Competition of Nitrite-Oxidizing Bacteria in Nitratation Reactor Systems and Their Kinetic Characteristics. Water research 40(5), 887-894.
Kim, S.-J. and Kwon, K.K., 2010. Handbook of Hydrocarbon and Lipid Microbiology. Timmis, K.N. (ed), Springer, Berlin, Heidelberg.
Kim, Y.M., Lee, D.S., Park, C., Park, D. and Park, J.M., 2011. Effects of Free Cyanide on Microbial Communities and Biological Carbon and Nitrogen Removal Performance in the Industrial Activated Sludge Process. Water research 45(3), 1267-1279.
Ledakowicz, S., Michniewicz, M., Jagiella, A., Stufka-Olczyk, J. and Martynelis, M., 2006. Elimination of Resin Acids by Advanced Oxidation Processes and Their Impact on Subsequent Biodegradation. Water Research 40(18), 3439-3446.
Li, H., Yang, M., Zhang, Y., Yu, T. and Kamagata, Y., 2006. Nitrification Performance and Microbial Community Dynamics in a Submerged Membrane Bioreactor with Complete Sludge Retention. Journal of biotechnology 123(1), 60-70.
Lin, H., 2012. Membrane Bioreactors for Industrial Wastewater Treatment : A Critical Review. Critical Reviews in Environmental Science and Technology 42(7), 677-740.
Liu, G., Bakker, G.L., Li, S., Vreeburg, J.H.G., Verberk, J.Q.J.C., Medema, G.J., Liu, W.T. and Van Dijk, J.C., 2014. Pyrosequencing Reveals Bacterial Communities in Unchlorinated Drinking Water Distribution System: An Integral Study of Bulk Water, Suspended Solids, Loose Deposits, and Pipe Wall Biofilm. Environmental science & technology.
Loy, A., Schulz, C., Lücker, S., Stoecker, K., Baranyi, C., Lehner, A., Wagner, M., Lu, S. and Scho, A., 2005. Microarray for Environmental Monitoring of the Betaproteobacterial Order 16s Rrna Gene-Based Oligonucleotide Microarray for Environmental Monitoring of the Betaproteobacterial Order "Rhodocyclales". Applied and environmental microbiology 71(3), 1373-1373.
Martin, J.W., Barri, T., Han, X., 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.
Mincer, T.J., Church, M.J., Taylor, L.T., Preston, C., Karl, D.M. and DeLong, E.F., 2007. Quantitative Distribution of Presumptive Archaeal and Bacterial Nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environmental microbiology 9, 1162-1175.
Misiti, T., Tandukar, M., Tezel, U. and Pavlostathis, S.G., 2013a. Inhibition and Biotransformation Potential of Naphthenic Acids under Different Electron Accepting Conditions. Water research 47(1), 406-418.
Misiti, T.M., Tezel, U., Tandukar, M. and Pavlostathis, S.G., 2013b. Aerobic Biotransformation Potential of a Commercial Mixture of Naphthenic Acids. Water research 47(15), 5520-5534.
Philippot, L. and Hallin, S., 2005. Finding the Missing Link between Diversity and Activity Using Denitrifying Bacteria as a Model Functional Community. Current opinion in microbiology 8(3), 234-239.
Prosser, I., 1989. Autotrophic Nitrification in Bacteria. Adv. Microb. Physiol 20, 125-192.
Qin, J., Oo, M., Tao, G. and Kekre, K., 2007. Feasibility Study on Petrochemical Wastewater Treatment and Reuse Using Submerged Mbr. Journal of Membrane Science 293(1-2), 161-166.

Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J. and Chu, K.-H., 2009. Biodegradation Potential of Wastewater Micropollutants by Ammonia-Oxidizing Bacteria. Chemosphere 77(8), 1084-1089.

Röling, W.F., Milner, M.G., Jones, D.M., Lee, K., Daniel, F., Swannell, R.J. and Head, I.M., 2002. Robust Hydrocarbon Degradation and Dynamics of Bacterial Communities During Nutrient-Enhanced Oil Spill Bioremediation. Applied and environmental microbiology 68(11), 5537-5548.

Schmidt, I., Sliekers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J.G., Jetten, M.S.M. and Strous, M., 2003. New Concepts of Microbial Treatment Processes for the Nitrogen Removal in Wastewater. FEMS Microbiology Reviews 27(4), 481-492.

Schramm, A., de Beer, D., van den Heuvel, J.C., Ottengraf, S. and Amann, R., 1999. Microscale Distribution of Populations and Activities Ofnitrosospira and Nitrospira Spp. Along a Macroscale Gradient in a Nitrifying Bioreactor: Quantification by in Situ Hybridization and the Use of Microsensors. Applied and environmental microbiology 65(8), 3690-3696.

Siripong, S. and Rittmann, B.E., 2007. Diversity Study of Nitrifying Bacteria in Full-Scale Municipal Wastewater Treatment Plants. Water research 41(5), 1110-1120.

Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M. and Hagen, M., 2014. Advanced Analytical Mass Spectrometric Techniques and Bioassays to Characterize Untreated and Ozonated Oil Sands Process-Affected Water. Environmental science & technology 48(19), 11090-11099.

Tewari, P., Singh, R., Batra, V. and Balakrishnan, M., 2012. Field Testing of Polymeric Mesh and Ash-Based Ceramic Membranes in a Membrane Bioreactor (Mbr) for Decentralised Sewage Treatment. Water SA 38(5), 727-730.

The Government of Alberta, 2015. Lower Athabasca Region-Tailings Management Framework for the Mineable Athabasca Oil Sands. The Government of Alberta (ed).

Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M. and Michel, G., 2011. Environmental and Gut Bacteroidetes: The Food Connection. Frontiers in microbiology 2.

Wang, C., Yediler, A., Lienert, D., Wang, Z. and Kettrup, A., 2003. Ozonation of an Azo Dye C.I. Remazol Black 5 and Toxicological Assessment of Its Oxidation Products. Chemosphere 52(7), 1225-1232.

Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Garcia-Garcia, E., Pun, J., Martin, J.W., Belosevic, M. and Gamal El-Din, M., 2013. Impact of Ozonation on Naphthenic Acids Speciation and Toxicity of Oil Sands Process-Affected Water to Vibrio Fischeri and Mammalian Immune System. Environmental science & technology 47(12), 6518-6526.

Wells, G.F., Park, H.-D., Yeung, C.-H., Eggleston, B., Francis, C.a. and Criddle, C.S., 2009. Ammonia-Oxidizing Communities in a Highly Aerated Full-Scale Activated Sludge Bioreactor: Betaproteobacterial Dynamics and Low Relative Abundance of Crenarchaea. Environmental microbiology 11(9), 2310-2328.

Yang, Y., Li, M., Michels, C., Moreira‐Soares, H. and Alvarez, P.J., 2014a. Differential Sensitivity of Nitrifying Bacteria to Silver Nanoparticles in Activated Sludge. Environmental Toxicology and Chemistry 33(10), 2234-2239.

Yang, Y., Quensen, J., Mathieu, J., Wang, Q., Wang, J., Li, M., Tiedje, J.M. and Alvarez, P.J.J., 2014b. Pyrosequencing Reveals Higher Impact of Silver Nanoparticles Than Ag+ on the Microbial Community Structure of Activated Sludge. Water research 48, 317-325.

Yergeau, E., Lawrence, J.R., Sanschagrin, S., Waiser, M.J., Korber, D.R. and Greer, C.W., 2012. Next-Generation Sequencing of Microbial Communities in the Athabasca River and Its Tributaries in Relation to Oil Sands Mining Activities. Applied and environmental microbiology 78(21), 7626-7637.

Zhang, H., Zhai, H., Ji, M., Su, X., Du, Z. and Liu, J., 2015. Long-Term Effect of Cr (Vi) on Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria in an Activated Sludge System. Desalination and Water Treatment 54(7), 1981-1989.

3 Treatment of raw and mildly ozonated OSPWs under decoupled anoxic and aerobic conditions: a comprehensive study[4]

3.1 Introduction

Bitumen is extracted from oil sands ores using a water intensive caustic hot-water process that has resulted in enormous volumes of oil sands process-affected water (OSPW) in Canada. Among the organic contaminants in OSPW, naphthenic acids (NAs) are of particular environmental concerns since they are corrosive and among the most toxic components of OSPW (acutely toxic to both aquatic and terrestrial species) (Allen 2008a, Brown and Ulrich 2015). Moreover, their complex molecular structures (i.e., long carbon chains and high cyclicity) contribute to their recalcitrance in the environment (Anderson et al. 2012, Gamal El-Din et al. 2011, He et al. 2012). NAs are a family of alicyclic and alkyl-substituted aliphatic carboxylic acids with a generalized formula of $C_nH_{2n+Z}O_x$, where n denotes carbon number, Z (a negative even integer) is the hydrogen deficiency resulting from cyclicity or double bond formation, and x represents the number of oxygen atoms. Species with $x = 2$ are referred as classical NAs, while those with $x \geq 3$ are termed as oxidized NAs (oxy-NAs) (Islam et al. 2014).

Among all the investigated processes for OSPW treatment, biodegradation is generally considered the most cost-effective and practical way to reduce NAs and detoxify OSPW through selectively removing NAs with low molecular weight and cyclicity (Han et al. 2008, Kannel and Gan 2012, Scott et al. 2005b). Most of biodegradation studies were conducted under aerobic conditions, demonstrating the effective aerobic degradation of NAs. For instance, Han et al.

[4] This chapter has been submitted to: Jinkai Xue, Yanyan Zhang, Yang Liu, Mohamed Gamal El-Din, 2016. Treatment of raw and ozonated oil sands process-affected water under decoupled anoxic denitrifying and aerobic nitrifying conditions: a comparative study. Submitted to: Biodegradation.

(2008) observed 48% of OSPW NAs removed in their aerobic batch suspension reactor after 98 days of operation. More recently, Choi et al. (2014), Hwang et al. (2013) and Shi et al. (2015) reported NA degradation of 18.5% – 43.1% using biofilm reactors under aerobic conditions.

It has been reported that ammonia oxidizing bacteria (AOB) improved the biodegradation of some recalcitrant organic compounds such as endocrine disrupting compounds and refractive petroleum hydrocarbons through the co-metabolism of ammonium monooxygenase (*amo*) (De Gusseme et al. 2009, Roh et al. 2009, Sayavedra-Soto et al. 2010). Misiti et al. (2013a) found that the commercial NAs mixture was reduced under the heterotrophic/nitrifying conditions when the initial NAs concentrations were higher than 80 mg/L although it is still unclear whether the activities of nitrifiers are beneficial to the degradation of NAs (Aga 2007). The indigenous NAs in OSPW are less biodegradable than commercial NAs (Scott et al. 2005a), which may have different behavior under nitrifying aerobic conditions. It is therefore interesting to perform a study to verify the contribution of nitrification to the aerobic degradation of OSPW indigenous NAs.

Despite the common evidence that higher oxidation potentials under aerobic conditions should favor the degradation of organic contaminants, some pollutants are better degraded by using other electron accepters instead of oxygen (Suarez et al. 2010). Previous studies have shown that some hydrocarbons (including aromatic compounds) could be degraded under denitrifying conditions. Among those identified denitrifiers capable of degrading hydrocarbons anaerobically (Heider et al. 1999), *Thauera aromatica* and *Azoarcus*-related species have been extensively studied for their abilities to degrade recalcitrant hydrocarbons (Heider et al. 1998, Heider and Fuchs 1997). Gunawan et al. (2014) reported that the removal of a surrogate NA (trans-4-methyl-1-cyclohexane carboxylic acid) coupled with denitrification was twice as fast as that under

aerobic conditions. In contrast, Misiti et al. (2013a) did not observe the degradation of commercial NAs under nitrate reducing conditions regardless of the presence of a degradable carbon source. It should be noted that the two aforementioned studies used surrogate or commercial NAs, which may not reflect the correlation between denitrification and OSPW indigenous NA degradation due to the different biodegradabilities of a commercial NAs mixture and OSPW indigenous NAs (Misiti et al. 2013b, Scott et al. 2005b). Therefore, it is necessary to investigate the effect of denitrifying conditions on OSPW indigenous NA degradation.

Furthermore, many of the previous studies revealed a remaining bio-recalcitrant fraction of OSPW NAs after biological treatment (Han et al. 2008, Scott et al. 2005b), making it clear that a certain pretreatment of OSPW to reduce this fraction prior to biological processes would be necessary to improve the removal efficiency. The most studied pretreatment method for OSPW is ozonation (Brown et al. 2013, Dong et al. 2015, Garcia-Garcia et al. 2011, Wang et al. 2013b), which was reported to preferentially decompose the most bio-persistent fraction of OSPW NAs and thus accelerating the subsequent aerobic biodegradation of OSPW (Martin et al. 2010). Although ozonation has been used as pretreatment for aerobic degradation, no study has ever been performed to investigate the effect of ozone pretreatment on subsequent biodegradation of OSPW under nitrification (aerobic) and denitrification (anoxic) condition.

A membrane bioreactor (MBR) system, which was configured with an anoxic tank followed by an aerobic tank with a membrane module submerged in, showed effective removal of NAs from both raw and ozonated OSPWs (Xue et al. 2016, Zhang et al. 2016). However, it is unclear how the anoxic tank and the aerobic tank contributed to the biodegradation of OSPW NAs. To date, there is no reported comparison study on anoxic and aerobic conditions for OSPW degradation. In addition, how mild-dose ozone pretreatment affects decoupled subsequent anoxic and aerobic

biodegradation of OSPW NAs needs to be investigated to design and optimize OSPW treatment processes. In the present study, batch reactors were operated under denitrifying anoxic and nitrifying aerobic conditions to treat raw and ozonated (utilized ozone dose of 30 mg O_3/L) OSPWs. The objectives of this study were 1) to investigate the effectiveness of anoxic and aerobic conditions on biodegradation of OSPW NAs; 2) to characterize and compare the microbial community characteristics under different conditions treating OSPW; and 3) to investigate the effect of mild-dose ozone pretreatment on subsequent anoxic and aerobic biodegradation of OSPW NAs. The results obtained from this study are expected to benefit the design and optimization of biological systems and onsite passive remediation of tailings water in the future.

3.2 Materials and Methods

3.2.1 Source waters and supplements

The OSPW source water was acquired from a tailings pond in Northern Alberta, Canada, in 2014. Ozonation of raw OSPW was performed using an AGSO 30 Effizon ozone generator (WEDECO AG Water Technology, Herford, Germany). A ceramic fine bubble gas diffuser was placed at the bottom of a 200 L reactor, through which the feed gas was added into the liquid phase of raw OSPW. During the ozonation process, two identical ozone monitors (HC-500, PCI-WEDECO) were adopted to control the ozone concentrations in the feed gas and off-gas lines continuously. A utilized ozone dose of 30 mg/L was applied, which is considerably mild compared with the doses (e.g., 90 mg O_3/L) used in previous studies (Gamal El-Din et al. 2011, Hwang et al. 2013). Based on our previous studies, a utilized ozone dose of 30 mg O_3/L could substantially increase the biodegradability of OSPW NAs while consuming less energy than

higher ozone doses (Dong et al. 2015). At the end, the ozonated OSPW was purged with nitrogen for 10 minutes to strip the residual ozone from the reactor.

Table 3-1. Characteristics of raw and ozonated OSPWs.

Parameter	Raw OSPW	Ozonated OSPW
pH	8.7±0.1	8.8±0.1
COD (mg/L)	209±1	197±1
BOD_5 (mg/L)	1.7±0.2	3.2±0.8
NO_3^--N (mg/L)	2.4±0.6	1.8±1.8
NO_2^--N (mg/L)	6.2±0.1	6.6±0.9
NH_3-N (mg/L)	5.2±0.1	5.8±0.7
Total Phosphate (mg P/L)	-	-
Classical NAs (mg/L)	25.2	12.7
Oxy-NAs (mg/L)	22.0	20.4

The characteristics of raw and ozonated OSPWs are tabulated in Table 3-1 in the Supporting Information. In order to ensure that bacteria have sufficient carbon source for growth, sodium acetate was added into the reactors. Modified Bushnell-Haas medium (BHM) (Scott et al. 2005b) was supplemented in feed OSPW (volumetric ratio BHM/OSPW=1/20) to ensure the sufficiency of trace elements for microbial growth. NH_4-N (NH_4Cl) and NO_3-N (KNO_3) were added to the corresponding reactors upon depletion. As a phosphorus source, NaH_2PO_4 was used.

Table 3-2. Compositions of the reactors (replication N = 5).

Feed type	Condition	Reactor ID	Composition of each replica reactor		
			OSPW (mL)	Nitrogen	Inoculum
Raw OSPW	Anoxic	RAN (1-5)	1000	50 mg NO_3-N/L	2 mL OSPW MBR anoxic sludge
	Aerobic	RAE (1-5)	500	50 mg NH_4-N/L	1 mL Raw OSPW MBR aerobic sludge
Ozonated OSPW	Anoxic	OAN (1-5)	1000	50 mg NO_3-N/L	2 mL OSPW MBR anoxic sludge
	Aerobic	OAE (1-5)	500	50 mg NH_4-N/L	1 mL OSPW MBR aerobic sludge

3.2.2 Bioreactors

1-L amber glass bottles were used as reactors for the four experimental treatments (five replicates of each): anoxic (denitrifying) reactors with raw OSPW, aerobic (nitrifying) reactor with raw OSPW, anoxic (denitrifying) reactors with ozonated OSPW, and aerobic (nitrifying) reactors with ozonated OSPW. The composition of each reactor is presented in Table 3-2. All anoxic reactors were filled with 1 L of OSPW, and purged with pure nitrogen gas for at least 5 min to eliminate dissolved and headspace oxygen prior to sealing them. A YSI 50B dissolved oxygen meter (YSI Inc.) was used to measure dissolved oxygen concentrations. For aerobic reactors, 500 mL of OSPW was added to allow oxygen diffusion from the headspace. The reactors were inoculated with the mixed liquor from the anoxic tank and aerobic tank of MBRs treating raw and ozonated OSPWs (Xue et al. 2016, Zhang et al. 2016) at a volumetric inocula/OSPW ratio of 1: 500, respectively. This MBR had been running for OSPW treatment for more than 1 year with the biomass concentration of 2900 – 3400 mg MLVSS/L.

Sodium acetate solution was added into each reactor to achieve a supplementary COD concentration of 250 mg/L so that the total COD level (sodium acetate + OSPW organic compounds) in the reactors was ~ 500 mg/L. Replenishments of external carbon source and nitrogen source are marked in Figure 3-1 and Figure 3-2. As marked with arrows in Figure 3-1 A, sodium acetate was only added in the aerobic reactors once at the beginning of the study to ensure the population of heterotrophs that potentially contribute to the biotransformation of OSPW NAs through co-metabolism with nitrifiers; whereas replenishment of sodium acetate was performed upon its depletion in the anoxic reactors (Figure 3-2 A) to maintain the abundance of denitrifiers that are well-known heterotrophs. To ensure the availability of NH_4-N to nitrifiers, NH_4Cl stock solution was added into the aerobic reactors with a level of 50 mg N/L once

depletion occurred. KNO_3 stock solution was transferred into the anoxic reactors with a concentration of 50 mg N/L. Inoculation, sampling, and replenishing for all anoxic reactors were performed in an anaerobic glove chamber to ensure the anoxic condition in the reactors. Nitrogen purging was conducted before recapping the reactors to remove dissolved oxygen that might be induced into the systems. Parafilm was applied after the anoxic bottles were tightly recapped to prevent air from entering the systems. To ensure the dissolution of oxygen into the mixed liquor, all the aerobic reactors were only loosely capped. All the bottles were placed in a shaker with a constant speed of 150 rpm to maintain the homogeneity of the mixed liquor. The temperature was maintained at room temperature (~20 °C) throughout the study.

3.2.3 Analyses

Reactors were well mixed prior to each sampling. A 10-mL aliquot was collected from all five replicates of each reactor type, and sufficiently mixed (50 mL total), creating a composite sample for each condition on each sample-collection day (indicated in Figure 3-1 and Figure 3-2). Water samples were filtered with a 0.22-μm membrane filter to remove suspended solids. Water chemistry parameters were measured in duplicates based on standard methods, including pH, chemical oxygen demand (COD), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), and nitrite nitrogen (NO_2-N). Standard Methods were followed when available (Federation and Association 2005). Ultra performance liquid chromatography coupled with high resolution mass spectrometry (UPLC/HRMS) (Choi et al. 2014) analysis was conducted to measure NAs concentration profiles. Molecular microbiological techniques including real-time quantitative polymerase chain reaction (qPCR) and 454 Pyrosequencing were performed to characterize microbial community structures in the reactors at different stages. Detailed information on molecular microbiological analyses is available elsewhere (Xue et al. 2016).

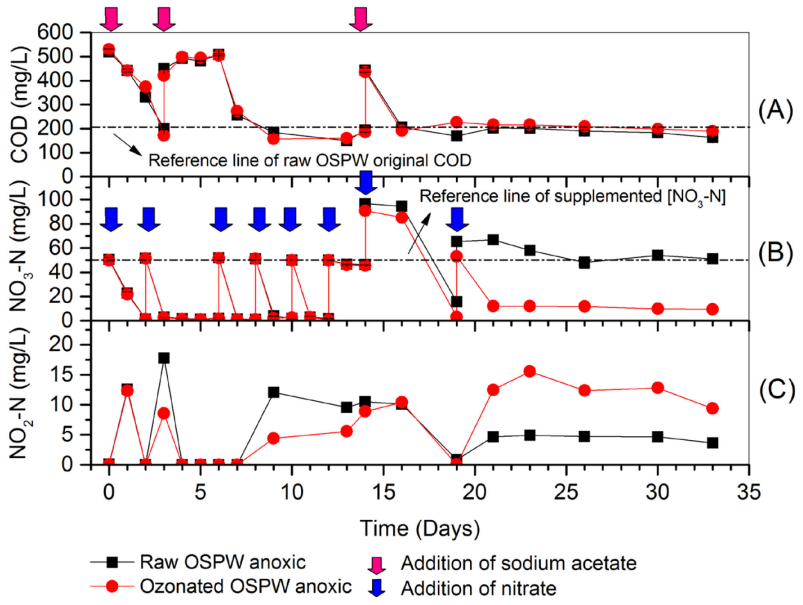


Figure 3-1. COD, NO_3-N, and NO_2-N concentrations in the anoxic reactors (each data point represents a combined sample from 5 replicates).

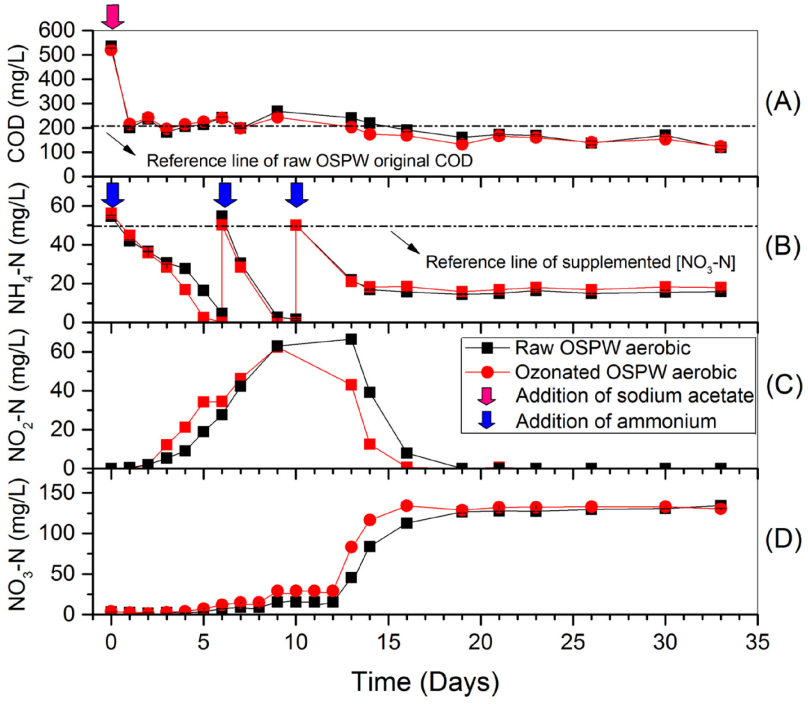


Figure 3-2. COD, NH_4-N, NO_3-N, and NO_2-N concentrations in the aerobic reactors (each data point represents a combined sample from 5 replicates).

3.3 Results and Discussion

3.3.1 Effect of ozonation on OSPW characteristics

Table 3-1 summarizes the characteristics of raw and ozonated OSPWs. Ozone pretreatment removed ~5.6% of COD from raw OSPW, and slightly improved the biodegradability of raw OSPW by increasing the BOD_5 concentration. With respect to NA removal, the mild ozonation pretreatment effectively degraded 57.2% of the total classical NAs and 7.3% of oxy-NAs from raw OSPW. As shown in Figure 3-3 A, B, C and D, ozonation effectively removed classical NAs with higher carbon numbers and higher cyclization degrees, which is consistent with previous studies (Martin et al. 2010, Pérez-Estrada et al. 2011, Wang et al. 2013b).

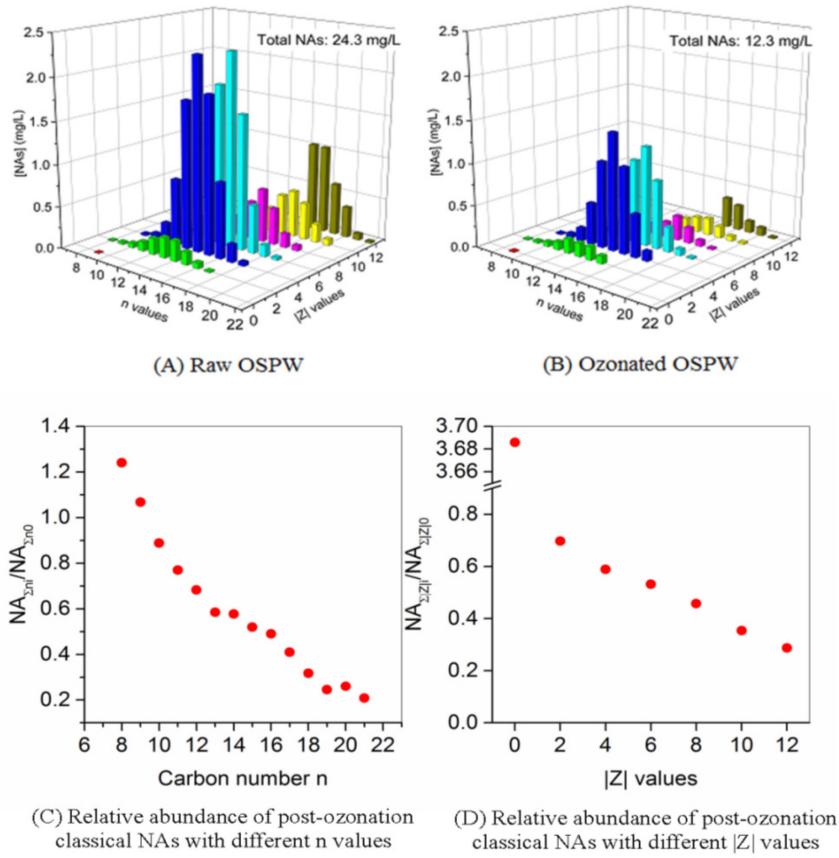


Figure 3-3. Effect of mild-dose ozone pretreatment on OSPW classical NAs.

Ozone is one of the strongest oxidizing chemical agents available, which can attack a wide spectrum of organic contaminants either directly or indirectly, through generating free hydroxyl radicals (·OH) (Dong et al. 2015). Under alkaline conditions, the ozonation process is dominated by indirect reactions via ·OH, which is much more oxidative than ozone molecules and capable of degrading aliphatic carbon chains by H atom abstraction (Glaze 1987).

It has been suggested that ·OH is the predominant oxidant during ozonation of OSPW, which usually has an alkaline pH (Pérez-Estrada et al. 2011). NAs with higher carbon numbers are more reactive towards ·OH due to the higher overall numbers of H atoms available for H abstraction (Pérez-Estrada et al. 2011). As for the effect of hydrogen deficiency (Z) or cyclicity, NAs with more rings are generally better decomposed by ·OH, which could be attributed to more tertiary carbon atoms associated with ring structure (Pérez-Estrada et al. 2011). Compared with primary and secondary carbon-centered radicals, tertiary carbon-centered radicals are more stable, making the H atoms on tertiary carbons easier to abstract (Wang et al. 2013b). Both oxo- and hydroxylated-NAs products have been reported after ozonation (Pérez-Estrada et al. 2011). The high energy consumption of the process prevents it from being economically feasible to use ozonation alone to fully mineralize organic pollutants. Instead, a usual practice is to combine lower-dose ozonation with biological process to attain good treatment efficiency without increasing the cost significantly (Oller et al. 2011).

3.3.2 COD removal, nitrification and denitrification performance

Figure 3-1 A, B and C display the concentrations of COD, NO_3-N, and NO_2-N of the anoxic reactors treating raw and ozonated OSPWs, respectively. It took three days for the microorganisms to reduce COD to a level of ~200 mg/L. The plateau of COD from Day 3 to Day 6 corresponded to the unavailability of nitrate over that period (Figure 3-1 B), which indicates

that denitrifiers were the major COD consumers in the reactors. Total COD concentrations (including sodium acetate and OSPW indigenous organic compounds) in all the reactors were reduced from 500 mg/L to ~250 mg/L within one day after the replenishment of NO_3-N on Day 6 (Figure 3-1 A). From Day 7 to 9, the total COD dropped to below the original COD level of raw/ozonated OSPW, indicating that the microorganisms started to degrade raw/ozonated OSPW organic compounds. OSPW NAs are conceivably more recalcitrant than sodium acetate. The COD concentration curves in both raw and ozonated OSPW anoxic reactors showed a plateau region between Day 9 and Day 13 despite of sufficiency of NO_3-N. The stable NO_3-N concentration of ~50 mg N/L from Day 12 to Day 14 (Figure 3-1 B) reveals that it is the bio-persistence of the OSPW organic substances rather than the insufficiency of NO_3-N that restricted further decrease in COD concentration in each reactor. In contrast to the minimal nitrate reduction in the raw OSPW anoxic reactors, Day 19 – 33 witnessed a better nitrate reduction efficiency in the ozonated OSPW anoxic reactors by leaving less NO_3-N, implying that ozonation pretreatment of OSPW enhanced the organic degradation by using nitrate as electron accepters.

Figure 3-2 A, B, C, and D present the concentrations of COD, NH_4-N, NO_2-N and NO_3-N, respectively, for raw and ozonated aerobic reactors. The total COD concentration in each reactor sharply dropped to the level of COD caused by OSPW organic compounds on Day 1, indicating the activeness of heterotrophs in the nitrifying aerobic sludge inoculum. From Day 2 to Day 8, the COD concentrations plateaued at around 200 mg/L. From Day 8, both raw and ozonated OSPW aerobic reactors demonstrated a decreasing trend in COD concentration.

In contrast to the rapid depletion of sodium acetate, it took the microorganisms about five days to consume the first addition of NH_4-N in both the raw and ozonated OSPW aerobic reactors. The

pKa of ammonium is 9.3 at 25 °C (Stumm and Morgan 2012), thus volatilization of ammonia in the reactors was ignored since the pronated non-volatile ammonium form dominated within the pH range of 5.5 – 8.9 of the aerobic reactors (Figure 3-3). As ammonium oxidizers became more abundant in the reactors, the second replenishment (on Day 6) of NH_4-N was consumed faster. However, the third replenishment of ammonium was incompletely consumed, remaining at a level of approximate 15 mg N/L in the raw and 18 mg N/L in the ozonated OSPW aerobic reactors. It is known that nitrification process is sensitive to pH variations with an optimum pH range of 7.0 – 8.0 (AWWA 2002). In the nitrifying reactors, the pH was decreased to a level of ~6.0 in each of the nitrifying aerobic reactors after Day 15 (Figure 3-4) because of alkalinity consumption by nitrification reactions.

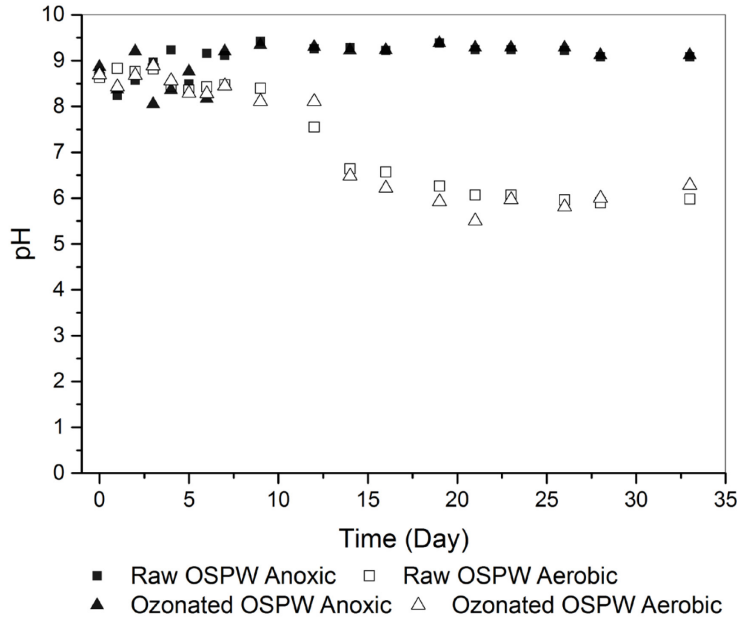


Figure 3-4. pH in the reactors over time.

Meanwhile, the accumulation of NO_2-N in the systems increased to over 60 mg N/L in reactors for both reactor types by Day 9 (Figure 3-2 C). As the nitrite oxidizers became adapted to the conditions in the reactors, the accumulated nitrite concentration in the raw and ozonated OSPW

aerobic reactors eventually dropped to a negligible level (Figure 3-2 C). The faster nitrite removal rate observed in the ozonated OSPW aerobic reactors might be attributed to the lower toxic effect of ozonated OSPW on nitrite oxidizers. The accumulated NO_3-N concentration in the reactors did not start to substantially increase until Day 12 when the accumulated nitrite began declining (Figure 3-2 D). The stabilized NO_3-N concentration in the reactors was around 130 mg N/L, which was congruent with the oxidized amount of NH_4-N.

It is thus clear that the creation of denitrifying anoxic conditions and nitrifying aerobic conditions was successful and they supported the degradation of organics in OSPW. Ozonation pretreatment improved the nitrification and denitrification efficiencies. However, none of the four studied conditions succeeded in completely mineralizing OSPW organics.

3.3.3 NA degradation

Table 3-3 presents the percentile removal rates of classical NAs and oxy-NAs in the reactors on Day 14 and Day 33.

3.3.3.1 Removal of classical NAs

It is clear that the nitrifying aerobic reactors showed better total classical NA degradation efficiency than their anoxic counterparts. The ozonated OSPW anoxic reactors did not show improved degradation of total classical NAs either on Day 14 or Day 33. As for the effect of ozonation on aerobic degradation of classical NAs, it is interesting to see that the ozonated OSPW aerobic reactors had out-performed the raw OSPW aerobic ones (35.8% vs. 30.0%) by Day 14, whereas the raw OSPW aerobic reactors demonstrated a higher performance (69.1% vs. 53.7%) by Day 33. After 33 days of operation, the overall total classical NA degradation rate by the ozonation/nitrifying aerobic biodegradation combination process was 76.5%.

Table 3-3. Concentrations and removal rates of NAs with O2, O3, O4, O5, and O6 in the four reactors.

Condition	O number	Concentration (mg/L)			Removal rate (%)		Half-life (days)
		Day 0	Day 14	Day 33	Day 14	Day 33	
Raw OSPW anoxic	2	24.3	19.1	18.8	21.4	22.6	89.1
	3	8.6	7.4	7.1	14.0	17.4	119.3
	4	10	8.2	7.8	18.0	22.0	92.1
	5	2.5	1.9	1.9	24.0	24.0	83.3
	6	0.9	0.6	0.6	33.3	33.3	56.4
	3 – 6	22.0	18.0	17.5	18.2	20.5	100.0
Raw OSPW aerobic	2	24.3	17.0	7.5	30.0	69.1	19.5
	3	8.6	7.3	6.8	15.1	20.9	97.4
	4	10	8.6	7.6	14.0	24.0	83.3
	5	2.5	2.0	1.7	20.0	32.0	59.3
	6	0.9	0.8	0.7	11.1	22.2	91.0
	3 – 6	22.0	18.6	16.8	15.5	23.6	84.8
Ozonated OSPW anoxic	2	12.3	10.8	10.4	12.2	15.4	136.3
	3	9.1	8.2	8.1	9.9	11.0	196.5
	4	8.1	7.1	6.7	12.3	17.3	120.5
	5	2.2	2.1	2.1	4.5	4.5	491.7
	6	1.0	0.7	0.7	30.0	30.0	64.1
	3 – 6	20.4	18.1	17.5	11.3	14.2	149.2
Ozonated OSPW aerobic	2	12.3	7.9	5.7	35.8	53.7	29.7
	3	9.1	8.1	7.8	11.0	14.3	148.4
	4	8.1	7.2	6.9	11.1	14.8	142.7
	5	2.2	2.0	2.0	9.1	9.1	240.0
	6	1.0	0.8	0.9	20.0	10.0	217.1
	3 – 6	20.4	18.1	17.6	11.3	13.7	154.9

In comparison, Hwang et al. (2013) reported that a continuous biofilm reactor removed 18.5% of parent NAs in OSPW. Some researchers achieved a NA degradation rate of 48% from raw OSPW after 98 days using batch suspended systems, suggesting that the half-life of OSPW NAs is around 44 – 240 days in their batch reactors (Han et al. 2008). It was also estimated that NAs have undergone slow biodegradation with an *in situ* half-life in a range of 12.8 – 13.6 years in tailing ponds (Han et al. 2009). The reactors in this study substantially shortened the half-life of OSPW classical NAs.

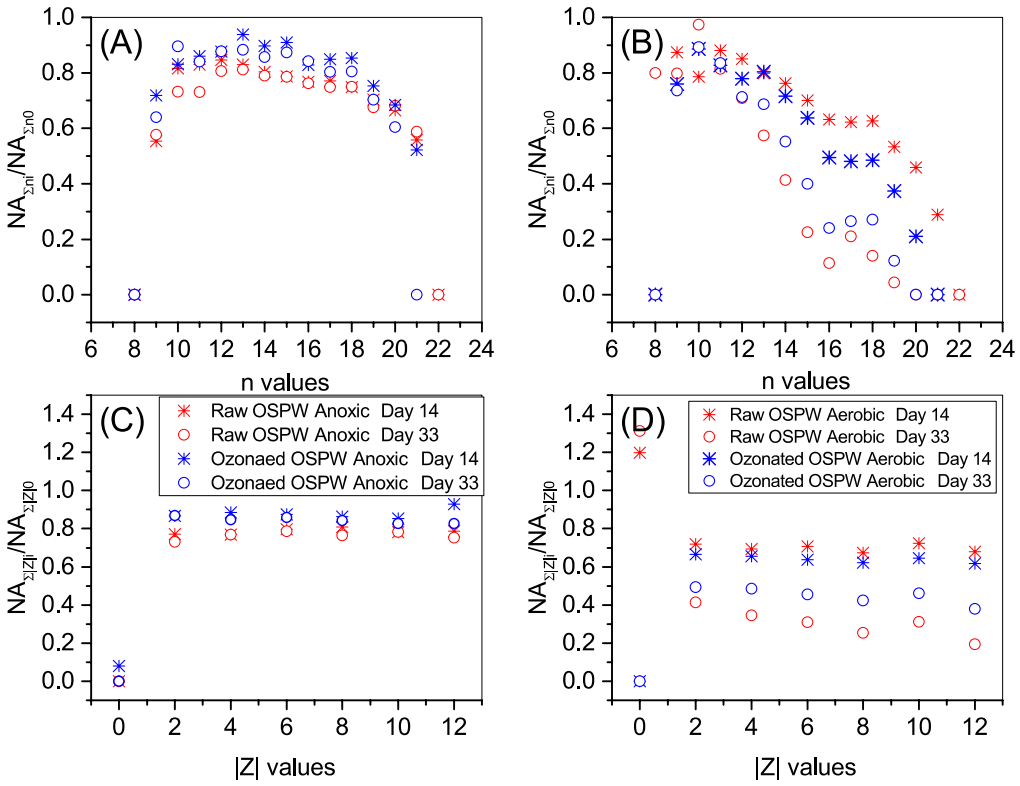


Figure 3-5. The relative residual abundances of NAs with different carbon number *n* ($NA_{\Sigma ni}/NA_{\Sigma n0}$) in the reactors on Day 14 and Day 33: (A) anoxic reactors, (B) aerobic reactors; and the relative residual abundances of NAs with different hydrogen deficiency Z ($NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$) in the reactors on Day 14 and Day 33: (C) anoxic reactors, (D) aerobic reactors.

Table 3-3 explicitly reveals that the majority of the total classical NA degradation had been accomplished in each of the anoxic reactors by Day 14. Taking into account the restricted denitrification within the period of Day 14 to 33, the poor degradation of classical NAs implies that denitrification process may be of importance for anoxic degradation of classical NAs. In contrast, steady biodegradation of NAs was taking place over the whole study period in those nitrifying aerobic reactors (Table 3-3).

To evaluate the biodegradation efficiency for classical NA species with different molecular carbon numbers (*n*) and hydrogen deficiencies (Z), Figure 3-5 A – D illustrate $NA_{\Sigma ni}/NA_{\Sigma n0}$ vs. *n* value and $NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$ vs. |*Z*| value for samples collected on Day 14 and Day 33. $NA_{\Sigma ni}$ denotes

the total concentration of NAs with the same *n* value in the sample collected on Day i; and $NA_{\Sigma n0}$ denotes the total concentration of NAs with the same *n* value in the untreated (Day 0) raw/ozonated OSPW. A higher $NA_{\Sigma ni}/NA_{\Sigma n0}$ ratio means a poorer NA degradation performance.

The anoxic reactors showed remarkably different patterns than their aerobic counterparts. Although the anoxic conditions effectively removed classical NAs with all *n* values, the degradation process was impeded somehow after Day 14. In contrast, Figure 3-5 B and D clearly indicate that the aerobic conditions worked better than their anoxic counterparts in terms of classical NA degradation. In addition, the second period of the study (Day 14 – 33) witnessed substantial aerobic removal of classical NAs, especially NAs with $15 \leq n \leq 21$. For instance, > 50% of the remaining NAs within this *n* range (Figure 3-5 B) were further removed in the raw OSPW aerobic reactors during the Day 14 – 33 period. Ozone pretreatment did not appreciably enhance subsequent anoxic biodegradation of classical NAs (Table 3-3 and Figure 3-5 A and C). In contrast, the enhancement of ozone pretreatment on subsequent aerobic degradation of NAs was considerable in terms of generally better removal rates of NAs within the first 14 days and lower final residual NAs concentrations after 33 days of incubation (Figure 3-5 and Table 3-3).

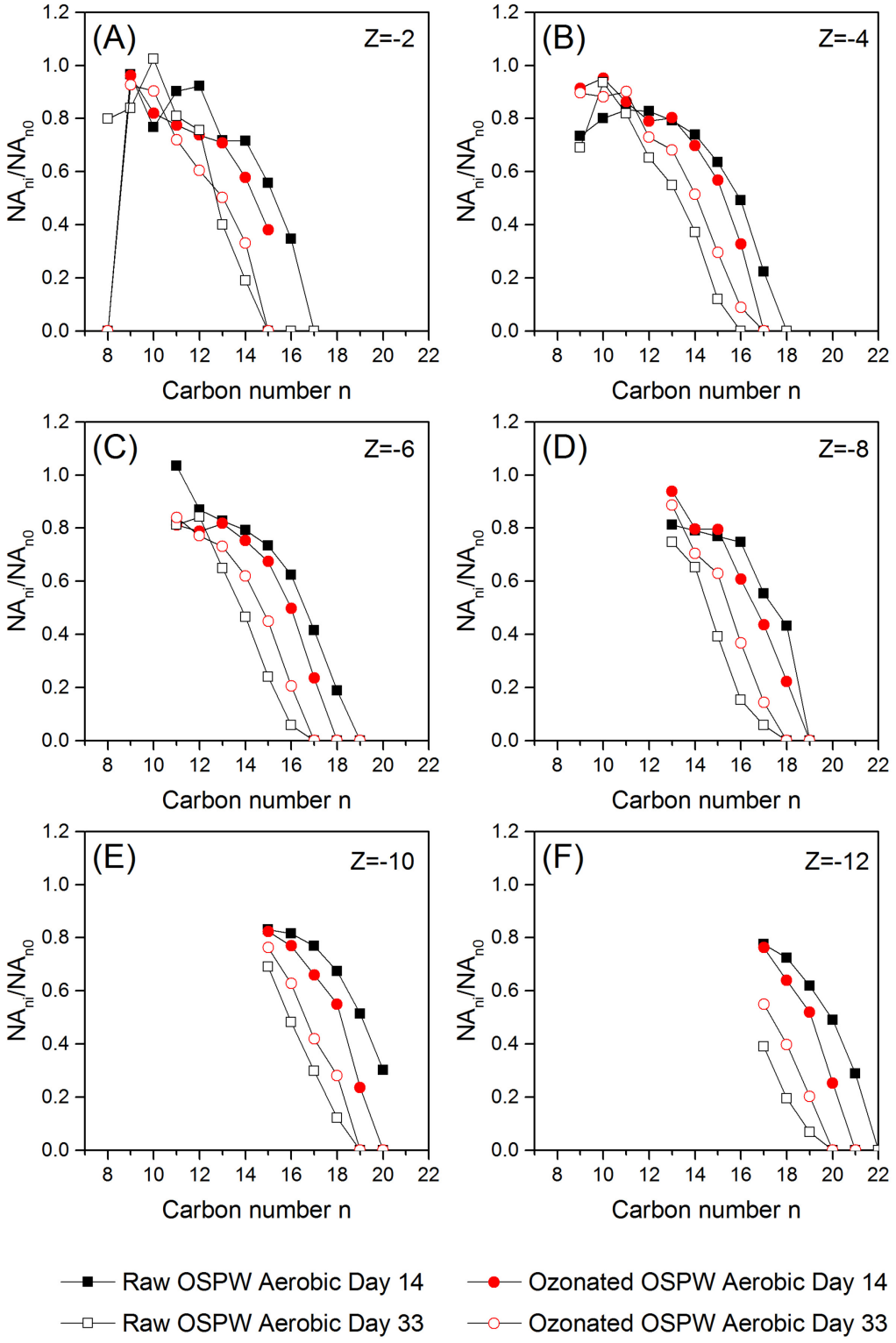


Figure 3-6. The relative remaining abundances of NAs with different carbon numbers (NA_{ni}/NA_{n0}) under each |Z| value in the reactors on Day 14 and Day 33.

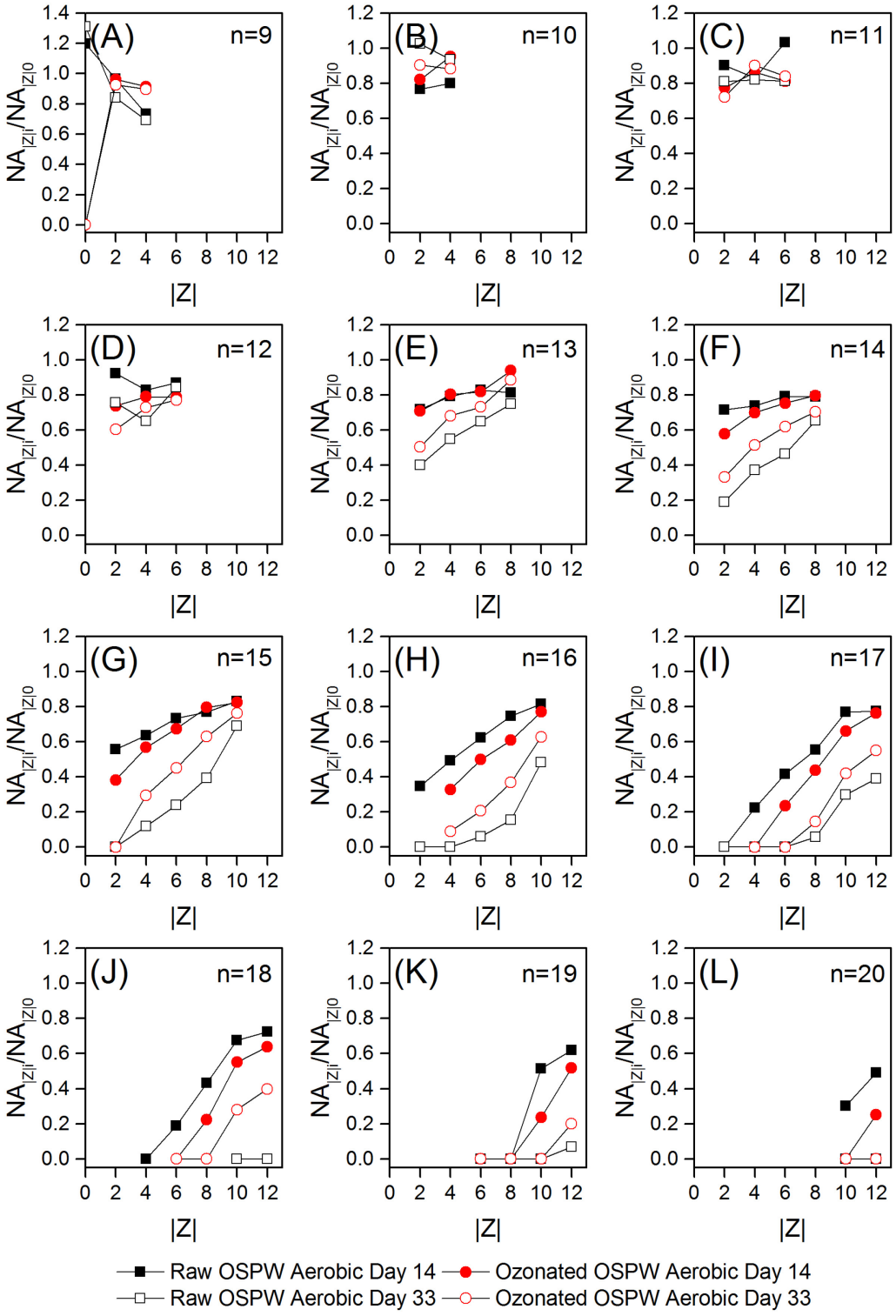


Figure 3-7. The relative remaining abundances of NAs with different |Z| values ($NA_{|Z|i}/NA_{|Z|0}$) under each carbon number *n* in the reactors on Day 14 and Day 33.

To better understand how ozone pretreatment, molecular carbon number (*n*), hydrogen deficiency (*Z*), and incubation duration affected the aerobic degradation of classical NAs, Figure 3-6 and Figure 3-7 are generated based on the NAs concentrations profiles. Figure 3-6 A – F illustrate NA_{ni}/NA_{n0} ratios for each $|Z|$ for samples collected on Day 14 and Day 33. NA_{ni} denotes the concentration of NAs with a certain *n* value in the sample collected on Day i; and NA_{n0} denotes the concentration of NAs with a certain *n* value in the untreated (Day 0) raw/ozonated OSPW. Similarly, Figure 3-7 A – L are for $NA_{|Z|i}/NA_{|Z|0}$ ratios for each *n* for samples collected on Day 14 and Day 33.

In Figure 3-5 B and D, and Figure 3-6, it is noticed that NAs with larger *n* values had generally been biodegraded to larger extents by Day 14 and Day 33. The relatively higher remaining abundance of NAs with smaller carbon numbers (e.g., C8 – 11) might be a result of bio-conversion of NAs with longer carbon chains (higher *n* values), which could be evidenced by the reappearance of C8 NAs in the Day 33 raw OSPW aerobic sample (Figure 3-6 A). All four samples included in Figure 3-6 generally demonstrated a strong negative correlation between NA_{ni}/NA_{n0} ratio and carbon number *n* within each *Z* series. Hence, it is suggested that NAs with higher carbon numbers could be better mitigated under aerobic conditions. Contradictorily, it was reported that the rate of biodegradation of OSPW NAs was inversely proportional to the number of carbons (especially $n > 17$) (Clemente et al. 2004, Han et al. 2009). The results in this study are also interesting compared with Han et al. (2008) and Wang et al. (2013b)'s study, where it was reported that the effect of carbon number (*n*) on biodegradation of NAs was not appreciable.

Furthermore, it is interesting that the aerobic reactors demonstrated excellent degradation of NAs that are more hydrophobic. Due to alkalinity consumption during nitrification process, a pH of

~6 was observed in the aerobic reactors after Day 14 (Figure 3-4). Since NAs typically have a pKa range of 5 – 6 (Headley et al. 2002), a significant fraction of NAs were likely to be converted into a protonated form that is more hydrophobic (Klamerth et al. 2015). In addition, NAs with higher carbon numbers tend to be more hydrophobic (Pourrezaei 2013). According to literature, bacteria with hydrophobic cell surfaces are able to uptake the substrates by direct contact to the accumulated hydrophobic pollutant phase, whereas bacteria with hydrophilic surfaces secrete bio-surfactants to facilitate uptake of hydrophobic contaminants (Peltola 2008). In addition, some bacteria may produce bound extracellular polymeric substances (EPS) to enhance the bioavailability of the substrates (Singh 2012). Therefore, organic compounds of higher hydrophobicity (or higher lipophilicity) could easily attach onto or penetrate the cell envelope, possibly leading to better biodegradation rates under certain circumstances (Parsons and Govers 1990). Recalling the reluctant nitrification performance of the aerobic reactors within the period of Day 14 to 33, the activities of nitrifiers might also be impeded by elevated intracellular toxic organic compounds from OSPW. Moreover, it should be taken into account that the microorganisms in the current study had been acclimatized for OSPW treatment for over 1 year (Xue et al. 2016).

Although the effect of cyclicity on NA degradation is not appreciable in Figure 3-5 D, most charts (E – L, C13 – 20) of Figure 3-7 demonstrate poorer degradation efficiencies for more cyclic (larger |Z| values) NAs, regardless of the sample source. This correlation between hydrogen deficiency and NA degradation efficiency is not clear for NAs with C9 – 12. That might be ascribed to the simultaneous degradation of low carbon number NAs and their generation from bioconversion of longer chained species. The abundance of acyclic ($|Z| = 0$) NAs in the raw OSPW aerobic system was raised, implying that some cyclized NAs in the raw OSPW

were converted into acyclic molecules by microorganisms. It has been reported that the biodegradation rate of NAs decreased with increasing cyclicity (higher |*Z*|) in both of commercial NAs and OSPW NAs (Han et al. 2008). Some other researchers found that biodegradation substantially decreased NAs with $|Z| < 6$ in untreated OSPW, leaving concentrations of NAs with $|Z| > 8$ relatively unchanged (Wang et al. 2013b). OSPW NAs of lower cyclicity are generally more hydrophobic (Pourrezaei 2013) and accordingly more accessible to microbes in aqueous environment, consequently more favorable for biodegradation. The worse biodegradability of NAs with higher cyclization might be attributed to steric hindrance caused by the ring structures, which prevents the tertiary carbon of the ring from being favored by microbial oxidation (Han et al. 2008).

Based on Figure 3-6 and Figure 3-7, the enhancement effect of ozone pretreatment on biodegradation of NAs is apparent for Day 14 samples, which is consistent with previous studies (Martin et al. 2010, Vaiopoulou et al. 2015). It was also noticed that ozone pretreatment especially enhanced the biodegradation of NAs with longer carbon chains on Day 14 (Figure 3-6 and Figure 3-7). However, the raw OSPW aerobic condition generally demonstrated better degradation efficiency by the end of the study though the second period (Day 14 – 33) of the study did witness substantial further degradation of most NAs species in ozonated OSPW as well. 100% of NA removal was observed for both raw and ozonated OSPW (Figure 3-6) when *n* is higher than 15 (Z = -2), 17 (Z = -4), 17 (Z = -6), 18 (Z = -8), 19 (Z = -10), and 20 (Z = -12) after 33 days. Hence, ozone pretreatment could accelerate the biodegradation of classical NAs under nitrifying aerobic condition for the acceleration effect did not last for a longer period (e.g., 33 days). It should be noted that the final concentrations of total classical NAs in the raw OSPW aerobic and ozonated OSPW aerobic reactors were 7.5 and 5.7 mg/L, respectively. Thus, the

remaining ozonated OSPW classical NAs may not necessarily be inherently refractory, but rather had an abundance lower than the threshold value for biodegradation (Misiti et al. 2013c). Therefore, it is suggested that the mild-dose ozone pretreatment effectively enhanced the subsequent aerobic biodegradation of OSPW classical NAs in terms of an accelerated degradation rate within a short term (i.e. 14 days in this study) and a much lower post-biodegradation residual concentration.

3.3.3.2 Removal of oxy-NAs

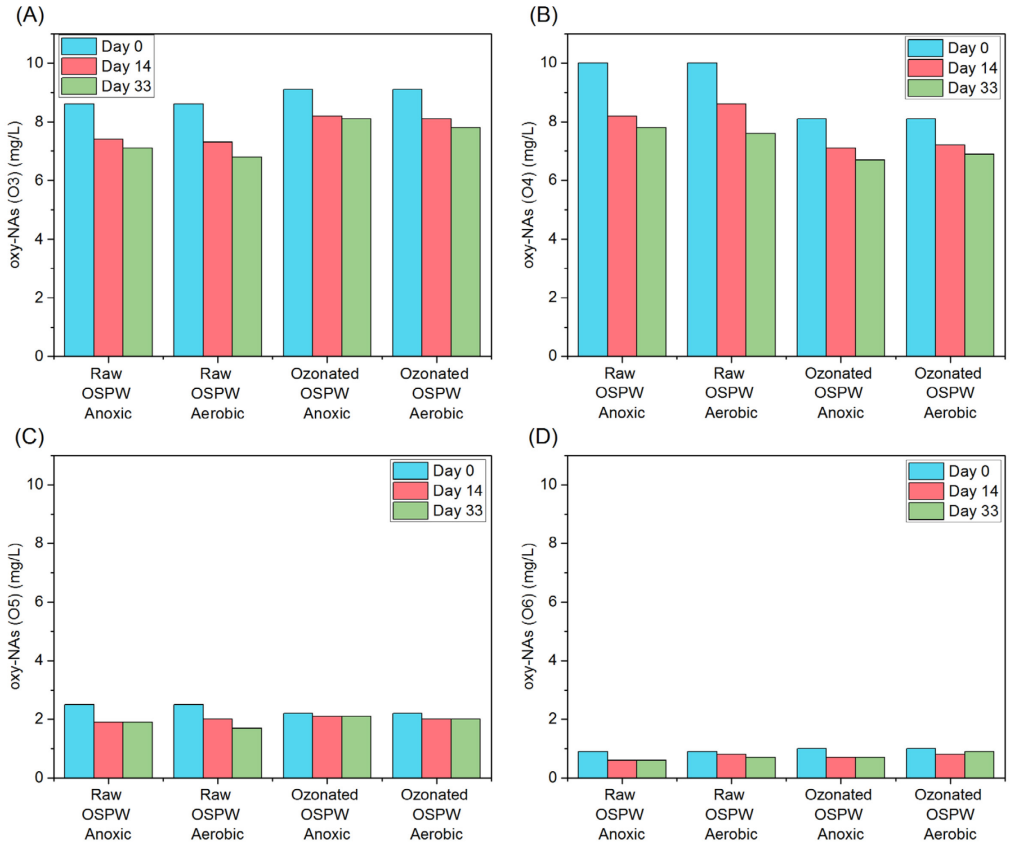


Figure 3-8. Concentrations of oxy-NAs with O3, O4, O5, and O6 on Day 0, Day 14, and Day 33: (A) oxy-NAs with O3; (B) oxy-NAs with O4; (C) oxy-NAs with O5; and (D) oxy-NAs with O6.

Table 3-3 demonstrates the concentration changes of the total oxy-NAs (including O3, O4, O5 and O6 species) over time in the reactors; and Figure 3-8 shows the concentrations of oxy-NAs

with different oxygen number (O3 – O6) under all four conditions on Day 0, 14, and 33. The ozonation treatment alone removed 7.3% of the total oxy-NAs from raw OSPW. Specifically, ozonation increased O3 and O6 oxy-NAs by 5.8% and 11.1%, respectively; and decreased O4 and O5 oxy-NAs by 19.0% and 12.0%, respectively.

The oxy-NA removal rate achieved by the combination of ozonation and biological treatment were 20.4% and 20.0% under aerobic and anoxic conditions, respectively. As opposed to the enhancedment of total classical NA removal by the combined process, ozonation did not appear to effectively improve the removal of the total oxy-NAs. In addition, the second period (Day 14 – 33) of the study did not witness a substantial removal of total oxy-NAs in any of the four reactors, indicating the recalcitrance of oxy-NAs.

As discussed previously, the nitrifying aerobic conditions achived much better classical NA removal rates than the anoxic conditions did. However, the two conditions showed quite similar efficiency in removing total oxy-NAs. Detailed information on concentrations and removal rates of oxy-NAs with different O numbers is presented in Figure 3-8 and Table 3-3. Each of the four reactors showed effective degradation for most oxy-NAs except the oxy-NAs with O5 in the ozonated OSPW anoxic reactor and oxy-NAs with O5 and O6 in the ozonated OSPW aerobic reactors ($\leq$ 10.0% removed).

In contrast, Han et al. (2008) reported that no concentration change of NAs with O3 or O4 had been observed after a 98-day aerobic biodegradation experiment; and Headley et al. (2009) found that O3 oxy-NAs appeared to be recalcitrant in their biological system. The effective O3 and O4 oxy-NA removal under the nitrifying aerobic conditions of this study indicates that nitrification may improve the degradation of oxy-NAs with O3 and O4. Previous studies suggested that oxy-

NAs are intermediates during aerobic biodegradation of classical NAs (Grewer et al. 2010, Han et al. 2009), among which O3 species are probably hydroxylated NAs (Han et al. 2008) and O4 are possibly dicarboxylic acids (Headley et al. 2009). Headley et al. (2009) observed dissipation of O4 and O5 NAs in their aquatic plant system, which is consistant with our results. However, no removal of O6 NAs was concluded in the previous study conducted by Headley et al. (2009). Contrarily, the four systems in this study achieved effective degradation of O6 NAs (mainly the species with lower |Z| values). Therefore, in addition to their efficiency in removing classical NAs, the reactors under the four conditions in this study were effective in degrading oxy-NAs with O3, O4, O5, and O6.

3.3.4 Microbial community characterization

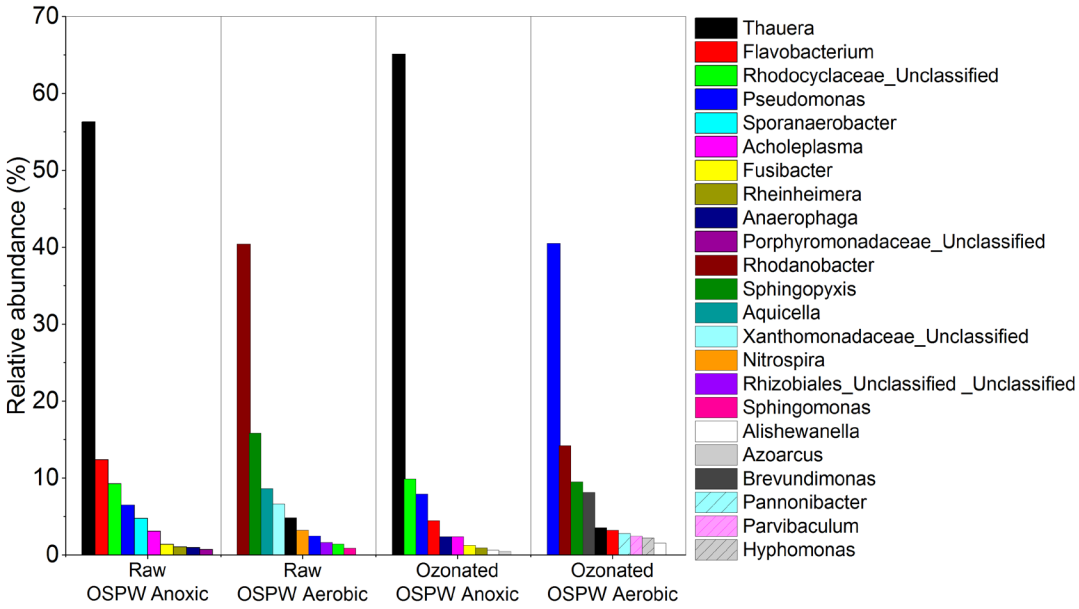


Figure 3-9. Relative abundances of the most abundant genera in each reactor type at the end of the incubation.

454 Pyrosequencing analysis was performed for microbial community characterization. The most abundant genera in each condition are identified and presented in Figure 3-9, which is

divided into four clusters for the raw OSPW anoxic, raw OSPW aerobic, ozonated OSPW anoxic, and ozonated OSPW aerobic reactors, respectively. The compositions of the four clusters differ from each other, indicating differences in the microbial community structures in the four reactors. *Thauera*, *Pseudomonas*, and *Flavobacterium* were commonly found in all four reactor types though in different abundances. *Acholeplasma* was only detected in the anoxic reactors, while *Sphingopyxis* only existed in the nitrifying aerobic reactors.

Most of the main genera in anoxic reactors were commonly available in both raw OSPW anoxic and ozonated OSPW anoxic reactors though the abundances of each genus differed for raw and ozonated OSPWs. In contrast, the microbial consortia difference between the raw OSPW aerobic and ozonated OSPW aerobic reactors were apparent. It is therefore suggested that the mild ozonation pretreatment of OSPW affected the microbial community structures of the subsequent biological processes. The ozone pretreatment decomposed those more recalcitrant and toxic substances that restrain the growth of some microorganisms; meanwhile, some bacteria indigenous to OSPW were deactivated during the ozonation process, which may result in the change of microbial community structure as well.

Although low abundance of *Nitrosomonas* (not included in Figure 6) was present in the raw OSPW aerobic (0.42%) and ozonated OSPW aerobic (0.22%) reactors, their ammonium monooxygenase (*amo*) might still contribute to the degradation of NAs through cometabolism. In fact, the abundances of *Nitrospira spp.* 16s rDNA and *amo* gene in the raw OSPW aerobic and the ozonated OSPW aerobic reactors were relatively stable over time despite the variations of the reactors' nitrification efficiency (Figure 3-10). The plausibly accumulated NAs in the nitrifier cells merely restrained the microbes instead of destructing them, indicating the bacteria's surprisingly good tolerance to OSPW NA toxicity. As the microorganisms were still alive, the

contribution of nitrifiers to degradation of NAs cannot yet be ruled out on the basis of suppressed nitrification performance.

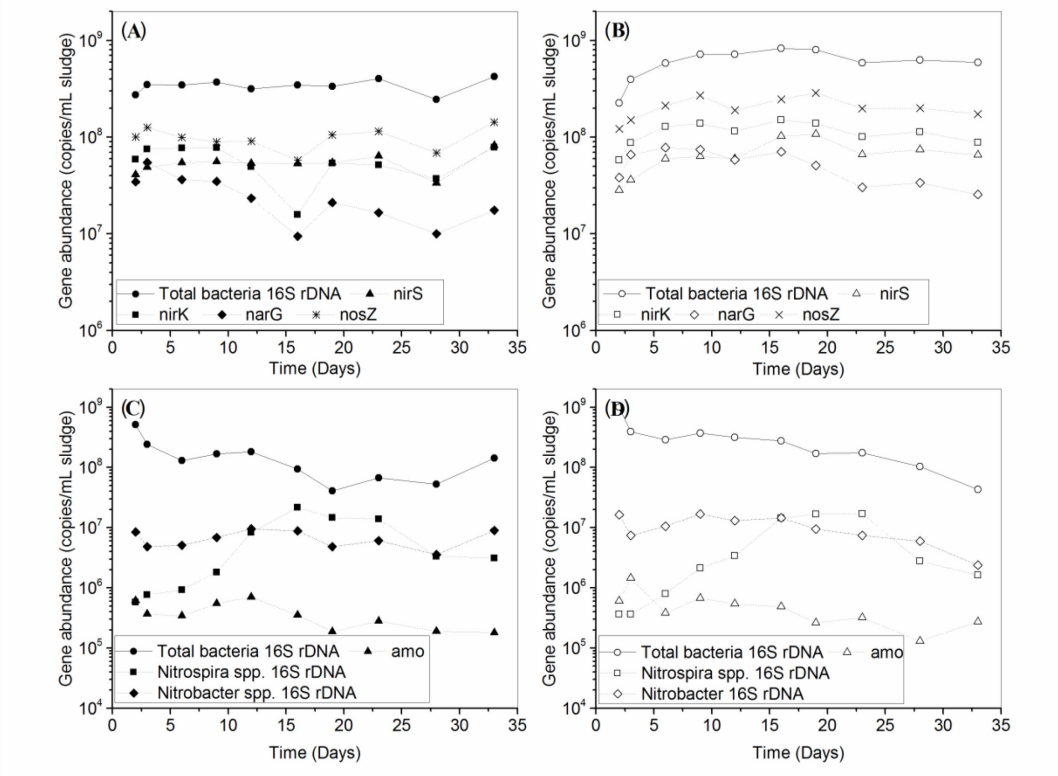


Figure 3-10. QPCR results: denitrifying genes in raw OSPW anoxic (A) and ozonated OSPW anoxic reactors (B); nitrifying genes in raw OSPW aerobic (C) and ozonated OSPW aerobic reactors (D).

All the dominating species found in the reactors are related to either organic contaminant degradation or nitrogen cycling, or both. Many genera (including *Thauera*) under the family *Rhodocyclaceae* contribute to nitrate reducing and degradation of recalcitrant organic compounds (Anders et al. 1995, Jiang et al. 2013, Mechichi et al. 2002, Oren 2014, Seviour and Nielsen 2010). Some *Pseudomona*s (i.e., *Pseudomonas fluorescens* and *Pseudomonas alcaligenes*) and *Flavobacterium* species are capable of reducing nitrate (or nitrite) and nitrous oxide (Hochstein and Tomlinson 1988); meanwhile degrading recalcitrant compounds including NAs (Cao et al. 2009, Rahman et al. 2003, Van den Tweel et al. 1988, Whitby 2010). Therefore,

denitrifiers that were dominating the anoxic reactors may also be the major degraders of raw and ozonated OSPW NAs. In addition, a number of genera (including *Rhodanobacter*) under *Xanthomonadaceae* are directly or indirectly related to oil or petroleum hydrocarbon degradation (Kanaly et al. 2002, Timmis et al. 2010). *Acholeplasma* has been reported to be able to degrade polycyclic aromatic hydrocarbons (PAH) anaerobically (Um 2004). Members of *Sphingopyxis*, *Brevundimonas* and *Pannonibacter* are capable of degrading hydrocarbons under aerobic conditions (Das 2014, Johnson et al. 2011, Shestakova et al. 2011, Timmis et al. 2010). *Rhizobiales* and *Aquicella* may also be capable of degrading PAHs and NAs (Biryukova et al. 2007, Ding et al. 2012, Headley and McMartin 2004). Previous studies found that *Brevundimonas* was only present in ozonated OSPW but not in fresh OSPW (Choi et al. 2014), the same results have been obtained in the current study, which confirms that ozonation process could affect the microbial structure in OSPW.

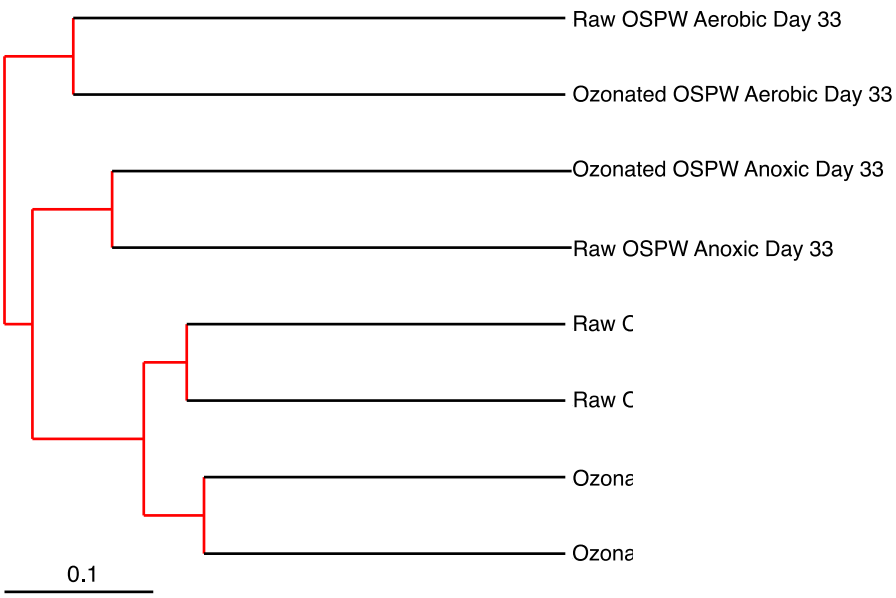


Figure 3-11. Bootstrapped tree.

The phenetic tree (bootstrapped tree) is constructed for microbial communities in the sludge samples collected on Day 1 and Day 33 on the basis of UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method (Figure 3-11). It is found that on Day 1 microbial communities in the raw OSPW anoxic and raw OSPW aerobic reactors clustered together while the microbial communities in the two reactors treating ozonated OSPW clustered together. That is reasonable since the inocula for the reactors were collected from the two continuous MBR systems treating raw and ozonated OSPW, which had internal recirculation between their anoxic tank and aerobic tank. After 33 days of batch operation, samples clustered on the basis of electron acceptor conditions rather than the source water types, which could be explained by the selective enrichment of aerobic and anoxic bacteria under their preferred condition.

3.4 Conclusions

Both of anoxic and aerobic conditions were found effective in degrading OSPW classical and oxy-NAs. Nitrifying aerobic reactors showed substantially higher removal efficiency of classical NAs. 22.6% and 69.1% of total classical NAs of raw OSPW were degraded by the raw OSPW anoxic and raw OSPW aerobic reactors, respectively. Regarding total oxy-NAs, removal rates of 20.4% and 23.6% were achieved by the raw OSPW anoxic and raw OSPW aerobic reactors, respectively. Ozone pretreatment effectively enhanced the subsequent aerobic biodegradation of OSPW classical NAs in terms of an accelerated degradation rate within a short term (i.e., 14 days in this study) and a much lower post-biodegradation final residual concentration after a longer period (i.e., 33 days in this study). After 33 days of operation, microbial species capable of degrading recalcitrant hydrocarbons were enriched in the reactors. The dominating genus in the raw OSPW anoxic and ozonated OSPW anoxic reactors was identified as *Thauera;* whereas *Rhodanobacter* and *Pseudomonas* dominated the raw OSPW aerobic and ozonated OSPW

aerobic reactors, respectively. It is suggested that the dominating denitrifiers were also involved in anoxic degradation of NAs; whereas the contribution of nitrifiers to aerobic degradation of NAs needs to be further investigated. The results obtained from this study may be beneficial to optimization of biological treatment processes (e.g., on-site bioremediation and engineered bioreactors) for OSPW treatment in the future. Although the OSPW NA removal efficiency of the anoxic reactors was lower than that of the aerobic reactors, the lower energy consumption by anoxic condition (no need of aeration) may make it a good choice to integrate anoxic and aerobic conditions into one system to better treat OSPW at lower costs.

References

Aga, D.S., 2007. Fate of pharmaceuticals in the environment and in water treatment systems, CRC Press.

Allen, E.W., 2008. 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.

Anders, H.-J., Kaetzke, A., KÄMPFER, P., Ludwig, W. and Fuchs, G., 1995. Taxonomic position of aromatic-degrading denitrifying pseudomonad strains K 172 and KB 740 and their description as new members of the genera Thauera, as Thauera aromatica sp. nov., and Azoarcus, as Azoarcus evansii sp. nov., respectively, members of the beta subclass of the Proteobacteria. International journal of systematic bacteriology 45(2), 327-333.

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., 2012. Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to Chironomus dilutus. Environmental science & technology 46(1), 486-493.

AWWA, 2002. Nitrification, United States Environmental Protection Agency, Washington DC.

Battimelli, A., Loisel, D., Garcia‐Bernet, D., Carrere, H. and Delgenes, J.P., 2010. Combined ozone pretreatment and biological processes for removal of colored and biorefractory compounds in wastewater from molasses fermentation industries. Journal of chemical technology and biotechnology 85(7), 968-975.

Beltrán, F.J., García-Araya, J.F. and Álvarez, P.M., 1999. Wine distillery wastewater degradation. 2. Improvement of aerobic biodegradation by means of an integrated chemical (ozone)-biological treatment. Journal of agricultural and food chemistry 47(9), 3919-3924.

Biryukova, O.V., Fedorak, P.M. and Quideau, S.A., 2007. Biodegradation of naphthenic acids by rhizosphere microorganisms. Chemosphere 67(10), 2058-2064.

Brown, L.D., Pérez-Estrada, L., Wang, N., El-Din, M.G., Martin, J.W., Fedorak, P.M. and Ulrich, A.C., 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. Chemosphere.

Brown, L.D. and Ulrich, A.C., 2015. Oil sands naphthenic acids: A review of properties, measurement, and treatment. Chemosphere 127, 276-290.
Cao, B., Nagarajan, K. and Loh, K.-C., 2009. Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. Applied microbiology and biotechnology 85(2), 207-228.
Choi, J., Hwang, G., Gamal El-Din, M. and Liu, Y., 2014. Effect of reactor configuration and microbial characteristics on biofilm reactors for oil sands process-affected water treatment. International Biodeterioration & Biodegradation 89(2014), 74-81.
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.
Das, S., 2014. Microbial Biodegradation and Bioremediation, Elsevier.
De Gusseme, B., Pycke, B., Hennebel, T., Marcoen, A., Vlaeminck, S.E., Noppe, H., Boon, N. and Verstraete, W., 2009. Biological removal of 17 alpha-ethinylestradiol by a nitrifier enrichment culture in a membrane bioreactor. Water research 43(9), 2493-2503.
Ding, G.-C., Heuer, H. and Smalla, K., 2012. Dynamics of bacterial communities in two unpolluted soils after spiking with phenanthrene: soil type specific and common responders. Frontiers in microbiology 3.
Dong, T., Zhang, Y., Islam, M.S., Liu, Y. and El-Din, M.G., 2015. The impact of various ozone pretreatment doses on the performance of endogenous microbial communities for the remediation of oil sands process-affected water. International Biodeterioration & Biodegradation 100, 17-28.
Gamal El-Din, M., Fu, H., Wang, N., Chelme-Ayala, P., Pérez-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. The 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., 2011. Ozone treatment ameliorates oil sands process water toxicity to the mammalian immune system. Water research 45(18), 5849-5857.
Glaze, W.H., 1987. Drinking-water treatment with ozone. Environmental science & technology 21(3), 224-230.
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? The Science of the total environment 408(23), 5997-6010.
Gunawan, Y., Nemati, M. and Dalai, A., 2014. Biodegradation of a surrogate naphthenic acid under denitrifying conditions. Water research 51, 11-24.
Han, X., MacKinnon, M.D. and Martin, J.W., 2009. Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS. Chemosphere 76(1), 63-70.
Han, X., 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.
He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., El-Din, M.G., Giesy, J.P. and Wiseman, S.B., 2012. Toxicity of untreated and ozone-treated oil sands process-affected

water (OSPW) to early life stages of the fathead minnow (Pimephales promelas). Water research 46(19), 6359-6368.
Headley, J. and McMartin, D., 2004. A Review of the Occurrence and Fate of Naphthenic Acids in Aquatic Environments. Journal of Environmental Science and Health, Part A 39(8), 1989-2010.
Headley, J.V., Peru, K.M., Armstrong, S.A., Han, X., Martin, J.W., Mapolelo, M.M., Smith, D.F., Rogers, R.P. and Marshall, A.G., 2009. Aquatic plant-derived changes in oil sands naphthenic acid signatures determined by low-, high-and ultra high-resolution mass spectrometry. Rapid Communications in Mass Spectrometry 23(4), 515-522.
Headley, J.V., Peru, K.M., McMartin, D.W. and Winkler, M., 2002. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. Journal of AOAC International 85(1), 182-187.
Heider, J., Boll, M., Breese, K., Breinig, S., Ebenau-Jehle, C., Feil, U., Gad'on, N., Laempe, D., Leuthner, B. and Mohamed, M.E.-S., 1998. Differential induction of enzymes involved in anaerobic metabolism of aromatic compounds in the denitrifying bacterium Thauera aromatica. Archives of microbiology 170(2), 120-131.
Heider, J. and Fuchs, G., 1997. Microbial anaerobic aromatic metabolism. Anaerobe 3(1), 1-22.
Heider, J., Spormann, A.M., Beller, H.R. and Widdel, F., 1999. Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiology Reviews 22, 459-473.
Hochstein, L.I. and Tomlinson, G.A., 1988. The enzymes associated with denitrification. Annual Reviews in Microbiology 42(1), 231-261.
Hwang, G., Dong, T., Islam, M.S., Sheng, Z., Pérez-Estrada, L.a., Liu, Y. and Gamal El-Din, M., 2013. The impacts of ozonation on oil sands process-affected water biodegradability and biofilm formation characteristics in bioreactors. Bioresource technology 130C, 269-277.
Islam, M.S., Moreira, J., Chelme-Ayala, P. and El-Din, M.G., 2014. Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Science of the total environment 493, 282-290.
Jiang, Y., Ulrich, A.C. and Liu, Y., 2013. Coupling bioelectricity generation and oil sands tailings treatment using microbial fuel cells. Bioresource technology.
Johnson, R.J., Smith, B.E., Sutton, P.a., McGenity, T.J., Rowland, S.J. and Whitby, C., 2011. Microbial biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching. The ISME journal 5(3), 486-496.
Kanaly, R.A., Harayama, S. and Watanabe, K., 2002. Rhodanobacter sp. strain BPC1 in a benzo [a] pyrene-mineralizing bacterial consortium. Applied and environmental microbiology 68(12), 5826-5833.
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.
Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and El-Din, M.G., 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. Science of the total environment 506, 66-75.
Martin, J.W., Barri, T., Han, X., 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.

Mechichi, T., Stackebrandt, E., Gad'on, N. and Fuchs, G., 2002. Phylogenetic and metabolic diversity of bacteria degrading aromatic compounds under denitrifying conditions, and description of Thauera phenylacetica sp. nov., Thauera aminoaromatica sp. nov., and Azoarcus buckelii sp. nov. Archives of microbiology 178(1), 26-35.
Misiti, T., Tandukar, M., Tezel, U. and Pavlostathis, S.G., 2013a. Inhibition and biotransformation potential of naphthenic acids under different electron accepting conditions. Water research 47(1), 406-418.
Misiti, T., Tezel, U. and Pavlostathis, S.G., 2013b. Fate and effect of naphthenic acids on oil refinery activated sludge wastewater treatment systems. Water research 47(1), 449-460.
Misiti, T.M., Tezel, U., Tandukar, M. and Pavlostathis, S.G., 2013c. Aerobic biotransformation potential of a commercial mixture of naphthenic acids. Water research 47(15), 5520-5534.
Oller, I., Malato, S. and Sánchez-Pérez, J., 2011. Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review. Science of the total environment 409(20), 4141-4166.
Oren, A., 2014. The Prokaryotes, pp. 975-998, Springer.
Parsons, J. and Govers, H., 1990. Quantitative structure-activity relationships for biodegradation. Ecotoxicology and environmental safety 19(2), 212-227.
Peltola, R., 2008. Bioavailability aspects of hydrophobic contaminant degradation in soils.
Pérez-Estrada, L.A., Han, X., Drzewicz, P., Gamal El-Din, M., Fedorak, P.M. and Martin, J.W., 2011. Structure–reactivity of naphthenic acids in the ozonation process. Environmental science & technology 45(17), 7431-7437.
Pourrezaei, P., 2013. Physico-chemical Processes for Oil Sands Process-affected Water Treatment, University of Alberta.
Rahman, K., Rahman, T., Lakshmanaperumalsamy, P., Marchant, R. and Banat, I., 2003. The potential of bacterial isolates for emulsification with a range of hydrocarbons. Acta biotechnologica 23(4), 335-345.
Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J. and Chu, K.-H., 2009. Biodegradation potential of wastewater micropollutants by ammonia-oxidizing bacteria. Chemosphere 77(8), 1084-1089.
Sayavedra-Soto, L.a., Gvakharia, B., Bottomley, P.J., Arp, D.J. and Dolan, M.E., 2010. Nitrification and degradation of halogenated hydrocarbons--a tenuous balance for ammonia-oxidizing bacteria. Applied microbiology and biotechnology 86(2), 435-444.
Scott, A.C., MacKinnon, M.D. and Fedorak, P.M., 2005a. Naphthenic acids in athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. Environmental Science and Technology 39(21), 8388-8394.
Scott, A.C., Mackinnon, M.D. and Fedorak, P.M., 2005b. Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. Environmental science & technology 39(21), 8388-8394.
Seviour, R.J. and Nielsen, P.H., 2010. Microbial ecology of activated sludge, IWA publishing.
Shestakova, N., Korshunova, A., Mikhailova, E., Sokolova, D.S., Tourova, T., Belyaev, S., Poltaraus, A. and Nazina, T., 2011. Characterization of the aerobic hydrocarbon-oxidizing enrichments from a high-temperature petroleum reservoir by comparative analysis of DNA-and RNA-derived clone libraries. Microbiology 80(1), 60-69.
Shi, Y., Huang, C., Rocha, K.C., El-Din, M.G. and Liu, Y., 2015. Treatment of oil sands process-affected water using moving bed biofilm reactors: with and without ozone pretreatment. Bioresource technology.

Singh, S.N., 2012. Microbial degradation of xenobiotics, Springer.
Stumm, W. and Morgan, J.J., 2012. Aquatic chemistry: chemical equilibria and rates in natural waters, John Wiley & Sons.
Suarez, S., Lema, J.M. and Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water research 44(10), 3214-3224.
Timmis, K.N., McGenity, T., Van Der Meer, J. and De Lorenzo, V., 2010. Handbook of hydrocarbon and lipid microbiology, Springer Berlin/Heidelberg.
Um, Y., 2004. Isolation and characterization of polycyclic aromatic hydrocarbon-degrading microorganisms under methanogenic conditions.
Vaiopoulou, E., Misiti, T.M. and Pavlostathis, S.G., 2015. Removal and toxicity reduction of naphthenic acids by ozonation and combined ozonation-aerobic biodegradation. Bioresource technology 179, 339-347.
Van den Tweel, W., Smits, J. and De Bont, J., 1988. Catabolism of dl-α-phenylhydracrylic, phenylacetic and 3-and 4-hydroxyphenylacetic acid via homogentisic acid in a Flavobacterium sp. Archives of microbiology 149(3), 207-213.
Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Garcia-Garcia, E., Pun, J., Martin, J.W., Belosevic, M. and Gamal El-Din, M., 2013. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. Environmental science & technology 47(12), 6518-6526.
Whitby, C., 2010. Microbial naphthenic Acid degradation. Advances in applied microbiology 70, 93-125.
Xue, J., Zhang, Y., Liu, Y. and El-Din, M.G., 2016. Treatment of oil sands process-affected water (OSPW) using a membrane bioreactor with a submerged flat-sheet ceramic microfiltration membrane. Water research 88, 1-11.
Zhang, Y., Xue, J., Liu, Y. and El-Din, M.G., 2016. Treatment of oil sands process-affected water (OSPW) using membrane bioreactor coupled with ozonation: A comparative study. Chemical Engineering Journal.

4 Optimization of operating conditions of an anoxic-aerobic membrane reactor (MBR) with a submerged flat-sheet ceramic microfiltration membrane for raw oil sands process-affected water (OSPW) treatment[5]

4.1 Introduction

The oil sands industry in Alberta, Canada has been generating enormous volumes of oil sands process-affected water (OSPW), resulting in a total tailings ponds area of 182 km^2 (including associated structures) by the year 2013 (CAPP 2014). OSPW is a complex mixture of suspended solids, salts and organic compounds. Among all the organic contaminants contained in OSPW, naphthenic acids (NAs) in particular have been reported as the major contributors to OSPW toxicity and persistency. They are known to be acutely toxic to a range of organisms, including microorganisms, algae, vertebrates and invertebrates (Anderson et al. 2012, Clemente and Fedorak 2005, Headley and McMartin 2004, Herman et al. 1994). Therefore, NAs are regarded as the target pollutants which need to be treated urgently (Choi and Liu 2014, Hwang et al. 2013, MacKinnon et al. 1993, Martin et al. 2010).

NAs are a wide group of aliphatic and alicyclic, alkyl-substituted carboxylic acids with a generalized formula $C_nH_{2n+z}O_x$, in which n refers to molecular carbon number; Z is zero or a negative even integer indicating hydrogen deficiency caused by the formation of rings or double bond equivalents (Headley and McMartin 2004, Holowenko et al. 2001); and x refers to the number of oxygen atoms. Species with $x = 2$ are classical NAs, while those with $x \geq 3$ are termed oxidized NAs (oxy-NAs) (Islam et al. 2014). It has been estimated that more than 20,000

[5] This chapter has been submitted: Jinkai Xue, Yanyan Zhang, Yang Liu, Mohamed Gamal El-Din, 2016. Dynamics of naphthenic acids and microbial community structures in a membrane bioreactor treating oil sands process-affected water: impacts of supplemented inorganic nitrogen and hydraulic retention time. Submitted to: Chemical Engineering Journal.

individual NAs are potentially associated with OSPW (Rowland et al. 2011). Hanging on the ore composition, extraction processes, the tailings age and NA quantification approaches, the OSPW NAs concentration reportedly varied greatly within the range of 20 – 120 mg/L (Allen 2008a, Clemente and Fedorak 2005, Han et al. 2008, Scott et al. 2005b). It has been suggested that NAs with low molecular weights and linearly grouped carbon rings may be more toxic in OSPW (Clemente et al. 2004, Frank et al. 2009, Headley and McMartin 2004, Holowenko et al. 2002); and NAs with ring structures tend to be more persistent in environment (Anderson et al. 2012). NAs are known to be acutely toxic to a range of organisms, including microorganisms, algae, vertebrates and invertebrates (Anderson et al. 2012, Clemente and Fedorak 2005, Headley and McMartin 2004, Herman et al. 1994), thus they could be detrimental to environment, especially to aquatic environments. It is therefore desirable to remove NAs from OSPW to mitigate its environment impact.

It has been suggested that biodegradation is responsible for the natural, but slow degradation of NAs in oil sands tailings ponds (Quagraine et al. 2005). However, it has been estimated that the *in situ* biodegradation half-life of NAs is within a range of 12.8 – 13.6 years (Han et al. 2009). To accelerate the biodegradation of OSPW NAs, it is crucial to develop engineered biological processes that are efficient in mitigating OSPW organic compounds. Choi and Liu (2014) performed a study on treatment of OSPW by using two sequencing batch reactors (SBRs) and achieved an acid extractable fraction (AEF) removal rate of 8.7% and 16.6% at a hydraulic retention time (HRT) of 24 h with the inoculation of activated sludge and mature fine tailings (MFT), respectively. Hwang et al. (2013) reported that 18.5% of the OSPW parent NAs was eliminated by OSPW indigenous microorganisms in a biofilm reactor with an HRT of 19 h.

Membrane bioreactor (MBR) process has been successfully applied for municipal and industrial wastewater treatment because of its compactness, high treatment capacity, low sludge production and excellent effluent quality compared with conventional activated sludge processes. To verify the feasibility of MBR for OSPW treatment, an anoxic-aerobic MBR system was performed with an HRT of 48 h for OSPW treatment. The MBR attained an NA removal rate of ~25% from OSPW after the startup period (Day 1– 426) (Xue et al. 2016). To further improve the MBR's performance for OSPW treatment, effort needs to be made to optimize the operating conditions.

HRT is one of the essential parameters in MBR operation. It not only affects the extent to which the target contaminants are degraded (Wen et al. 2010), but also profoundly impacts membrane fouling development (Fallah et al. 2010, Shariati et al. 2011). Longer HRTs are usually required to treat industrial wastewater, especially those containing complex organic contaminants (Viero and Sant'Anna 2008). However, the effect of HRT on degradation of organic pollutants was still unclear as indicated by several studies (Chang et al. 2006, Qin et al. 2007, Shariati et al. 2011, Sipma et al. 2010). On the other hand, a shorter HRT is favorable as it reduces the required reactor volume (less investment) for the same treatment capacity. In addition, HRT has an indirect yet profound impact on membrane fouling through altering sludge properties (i.e., the concentration of MLSS, the production of extracellular polymeric substances (EPS) and the viscosity of sludge) (Meng et al. 2007, Qu et al. 2013). Due to increased membrane flux, shortened HRTs usually result in severer membrane fouling (Fallah et al. 2010, Meng et al. 2007, Shariati et al. 2011), which could increase the operating cost substantially. Therefore, a balance between contaminant removal performance and operating cost must be considered for HRT selection (Viero and Sant'Anna 2008).

Ammonia monooxygenase (*amo*) produced by ammonia oxidizing bacteria (AOB) is able to degrade a wide spectrum of refractory organic substrates through co-metabolisms (De Gusseme et al. 2009, Roh et al. 2009, Tran et al. 2009). Deni and Penninckx (1999) reported that *in vitro* AOB could partially oxidize hydrocarbons. Verstraete and Philips (1998) used nitrifiers as indirect bio-catalysis to remove recalcitrant organic compounds. However, nitrifiers are not necessarily stimulated by higher NH_4-N concentrations as free ammonia (FA) is inhibitory to many microbial activities including nitrification (Anthonisen et al. 1976). Given the alkaline nature of OSPW, effect of FA should not be ignored. Therefore, it is needed to find out an optimal supplemented NH_4-N concentration to maximize the MBR's performance of NA degradation.

After the start-up stage of the reactor, efforts were made to optimize the system's NA degradation performance by changing the feed water nitrogen concentrations (NH_4^+ and NO_3^-) and HRT. The objectives of this study were: 1) to explore the optimal operating conditions to improve the MBR's NA degradation efficiency; and 2) to characterize the microbial community structure in the MBR with the aim of uncovering the most effective bacterial groups associated with NA removal.

4.2 Methodology

4.2.1 Source waters, external carbon source and supplemented nutrients

The OSPW used in this study was collected from the same tailings pond in Northern Alberta region. Characteristics of the water are given in Table 4-1. Raw OSPW was received in 200-liter barrels and preserved in dark at 4 °C prior to treatment. To facilitate the growth of microorganisms in the system, sodium acetate (CH_3COONa) was supplied as an external carbon

source at a constant organic loading rate (OLR) of 125 g COD/(m^3·d) throughout the whole operation. To ensure the sufficiency of nitrogen, phosphorus, and other trace nutrients, supplemented KNO_3, NH_4Cl, NaH_2PO_4, and modified Bushnell-Haas medium (BHM) (Clemente et al. 2004) were added in the feed water. To avoid undesirable microbial growth in the feed tank, a stock solution of sodium acetate, and a stock solution of NO_3-N, NH_4-N and phosphate were continuously injected into the MBR at a pre-set rate by using a syringe pump. Detailed information on system start-up/sludge acclimatization is available in our previously published report (Xue et al. 2016).

Table 4-1. Characteristics of OSPW.

Parameter	Value
pH	8.8±0.2
Chemical oxygen demand (COD)	224±46 mg/L
5 day biochemical oxygen demand (BOD_5)	5.4±0.8 mg/L
NH_4-N	1.2±1.4 mg/L
NO_3-N	4.3±1.9 mg/L
Total suspended solids (TSS)	36.7±1.7 mg/L
Total alkalinity	598.4±55.4 mg/L as $CaCO_3$
Classical NAs	49.9±4.0 mg/L
Oxy-NAs	39.8±4.2 mg/L

4.2.2 MBR configuration

The configuration of the MBR is illustrated in Figure 4-1. Information on system inoculation and start-up is described elsewhere (Xue et al. 2016). The MBR was configured with a feed tank, an anoxic tank, an aerobic tank with an immersed membrane module, and a permeate tank. A stirrer was equipped in the feed tank to ensure the homogeneity of the feed water. The suspension of mixed liquor in the anoxic tank was actualized through mechanical agitation with an overhead stirrer. A flat sheet ceramic microfiltration membrane (Meidensha Corporation, Japan) with a pore size of 0.1 μm and an effective area of 128 cm^2 was immersed in aerobic tank. Dissolved

oxygen (DO) concentrations in the anoxic tank and the aerobic tank were maintained at a level of < 0.3 mg/L and > 4.0 mg/L, respectively. The retentate in the aerobic tank was recirculated to the anoxic tank by overflow at a recycling ratio of 2.

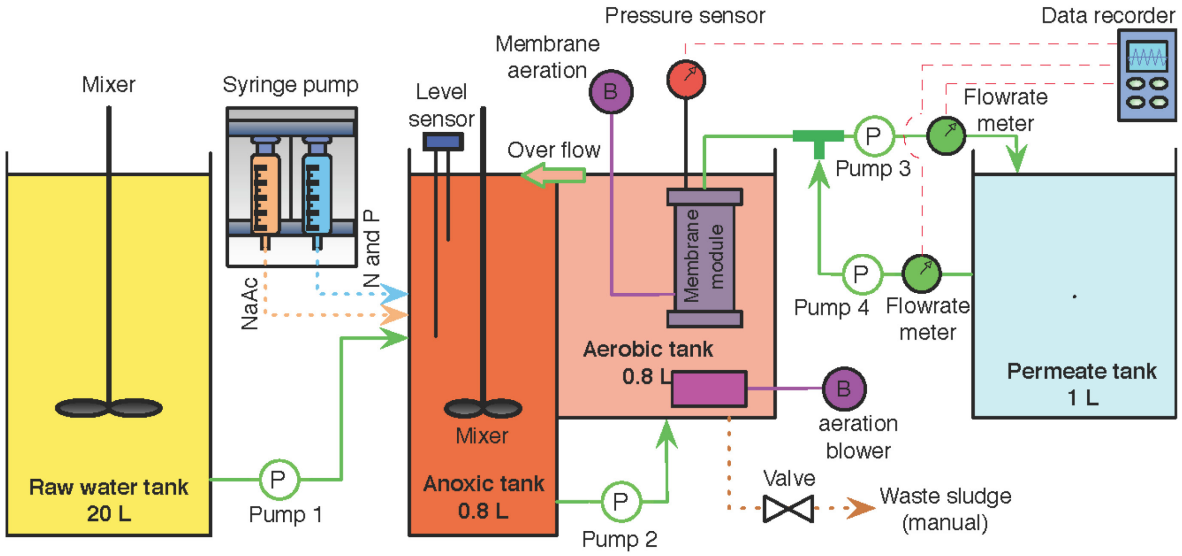


Figure 4-1. Schematic diagram of the MBR.

4.2.3 Operating conditions

Table 4-2. Operating conditions examined.

Stage	Period days	Supplemented nitrogen		HRT hours
		NH_4-N mg/L	NO_3-N mg/L	
A25H48	300 — 449	25	50	48
A75H48	449 — 546	75	0	48
A50H48	546 — 630	50	25	48
A25H72	630 — 680	25	50	72
A25H12	680 — 712	25	50	12
A25H24	712 — 742	25	50	24

The different operating conditions examined in this study are tabulated in Table 4-2. Despite the changes made to NO_3-N and NH_4-N concentrations during this optimizing period, total inorganic

nitrogen (TIN, i.e., nitrate, nitrite and ammonium nitrogen) concentration supplemented to the system was maintained at 75 mg N/L throughout the study. The system automatically performed a 30-second backwashing followed by 9.5 minutes of membrane filtration, which comprised a 10-minute working phase. The relaxation duration between two working phases was adjusted to achieve a desired equivalent HRT. A Yokogawa Daqstation DX1000 recorder was connected with the flowmeter and pressure sensor to continuously monitor the permeate flowrate, backwash flowrate and transmembrane pressure (TMP). According to the manufacturer's suggestion, chemical cleaning (soaking the membrane module in 0.1% HClO solution for 1 h) was applied once TMP exceeded -35 kPa. Sludge in the MBR was wasted through weekly sampling (30 mL from each tank for solids content measurements); and the calculated solid retention time (SRT) was ~187 days.

4.2.4 Analyses

4.2.4.1 Water chemistry

Samples were collected weekly from the feed by using a disposable syringe, and from the permeate by leaving the permeate tubing in a clean glass bottle till a volume of 100 mL was achieved. Right after sample collection, Thermo Scientific™ Target2™ Nylon Syringe Filters (0.22 μm) were used to filter water samples prior to analyses. Traditional water chemistry parameters including pH, chemical oxygen demand (COD), nitrate nitrogen (NO_3-N) concentration, ammonium nitrogen (NH_4-N) concentration, and nitrite nitrogen (NO_2-N) concentration were measured for the feed and permeate samples in duplicates according to standard methods (Federation and Association 2005). To measure the mixed liquor volatile suspended solids (MLVSS) concentration (an indicator of microbial growth), a 30 mL sludge sample was collected weekly from the middle depth of the anoxic and aerobic tank by using a

disposable syringe. The aforementioned analyses were performed right after the samples were collected and pre-filtered. Dissolved oxygen (DO) concentrations in the anoxic and aerobic tanks were monitored through plugging the DO meter (YSI 50B Dissolved Oxygen Meter) probe into the reactor tanks to ensure that anoxic and aerobic conditions were achieved in corresponding tanks.

4.2.4.2 NA measurement

To assess the MBR performance on OSPW NA degradation, ultra-performance liquid chromatography coupled with high resolution mass spectrometry (UPLC/HRMS) (Islam et al. 2014) was performed to measure classical and oxidized NAs concentrations of the feed and permeate samples at the end of each operating condition stage. With respect to permeate sampling, a volume of 10 mL was collected from totally 2.4 L of permeate water produced over three consecutive days for UPLC/HMRS analysis. The second of the three days was used as the nominal sampling date.

4.2.4.3 MiSeq sequencing

A PowerSoil® DNA Isolation Kit from Mo-Bio Laboratories, Inc. (CA, USA) was used to extract DNA from collected sludge samples according to the manufacturer's manual. In addition, the fouling layer on membrane surfaces was carefully scraped off by using a spatula and collected for DNA extraction. Extracted DNA samples were diluted to a concentration of ~20 ng/μL before sending to a commercial laboratory (Research and Testing Laboratory, Texas, US) for Illumina MiSeq sequencing. Samples were sequenced using Illumina MiSeq sequencing instruments and reagents according to the manufacturer's guidelines. Primers used for MiSeq sequencing were 28F (GAGTTTGATCNTGGCTCAG) and 388R (TGCTGCCTCCCGTAGGAGT). Raw sequence data were processed with the Quantitative

Insights Into Microbial Ecology (QIIME, http://qiime.org) software package with default settings. Operational taxonomic units (OTUs) were picked through using open-reference clustering algorithm. A similarity of 97% was used for sequence clustering. Both α diversity (diversity within a sample) and β diversity (diversity among a group of samples) were performed based on the OTU table generated. Parameters including Chao1, observed species, and Shannon were computed to measure α diversity. And β diversity was conducted through building weighted and unweighted UniFrac distance matrices on the basis of a phylogenetic tree. Principal coordinate analysis (PCoA) was performed to examine the correlations among microbial communities from different samples. 2.4.4 Statistical method

Paired two sample t-test was used in this study to compare the MBR performance under different operating conditions with the significance level of 0.05. Pearson correlation coefficient (r_p) was used to estimate linear correlations. The coefficient r_p is a numerical value within the range of -1 and 1 that expresses the strength of the linear relationship between two parameters. An r_p closer to 1 indicates a strong positive correlation. An r_p of 0 signalize that there is no relationship. Values closer to -1 indicate a strong negative relationship between the two variables.

4.3 Results and discussion

4.3.1 COD and nitrogen removal, and biomass growth

Table 4-3. Permeate COD and inorganic nitrogen under different supplemented NH_4-N concentrations (HRT = 48 h).

Supplemented NH_4-N	COD	COD removal	NH_4-N	NH_4-N removal	TIN	TIN removal
mg/L	mg/L	%	mg/L	%	mg/L	%
75	211±28	-5.4±9.4	0.16±0.21	99.8±0.3	11.1±10.6	86.2±13.1
50	206±23	-3.3±12.5	0.06±0.12	99.9±0.2	26.9±11.1	67.0±13.3
25	221±21	1.3±12.5	0.39±0.96	98.4±4.0	5.6±3.5	93.0±4.2

Table 4-4. Permeate COD and inorganic nitrogen at different HRTs (25 mg NH_4-N/L).

HRT	COD	COD removal	NH_4-N	NH_4-N removal	TIN	TIN removal
h	mg/L	%	mg/L	%	mg/L	%
72	239±19	5.8±13.6	0.10±0.17	99.6±0.7	15.5±6.6	80.7±8.0
48	221±21	1.3±12.5	0.39±0.96	98.4±4.0	5.6±3.5	93.0±4.2
24	226±30	10.9±2.0	0.18±0.21	99.4±0.8	12.2±1.4	84.6±1.8
12	209±14	10.7±3.5	0.06±0.09	99.8±0.4	20.4±6.9	74.7±8.3

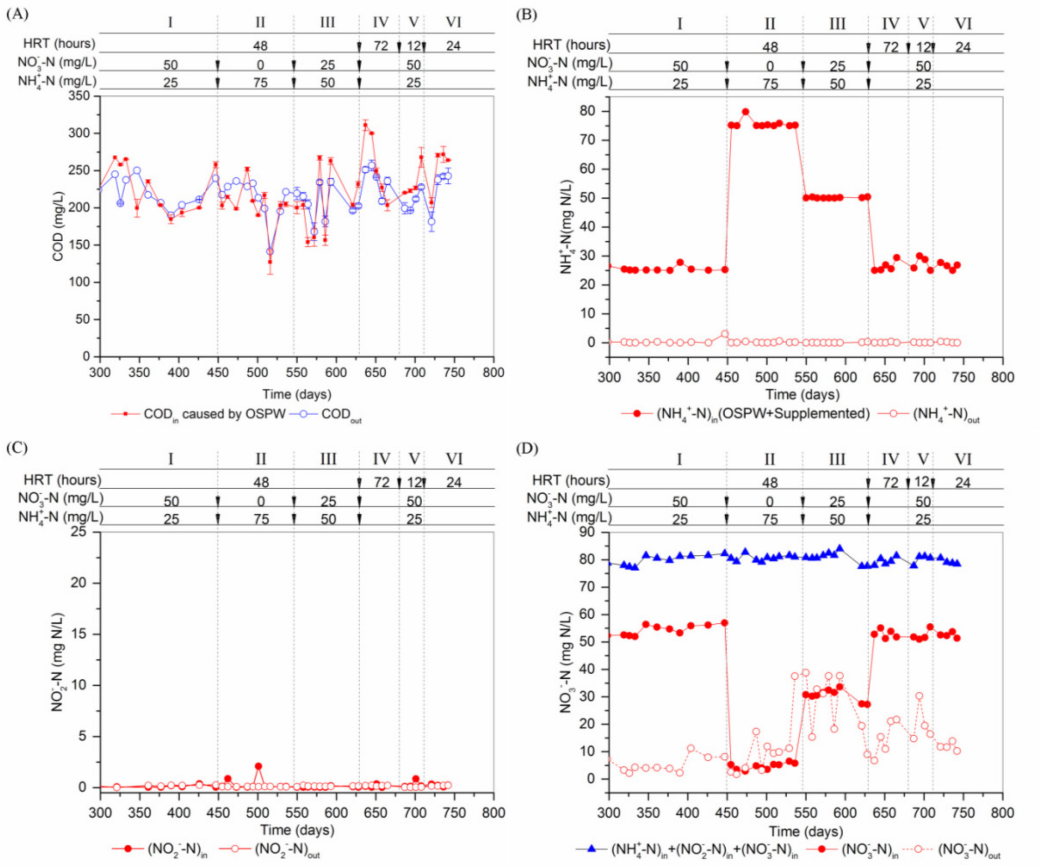


Figure 4-2. Water chemistry parameters over time: (A) COD; (B) NH_4-N; (C) NO_2-N; and (D) NO_3-N.

(Note: Since NH_4-N and NO_3-N were supplemented directly into the MBR anoxic tank with pumped syringes, influent NH_4-N in chart B is a sum of OSPW indigenous and supplemented NH_4-N. Similarly, influent NO_3-N concentration in chart D is a sum of OSPW indigenous and supplemented NO_3-N.)

Table 4-3 summarizes the permeate COD, NH_4-N and TIN concentrations under different supplemented NH_4-N concentrations at an HRT of 48 h. As for the permeate water quality under

different HRTs, Table 4-4 is presented. Figure 4-2 A, B, C and D demonstrate COD, NH_4-N, NO_2-N and NO_3-N concentrations over time, respectively. The permeate COD level throughout the study was close to that of the original OSPW COD. To better understand COD changes during the MBR process, t-test was conducted between original OSPW COD and permeate COD concentrations within each of the six stages. Compared with original OSPW COD, t-test P values for MBR permeate COD are 0.162, 0.851, 0.519, and 0.289 at A75H48, A50H48, A25H48, and A25H72, respectively. Thus, the MBR process did not significantly change the OSPW indigenous COD ($P >> 0.05$) over the four stages. In contrast, MBR permeate at A25H24 and A25H12 showed significant COD reduction ($P < 0.05$) with a P of 0.017 and 0.002, respectively, implying the operating conditions used at A25H24 and A25H12 were more favorable for OSPW indigenous COD degradation. An average COD degradation rate of 10.9% and 10.7% was achieved in the MBR at A25H24 and A25H12, respectively. The better COD removal in those two stages might result from enhancement of rejection by biofouling layer (Khor et al. 2007) given that more frequent severe membrane fouling were witnessed within the two periods (Figure 6-3).

Despite that the total concentrations of NH_4-N (including OSPW indigenous and supplemented NH_4-N) varied over the whole operation duration, the MBR constantly produced a permeate NH_4-N concentration of ~0 mg/L under all operating conditions, indicating its excellent ammonium oxidation stability (Table 4-3 and Table 4-4). NO_2-N was also closely monitored over time. The NO_2-N concentration in the MBR permeate was found to be negligible ($\leq$ 0.25 mg N/L) throughout the study (Figure 4-2 C), reflecting the insusceptibility of nitrite oxidizing microorganisms against operating condition variations. With respect to TIN removal performance of the MBR, the MBR showed more fluctuations as the operating condition

changed, suggesting that denitrifiers might be more susceptible to changes of operating conditions and need more time to adjust themselves.

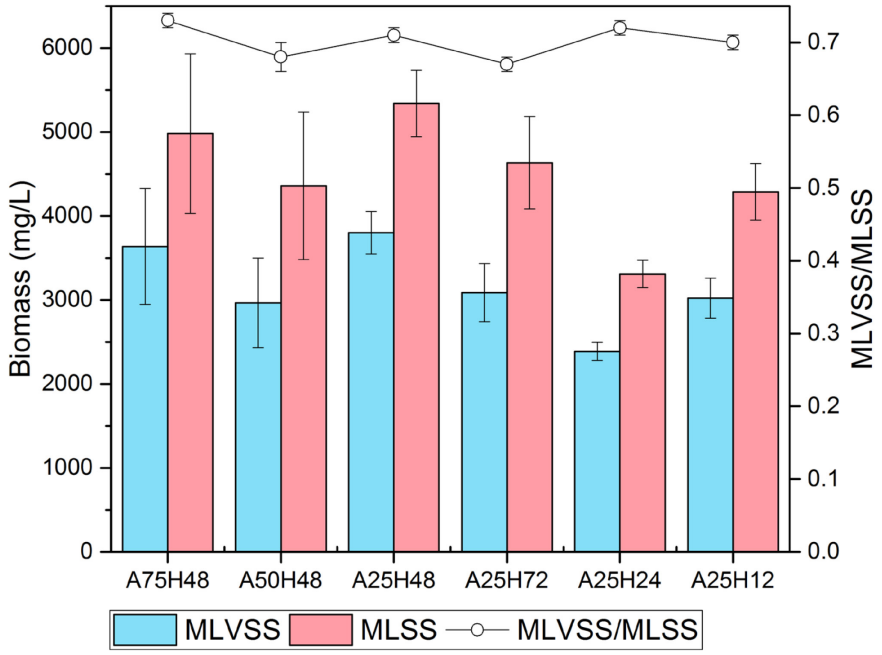


Figure 4-3. Biomass (MLSS and MLVSS) concentrations and MLVSS/MLSS ratio under different operating conditions.

Figure 4-3 describes MLSS and MLVSS concentrations and MLVSS/MLSS ratios under different operating conditions. As recirculation was performed from the aerobic tank to the anoxic tank at a rate of 2-fold the influent flowrate, an average value of the measured solids concentrations in the anoxic and aerobic tank was calculated for each sampling day and presented in the figure. The MLVSS/MLSS ratio was relatively stable within a range of 0.63 – 0.74 over the whole study in spite of different operating conditions. The typical MLVSS/MLSS ratio is 0.8 – 0.9 in an activated sludge system (Metcalf et al. 2003). The lower MLVSS/MLSS ratio in this study could be attributed to the long SRT. In Figure 4-3, the bars for MLSS and MLVSS behaved in great congruity. Thus, MLVSS is picked to describe how biomass

concentration changed over time under each operating condition. When the HRT was shortened to 24 hours at A25H24 and 12 hours at A25H12, the MLVSS concentration fell to an average level of ~2390 mg/L and ~3020 mg/L, respectively. The bacterial growth in the system was likely to be impacted by the intensified OSPW organic loading at shorter HRTs.

4.3.2 Classical and oxidized NA removal performance

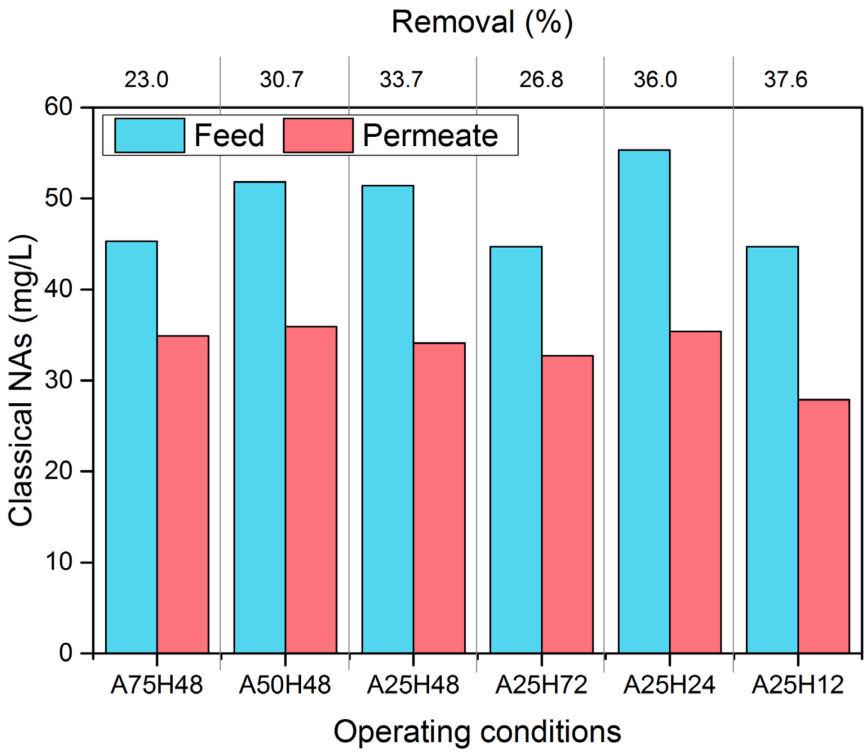


Figure 4-4. Total classical NAs concentrations of the MBR feed and permeate under different operating conditions.

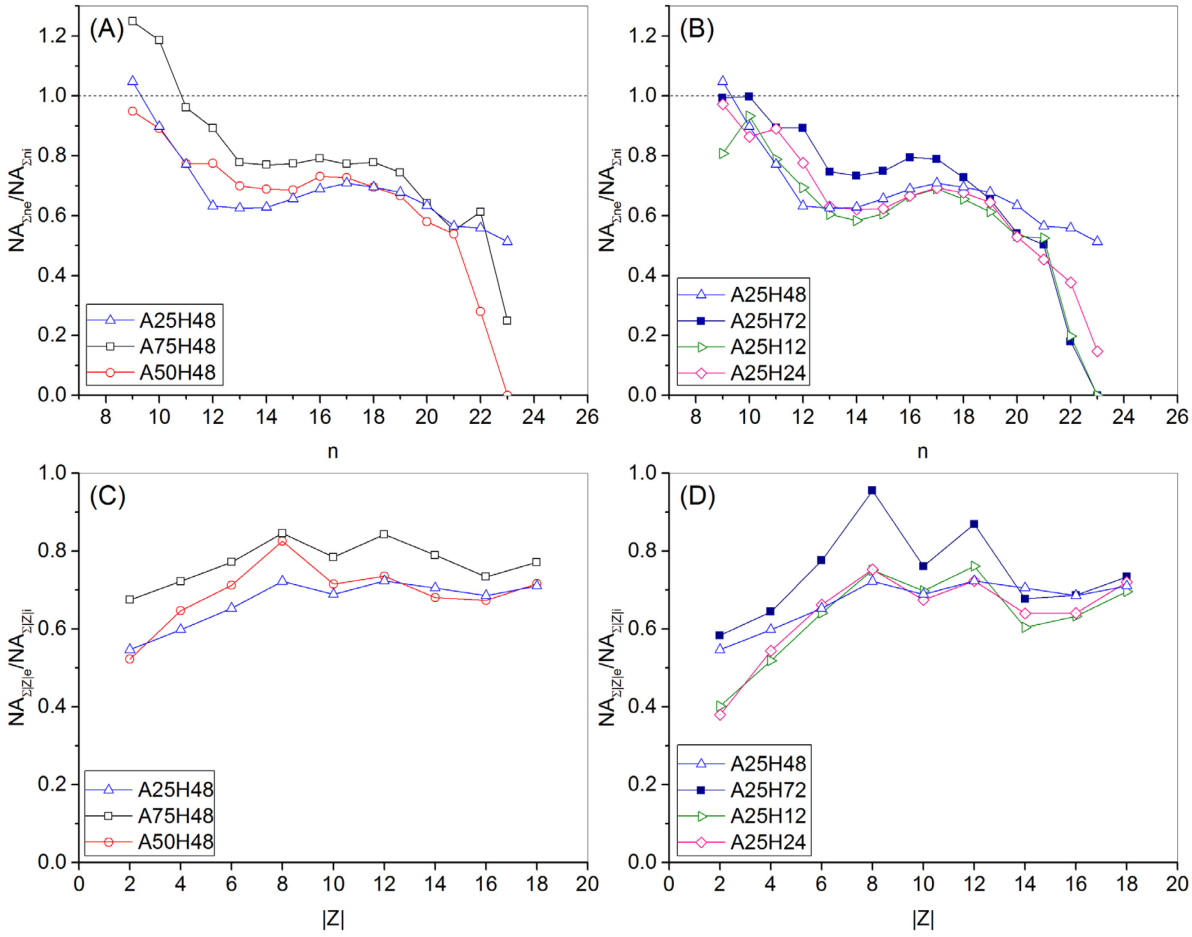


Figure 4-5. The relative abundance of remaining classical NAs with a carbon number *n* ($NA_{\Sigma ne}/NA_{\Sigma ni}$) in the MBR permeate under different operating conditions: (A) HRT was fixed at 48 h while supplemented nitrogen composition was changed; (B) Supplemented nitrogen composition was fixed as 25 mg NH_4-N/L and 50 mg NO_3-N/L while HRT was changed. And the relative abundance of remaining classical NAs with different hydrogen deficiencies |*Z*| ($NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$) in the MBR permeate under different operating conditions: (C) HRT was fixed at 48 h while supplemented nitrogen composition was changed; (D) Supplemented nitrogen composition was fixed as 25 mg NH_4-N/L and 50 mg NO_3-N/L while HRT was changed.

Figure 4-4 presents total classical NAs concentrations in the feed and permeate under different operating conditions. To examine the degradation efficiency of NAs with different carbon number *n* and different hydrogen deficiency *Z* under each operating condition, Figure 4-5 A, B, C, and D are prepared. A relative abundance of remaining classical NAs ($NA_{\Sigma ne}/NA_{\Sigma ni}$) is used to indicate the extent of biodegradation of NAs with each *n*, in which $NA_{\Sigma ne}$ denotes the total

concentration of NAs with a carbon number of *n* in the MBR effluent; while $NA_{\Sigma ni}$ is the total concentration of NAs with a carbon number of *n* in the MBR influent. Therefore, a lower $NA_{\Sigma ne}/NA_{\Sigma ni}$ means a better removal rate of the group of NAs with a certain carbon number *n*. Similarly, a relative abundance of remaining classical NAs ($NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$) is used to indicate the extent of biodegradation of NAs with each $|Z|$. To better disclose how molecular carbon number *n* and hydrogen deficiency/cyclicity affected the degradation of NAs, Figure 4-6, Figure 4-7, Figure 4-8 and Figure 4-9 are plotted based on the NAs concentrations profiles.

Although the stages differed in operating conditions, similar patterns could be seen for $NA_{\Sigma ne}/NA_{\Sigma ni}$ over the *n* range in Figure 4-5 A and B. For C9 – 12 and C19 – 23, a higher $NA_{\Sigma ne}/NA_{\Sigma ni}$ ratio is correlated with a lower carbon number *n*; whereas a plateau or a slightly climbing trend of the $NA_{\Sigma ne}/NA_{\Sigma ni}$ ratio is observed when *n* increases from 13 to 18 in Figure 4-5 A and B. As demonstrated in Figure 4-6 and Figure 4-7, species with more carbon numbers were generally better degraded within each *Z* series. In contrast, previous studies indicated that the molecular carbon number *n* has either negatively proportional correlation to or minor influence on biodegradation of NAs (Clemente et al. 2004, Han et al. 2008, Wang et al. 2013b). This discrepancy evidences that the capabilities of biological systems are considerably influenced by their configurations.

In regards to Figure 4-5 C and D, similar lines are obtained for different operating conditions. For $|Z| = 2$ to 8, the $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$ ratio is positively proportional to $|Z|$; whereas the $|Z|$ range of 9 to 18 witnesses a relatively stable region. The $NA_{\Sigma ne}/NA_{\Sigma ni} - n$ relation and $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i} - |Z|$ relation found in this study are consistent with what has been reported in our previous article (Xue et al. 2016). It is noticed that within each individual *n* series, an increased degree of cyclization (a higher $|Z|$) generally resulted in poorer removal efficiency (Figure 4-8 and Figure

4-9). This trend is not surprising as the biodegradation rate of NAs decreased with increasing cyclicity (higher $|Z|$) in both of commercial NAs and OSPW NAs according to previous research (Han et al. 2008).

NAs with larger n and lower cyclicity are generally more hydrophobic (Pourrezaei 2013). Organic compounds of higher hydrophobicity (or higher lipophilicity) could easily attach onto or penetrate the cell envelope, possibly leading to better biodegradation rates under certain circumstances (Parsons and Govers 1990, Peltola 2008, Singh 2012). Moreover, for those particular species, stronger hydrophobic interactions of NAs-NAs, NAs-flocs, and NAs-fouling layer were expected, which would result in elevated local concentrations of hydrophobic NAs. And the locally elevated concentrations of NAs would benefit overall biodegradation as a threshold level of NAs exists for microorganisms to initiate the biodegradation process (Misiti et al. 2013c).

Oxy-NAs concentrations of the MBR feeds and permeates were also monitored at different stages (Figure 4-10). Di-oxidized (O4), tri-oxidized (O5), and tetra-oxidized (O6) NAs were all found to be degraded to a certain extent at each stage. In contrast, more mono-oxidized (O3) NAs were detected after the MBR biological process at all stages except A25H48 and A25H12. It was suggested that O3 and O4 oxy-NAs are intermediates during aerobic biodegradation of classical NAs (Grewer et al. 2010, Han et al. 2009, Martin et al. 2010). O3 species are probably hydroxylated NAs (Han et al. 2008) and O4 are possibly dicarboxylic acids (Headley et al. 2009). Dissipation of O4 and O5 oxy-NAs was detected in Headley et al. (2009)'s study, which is in accordance to the results in this study. In contrast, some other researchers reported unappreciable changes of O3 and O4 oxy-NAs after 98 days (Han et al. 2008) and 84 days

(Brown et al. 2013) of incubation. Therefore, the MBR in this study demonstrated competent oxy-NA degradation performance.

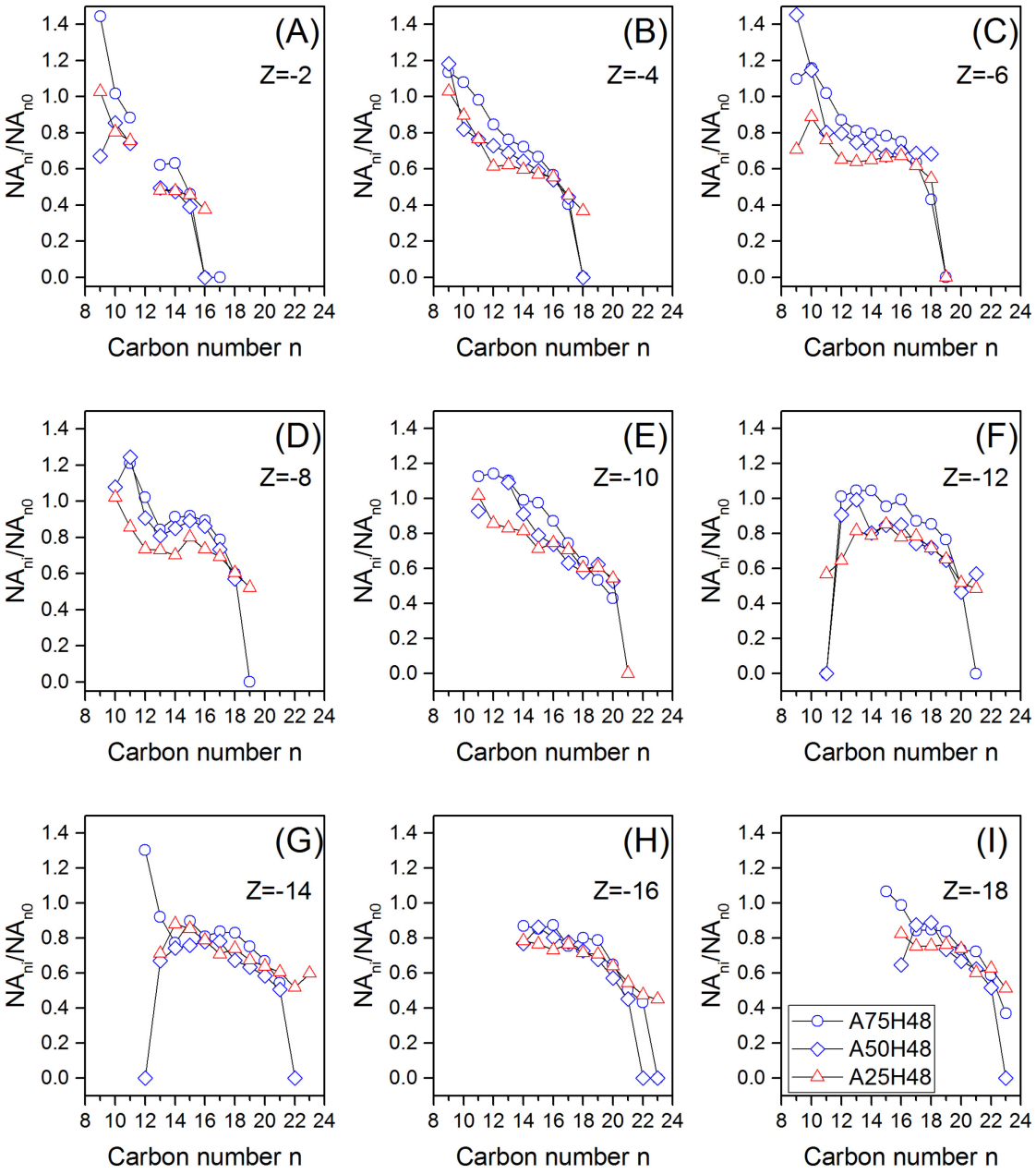


Figure 4-6. The relative abundance of remaining classical NAs with different carbon numbers (NA_{ni}/NA_{n0}) within each Z series of the permeate samples under different supplemented NH_4-N concentrations.

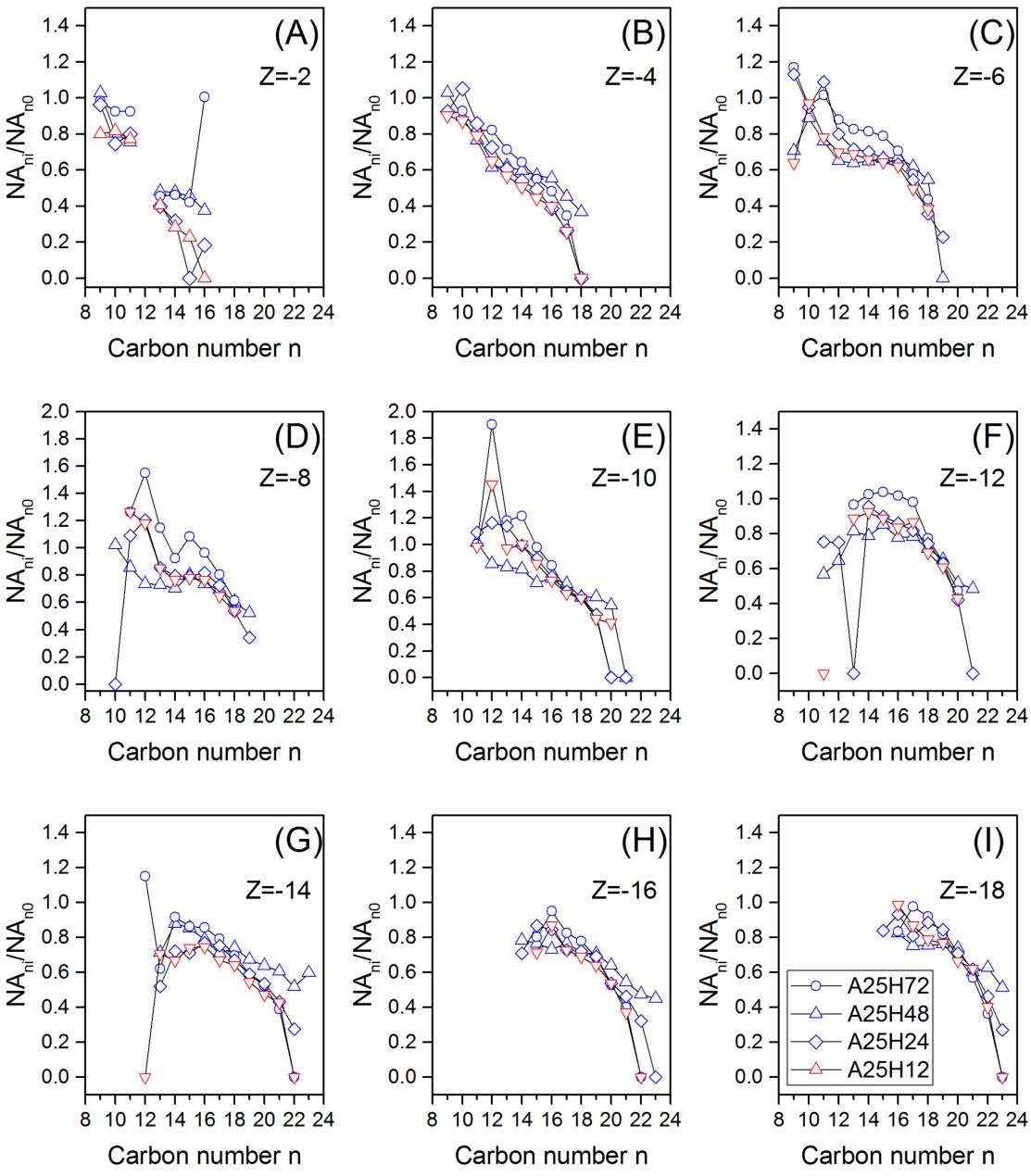


Figure 4-7. The relative abundance of remaining classical NAs with different carbon numbers (NA_{ni}/NA_{n0}) within each Z series of the permeate samples at different HRTs.

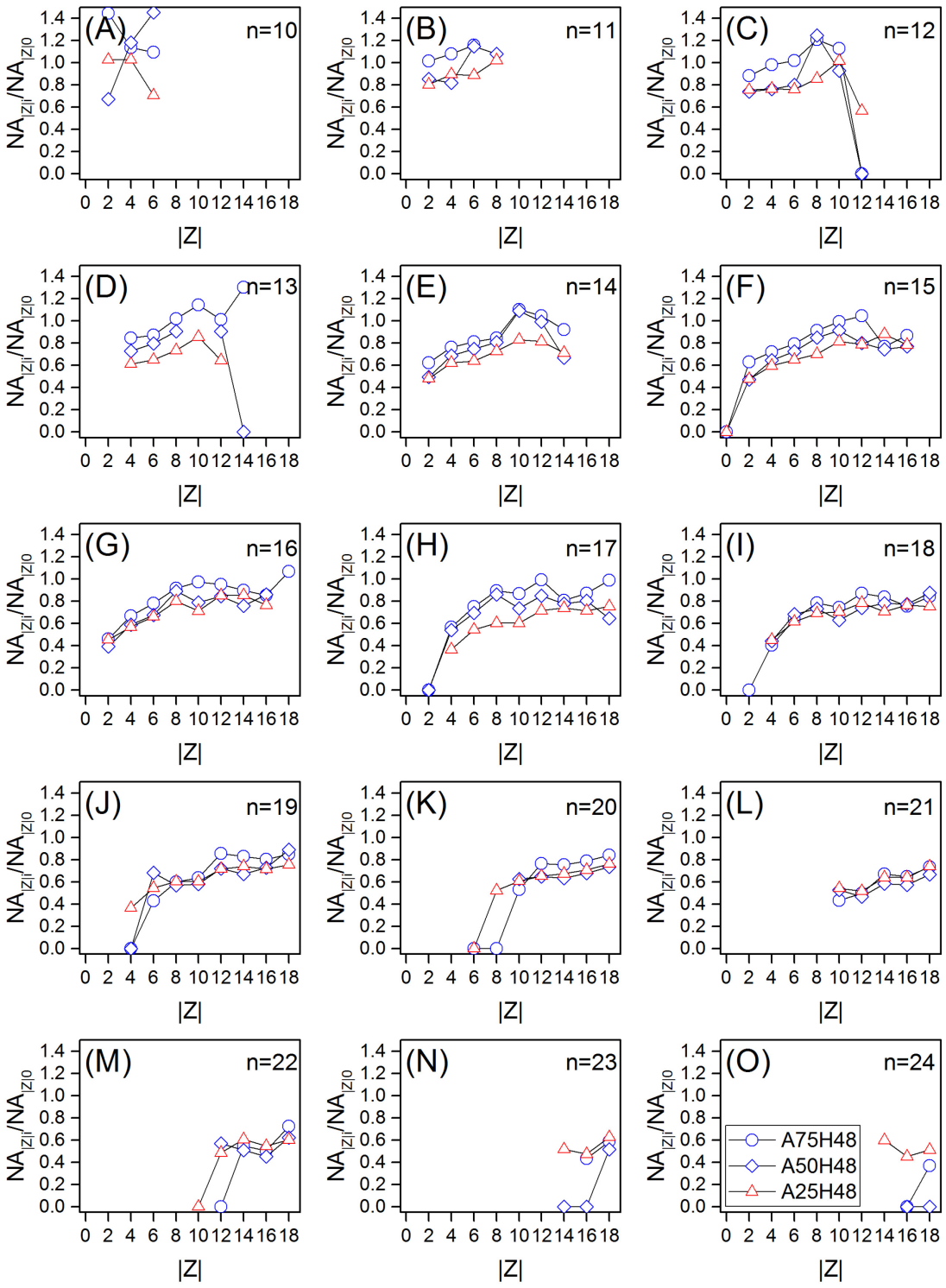


Figure 4-8. The relative abundance of remaining classical NAs with different hydrogen deficiencies (NA_{Zi}/NA_{Z0}) within each *n* series of the permeate samples under different supplemented NH_4-N concentrations.

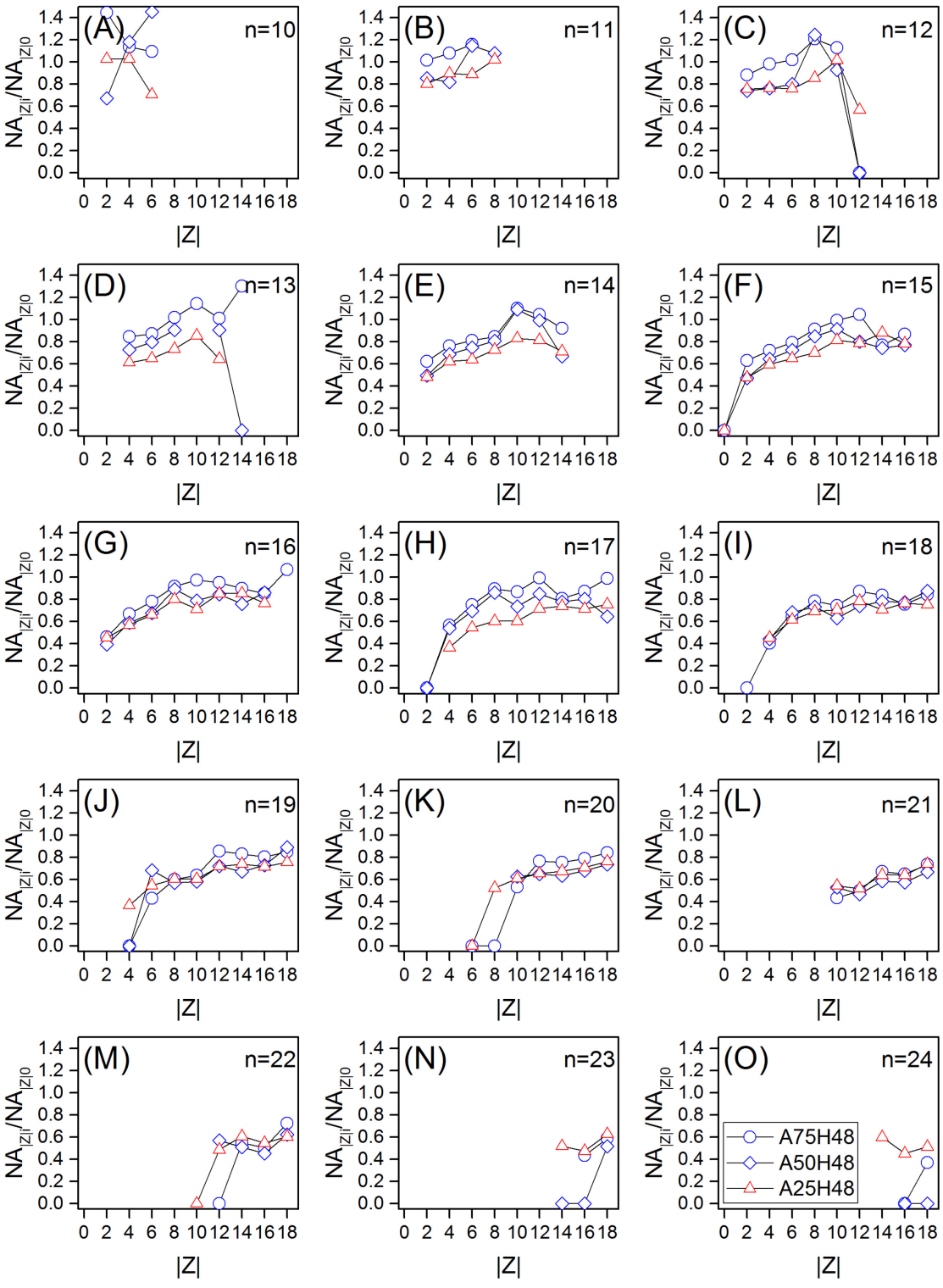


Figure 4-9. The relative abundance of remaining classical NAs with different hydrogen deficiencies (NA_{Zi}/NA_{Z0}) within each *n* series of the permeate samples at different HRTs.

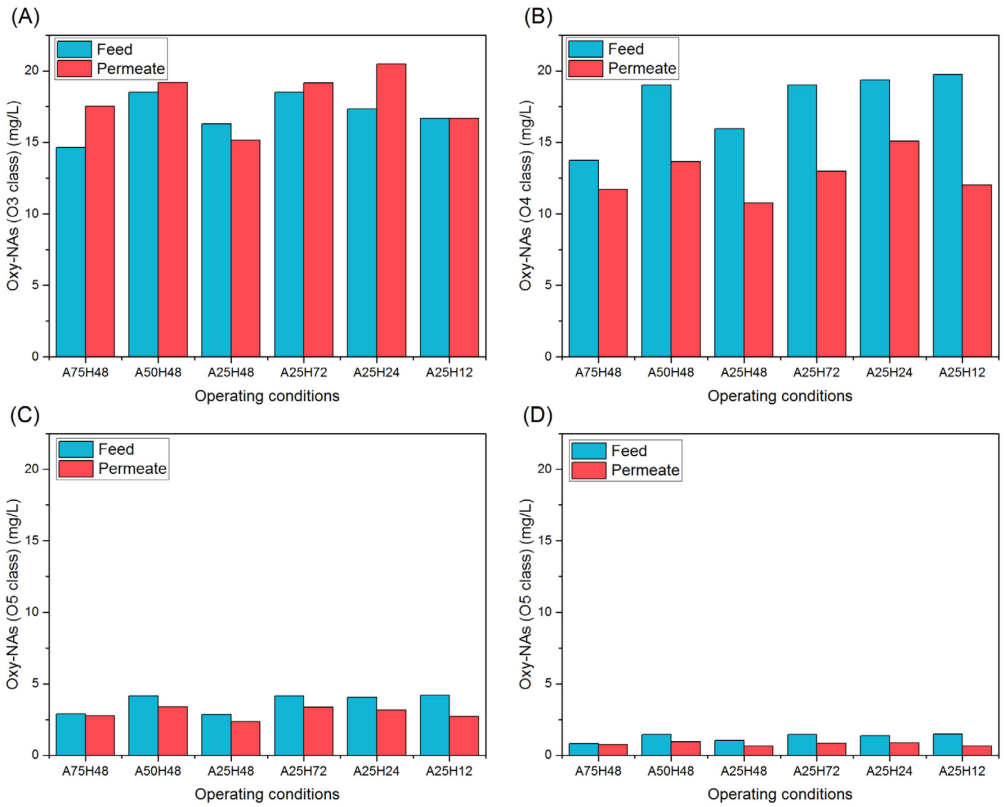


Figure 4-10. Oxy-NAs concentrations of the MBR feed and permeate under different operating conditions: (A) oxy-NAs with O3; (B) oxy-NAs with O4; (C) oxy-NAs with O5; and (D) oxy-NAs with O6.

4.3.2.1 Effect of supplemented nitrogen composition

In addition to volatilization and biomass assimilation, the majority of NH_4-N was oxidized into NO_3-N by nitrifiers (Table 4-3, Figure 4-2 B). Discussion in the following paragraphs will therefore be focused on how different influent NH_4-N concentrations affected the MBR's efficiency in degrading OSPW NAs when the HRT was 48 h.

a. Classical NA removal

The removal rates of total classical NAs at A75H48, A50H48 and A25H48 were 23.0%, 30.7% and 33.7%, respectively (Figure 4-4). The lines for A75H48 in Figure 4-5 A and C demonstrate a substantial separation over those for A50H48 and A25H48, indicating poorer NA degradation at

A75H48. Based on the removal efficiency of classical NAs in Figure 4-4, $NA_{\Sigma ne}/NA_{\Sigma ni}$ and $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$ in Figure 4-5, A75H48 showed the lowest effectiveness for OSPW treatment, which may be attributed to the toxicity of FA caused by high ammonia concentration. It is known that a higher total ammonia (NH_3-N and NH_4-N) concentration under an alkaline pH environment results in a higher FA concentration, which has been reported to be inhibitory to nitrifiers and other microbes (Calli et al. 2005, Yang et al. 2004). Based on Ford et al. (1980)'s study, an FA concentration of ~13.5 mg/L is estimated in the MBR at A75H48 stage.

In Figure 4-5 A, the two lines of A50H48 and A25H48 are closely weaved over the *n* range. T-test suggests that the difference between the two lines is insignificant ($P = 0.3360 > 0.05$). For NAs of C12 – 17, A25H48 removed more NAs than A50H48 did; whereas the latter achieved higher removal of C9 and C20-23 NA species. Moreover, it is interesting to notice the residual abundance of C23 NAs at A75H48 was lower than that at A25H48. Therefore, it is suggested that higher concentrations of influent NH_4-N might be helpful to degradation of NAs with higher carbon numbers (i.e., $n > 21$ in this study). Provided the poorer overall NA degradation at A75H48, it is not surprising to see that its $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$ line is located noticeably higher than the other two stages' in Figure 4-5 C. T-test returned a $P = 0.1659 > 0.05$ for $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$ values of A50H48 and A25H48, indicating that the degradation efficiencies of NAs under the two operating conditions were not significantly different. However, it is noticed that for |Z| of 4, 6, 8 and 10, A50H48 resulted in more ($P = 0.034 < 0.05$) residual NAs than A25H48 (Figure 4-5 C). It is therefore suggested that a supplemented NH_4-N concentration of 25 mg/L is more beneficial to classical NA degradation than the other two examined NH_4-N concentrations.

b. Oxy-NA removal

Figure 4-10 illustrates oxy-NAs concentrations of the MBR feeds and permeates at different stages. Among the three stages with an HRT of 48 h, A25H48 was the only stage that reduced the O3 oxy-NAs whereas A75H48 and A50H48 increased the abundance of that group of oxy-NAs by 20% and 4%, respectively. As for O4 oxy-NAs, all three 48-h HRT stages effectively reduced its concentration with a performance ranking of A25H48 (33%) > A50H48 (28%) > A75H48 (15%). Regarding the removal of O5 and O6 oxy-NAs, A25H48 demonstrated either similar or higher performance than A50H48; while A75H48 showed the poorest degradation ability for both of the two groups. The supplemented NH_4-N concentration of 25 mg N/L is therefore considered the best for oxy-NA degradation among the three examined supplemented NH_4-N levels.

Therefore, based on the higher removal rates of total classical NAs (33.7%) and total oxy-NAs (20%), the nitrogen composition used at A25H48 (25 mg NH_4-N/L and 50 mg NO_3-N/L) was selected for further trials on different HRTs.

4.3.2.2 Effect of HRT

A25H72, A25H48, A25H24 and A25H12 were the four stages sharing the same supplemented inorganic nitrogen composition (25 mg NH_4-N/L and 50 mg NO_3-N/L). Those four stages are compared to evaluate the effect of HRT on OSPW NA degradation in the MBR.

a. Classical NA removal

HRT of 72 h resulted in the lowest degradation of total classical NAs. Discussion is thus focused on comparing NA degradation rates at the rest HRTs, which are 48, 24 and 12 h. To better examine the effect of HRT on classical NA degradation, Figure 4-5 B and D are constructed.

$NA_{\Sigma ne}/NA_{\Sigma ni}$ at HRT of 12 h was significantly lower than those at HRT of 48 h ($P = 0.038 < 0.05$) and 24 h ($P = 0.040 < 0.05$). Among the four HRTs examined, the 48-h HRT showed the poorest removal efficiency of NAs with higher carbon numbers (C19 – 23), whereas 72-h HRT and 12-h HRT demonstrated a similarly excellent removal rate of C20 – 23 NAs. With respect to removal of NAs with different cyclization degrees, Figure 4-5 D shows that the 72-h HRT returned the worst removal efficiency for NAs with a |Z| within the range of 2 to 12. For |Z| = 2, 4, 14 and 16, HRT of 48 h showed higher $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$ ratios than the 24-h and 12-h HRTs, indicating a poorer removal efficiency. For the rest |Z| values, the difference among the HRTs of 48 h, 24 h and 12 h was marginal. Within the whole |Z| range, the difference of $NA_{\Sigma|Z|e}/NA_{\Sigma|Z|i}$ ratios between the HRTs of 24 h and 12 h was insignificant ($P = 0.671 > 0.05$), indicating that the HRTs of 24 h and 12 h made no difference in degrading NAs sharing the same cyclization degree. Therefore, the HRT of 12 h is considered as the optimal among the four investigated HRTs.

b. Oxy-NA removal

Among the four stages, A25H24 showed the least removal rate of total oxy-NAs (O3 – 6), which was 6%; whereas A25H12 exhibited the highest overall removal of oxy-NAs (23.9%). Owing to their substantial abundance, O3 and O4 oxy-NAs are conceivably worth more attention than O5 and O6 species. Regarding the removal of O3 oxy-NAs, the performance ranking is listed as A25H48 (7%) > A25H12 (0%) > A25H72 (-3%) > A25H24 (-18%), where a negative sign indicates increase. In contrast, the ranking for removal of O4 oxy-NAs is A25H12 (39%) > A25H48 (33%) > A25H72 (32%) > A25H24 (22%). With respect to degradation of O5 and O6 oxy-NAs, A25H12 was arguably the outperformer among the four stages. No clear correlation between HRT and oxy-NA degradation rate could be elucidated based on the data in this study.

Oxy-NAs may be created from species with different oxygen numbers, carbon numbers (*n*) or hydrogen deficiencies (Z), and in the meantime, be decomposed into other species by microorganisms during the course of incubation (Martin et al. 2010). Therefore, an HRT of 12 h is considered as the optimal on the basis of overall classical NA and oxy-NA removal.

Furthermore, it is not unconceivable that NA species with diverse molecular structures may have different bio-persistence, thus requiring different optimal operating conditions (Figure 4-6, Figure 4-7, Figure 4-8 and Figure 4-9). Hundreds of different NAs, including classical and oxidized, were involved in the system, making it intricate to decipher the kinetics underlying. Previous researchers have reported enhancement of contaminant rejection performance by membrane biofouling. It has been suggested that formation of biofouling may promote the microfiltration (MF) membrane rejection performance up to the nano-filtration (NF) efficiency (Khor et al. 2007). And solutes with higher molecular weights and more complex molecular structures are expected to be better rejected by NF membrane (Bellona et al. 2004). In addition, it is apparent that the negatively charged deprotonated NA molecules confronted electrostatic repulsion from the membrane fouling layer (Bellona et al. 2004). Thus, NAs could be either repelled or adsorbed by the fouling layer, depending on the hydrophobic interaction and electrostatic repulsion. Moreover, locally elevated abundance of organic pollutants in the sludge and fouling layer might be helpful for biodegradation (Sipma et al. 2010). It is suggested that the fouling layer formed on membrane surface helped reject NAs and thus elevated the NAs concentration in the MBR sludge and enriched NAs on membrane surface, which eventually enhanced the biodegradation of NAs. Hence, organic and biofouling in the MBR was not necessarily unfavorable in light of the improved NA degradation. It is therefore imperative to contain membrane fouling within a certain level to help mitigation of recalcitrant organic

pollutants without significantly increasing the demand of chemical cleaning and membrane replacement.

4.3.3 Microbial community characterization

The results of Illumina MiSeq sequencing are illustrated in Figure 4-11. Out of great similarity caused by the internal recirculation between the anoxic and aerobic tanks, the OTU numbers of the two compartments were averaged for following analyses. As is shown in Figure 4-11 A, a bar chart has been generated for the 10 most abundant orders at each stage. *Rhodocyclales*, *Burkholderiales* and *Nitrosomonadales* of the phyla *Proteobacteria*, *Cytophagales*, [*Saprospirales*] and *Flavobacteriales* of the phyla *Bacteroidetes*, *Nitrospirales* of the phyla *Nitrospirae*, and RB41 of the phyla *Acidobacteria* were arguably the most abundant microorganisms in the MBR throughout the whole operation.

Researchers have reported dominating *β-Proteobacteria* in oil sands tailings ponds and Athabasca river sediments as degrader of NAs and aromatic hydrocarbons (Yergeau et al. 2012), which helps explain the high abundance of *β-Proteobacteria* detected in this study. *Rhodocyclales* are responsible for denitrification and degradation of aromatic hydrocarbons (Loy et al. 2005). *Burkholderiales*, *Cytophagales,* and *Nitrosomonadales* have been linked to degradation of bio-recalcitrant hydrocarbons including NAs (Bell et al. 2013a, Pérez‐Pantoja et al. 2012, Röling et al. 2002, Wang et al. 2015). *Flavobacteriales* have been documented to be able to metabolize recalcitrant organic compounds, including polycyclic aromatic hydrocarbons (PAHs) (Cao et al. 2009, Van den Tweel et al. 1988) and NAs (Whitby 2010). The high population of *Nitrosomonadales* (ammonium oxidizers) and *Nitrospirales* (nitrite oxidizers) justifies the excellent nitrification performance of the MBR over the whole operation period.

The relative abundance of *Rhodocyclales* (Figure 4-11 A) of sludge samples over the stages is positively correlated to the MBR's denitrification performance with a Pearson correlation coefficient r_p of 0.790. In addition, the relative abundances of *Rhodocyclales* and the MBR's total classical NA removal rates over the six stages showed a moderate positive correlation with an r_p of 0.608, indicating that *Rhodocyclales* might play a role in overall degradation of total classical NAs. The higher *Rhodocyclales* abundances at stages A25H48, A25H24 and A25H12 further evidenced that an NH_4-N concentration of 25 mg/L was favorable for degradation of total classical NAs. However, further study needs to be done to confirm the correlation between *Rhodocyclales* and degradation of total classical NAs.

Correlation between relative abundance of each of the listed orders and removal rates of classical NAs with each *n* number over the whole operation duration has been evaluated by calculating r_p and tabulated in Table A1 in Appendix. The correlation coefficients between *Rhodocyclales'* relative abundance and removals of C12 – 17 classical NAs were all over 0.7. As for classical NAs with higher molecular carbon numbers (C19 – 23), relative abundance of *Cytophagales* showed strong positive correlations to their removal rates ($r_p \geq 0.755$). In addition, relative abundances of *Burkholderiales*, RB41, and *Deinococcales* showed moderate positive correlation with removal rate of C23 classical NAs.

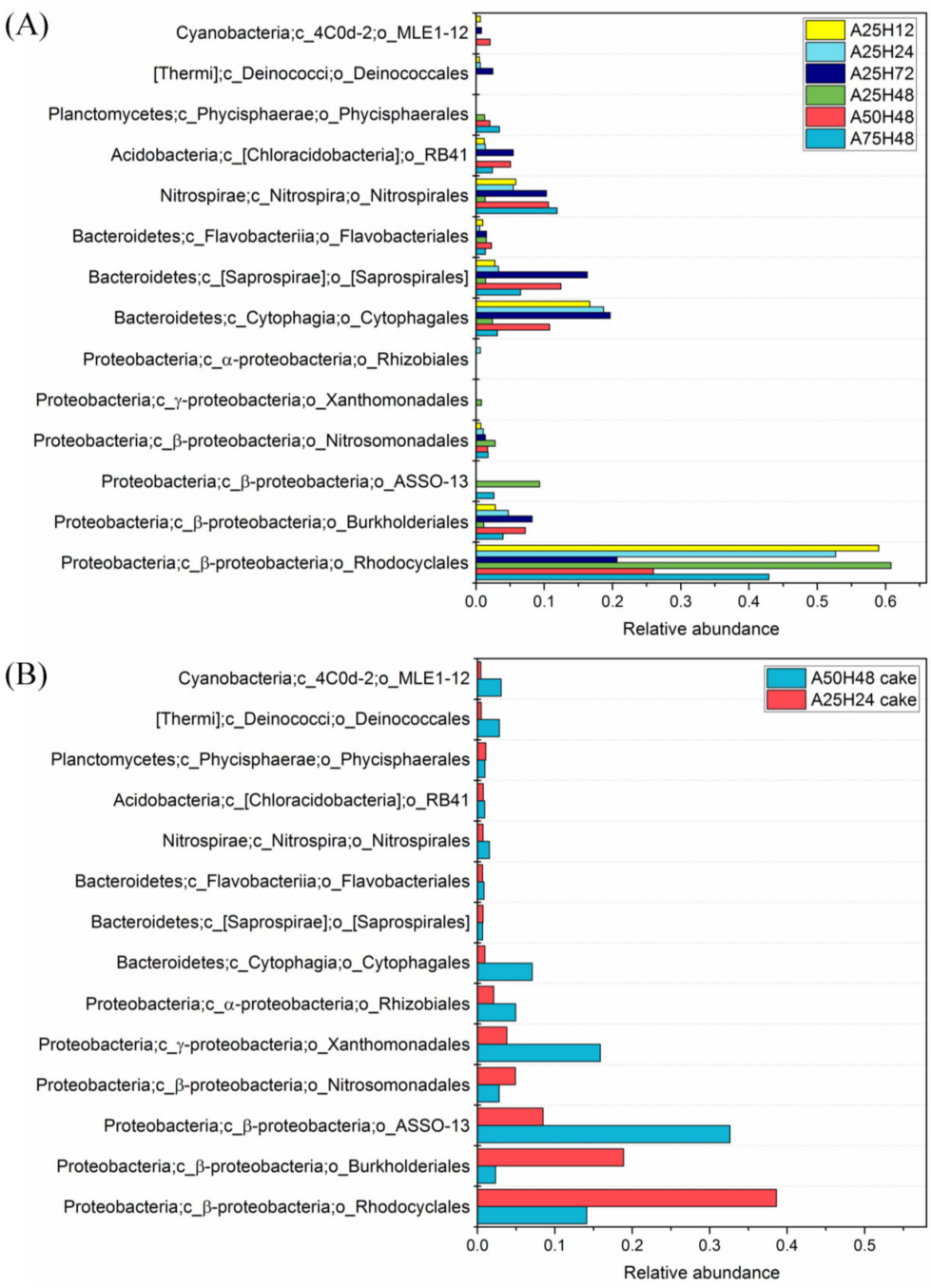


Figure 4-11. Microbial community compositions: 10 most abundant orders detected in (A) sludge samples from all operating stages; (B) membrane cake layer samples at stage A50H48 and A25H24.

(Note: Square brackets indicate taxonomic changes recommended by Greengenes.)

Moreover, correlations between relative abundance of each of the listed orders and removal rates of classical NAs with different |Z| values over the whole operation duration have been assessed and tabulated in Table A2. *Rhodocyclales* showed a strong positive correlation to removal of NAs with a |*Z*| of 6 and 8, whereas *Cytophagales* were found to be positively correlated to degradation of NAs with a |Z| of 14 and 16. Therefore, it is suggested that degradation of NAs with different molecular structures were completed by different microbial species either synergistically or independently. To confirm the effect and specificity of a certain microbial group to degradation of certain groups of NAs, further effort should be made by using isolated bacterial cultures and model compounds.

Figure 4-11 B is presented for relative abundances of the top 10 bacterial orders of the two samples collected from membrane cake layers when TMP exceeded the critical level (-35 kPa). All the major orders were commonly found in both of sludge samples and their corresponding cake layer samples though the suspended and the cake layer communities differed in relative abundances of microbes. Some bacteria, such as *Rhodocyclales* and *Cytophagales*, were found more abundant in the sludge samples, whereas some others, such as *Nitrospirales* and ASSO-13, were more abundant in the cake layer samples. In addition, the abundances of most of the listed bacteria in suspended matrix and cake layer varied in a similar trend as the MBR's operating conditions changed. For instance, Stage A25H24 witnessed more *Rhodocyclales* in both of sludge and cake layer microbial communities than Stage A50H48 did. It is therefore suggested that the suspended growth community affected the microbial community on membrane cake layer. Furthermore, the similar microbial community compositions of the sludge samples and their corresponding cake layer counterparts imply that the microorganisms inhabiting membrane

cake layer might contribute to degradation of NAs given that biofilm systems have proven their effectiveness for OSPW NA degradation (Choi et al. 2014).

Table 4-5. Alpha diversity parameters of microbial communities under different operating conditions.

Stage	**Parameters** (N = 14640 sequences/sample)		
	Chao1[a]	**Observed species**[b]	**Shannon**[c]
A75H48	1239.3	551	5.29
A50H48	882.5	480	5.16
A25H48	1055.2	485	4.39
A25H72	948.9	496	4.88
A25H24	890.1	439	4.80
A25H12	732.0	427	4.31

a. Chao1 index is usually used to estimate the total number of species within a community;
b. Observed species refers to the total count of unique OTUs in the sample.
c. Shannon index is a value of evenness that combines species richness and abundance to describe how different species are numerically distributed within a community.

To examine α diversity within each sludge sample at different operating conditions, Chao1, observed species, and Shannon indices were computed through rarefaction at a cutoff of 3% (Table 4-5). Chao1 and observed species indices both indicate that A25H12 had the lowest species richness among the six stages. In the meantime, A25H12 showed the lowest Shannon index, implying the lower evenness or poorer richness or both of the community. It is interesting to notice that 5% of the total count of genera constituted over 70% of the total bacterial abundance (Table 4-6) for every operating condition, suggesting that the majority of the species richness was originated from numerically rare microbial species. Given that the A25H12 stage demonstrated the best classical and oxidized NA removal rates, it is suggested that rare microbial species might not contribute to the MBR's NA degradation performance though their existence largely affected the species richness of the community. This is in accordance to previous research (Ma et al. 2013a).

Table 4-6. Total abundances of the 5% most abundant identified genera under each operating condition.

Stages	Count of total genera	Count of the 5% most abundant genera	Total abundance of the 5% most abundant genera
A75H48	177	9	79%
A50H48	151	8	75%
A25H48	181	9	90%
A25H72	132	7	70%
A25H24	98	5	73%
A25H12	122	6	83%

4.4 Conclusions

After more than 2 years of continuous operation, the MBR's performance on OSPW NA degradation was successfully improved through changing the supplemented inorganic nitrogen composition and HRT. The study showed that higher supplemented NH_4-N concentrations or longer HRTs did not guarantee better NA removal. Instead, an NH_4-N concentration of 25 mg/L and an HRT of 12 h demonstrated the best removal rates of total classical NAs (37.6%) and total oxy-NAs (23.9%), indicating A25H12 was the optimal among the six examined operating conditions. It should be noted that different optimal operating conditions may exist for best degradation of different NAs groups. In addition, classical NAs with larger carbon numbers were generally better mitigated in the MBR; and species with higher cyclicity were more persistent to microorganisms. Moreover, MiSeq sequencing analysis revealed that orders of *Proteobacteria* (i.e., *Rhodocyclales*, *Burkholderiales*, and *Nitrosomonadales*), *Bacteroidetes* (i.e., *Cytophagales*, *[Saprospirales]*, and *Flavobacteriales*), and *Nitrospirae* (i.e., *Nitrospirales*) were the dominating microbial groups over the whole study duration though their relative abundances varied as the

operating conditions changed. Furthermore, statistical calculations indicate strong positive correlations between certain microbial orders and NAs with particular molecular structures. Further effort needs to be made through using model compounds and isolated bacteria cultures to examine and confirm the contribution and specificity of those microbes to degradation of NAs with certain molecular structural features.

References

Allen, E.W., 2008. 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.

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., 2012. Effectiveness of Ozonation Treatment in Eliminating Toxicity of Oil Sands Process-Affected Water to Chironomus Dilutus. Environmental science & technology 46(1), 486-493.

Anthonisen, A., Loehr, R., Prakasam, T. and Srinath, E., 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. Journal (Water Pollution Control Federation), 835-852.

Bell, T.H., Yergeau, E., Maynard, C., Juck, D., Whyte, L.G. and Greer, C.W., 2013. Predictable Bacterial Composition and Hydrocarbon Degradation in Arctic Soils Following Diesel and Nutrient Disturbance. The ISME journal 7(6), 1200-1210.

Bellona, C., Drewes, J.E., Xu, P. and Amy, G., 2004. Factors Affecting the Rejection of Organic Solutes During Nf/Ro Treatment—a Literature Review. Water research 38(12), 2795-2809.

Brown, L.D., Pérez-Estrada, L., Wang, N., El-Din, M.G., Martin, J.W., Fedorak, P.M. and Ulrich, A.C., 2013. Indigenous Microbes Survive in Situ Ozonation Improving Biodegradation of Dissolved Organic Matter in Aged Oil Sands Process-Affected Waters. Chemosphere.

Calli, B., Mertoglu, B., Inanc, B. and Yenigun, O., 2005. Effects of High Free Ammonia Concentrations on the Performances of Anaerobic Bioreactors. Process Biochemistry 40(3), 1285-1292.

Cao, B., Nagarajan, K. and Loh, K.-C., 2009. Biodegradation of Aromatic Compounds: Current Status and Opportunities for Biomolecular Approaches. Applied microbiology and biotechnology 85(2), 207-228.

CAPP, 2014. The Facts On: Oil Sands, Canadian Association of Petroleum Producers, oilsandstoday.ca.

Chang, J.-S., Chang, C.-Y., Chen, A.-C., Erdei, L. and Vigneswaran, S., 2006. Long-Term Operation of Submerged Membrane Bioreactor for the Treatment of High Strength Acrylonitrile-Butadiene-Styrene (Abs) Wastewater: Effect of Hydraulic Retention Time. Desalination 191(1), 45-51.

Choi, J., Hwang, G., Gamal El-Din, M. and Liu, Y., 2014. Effect of Reactor Configuration and Microbial Characteristics on Biofilm Reactors for Oil Sands Process-Affected Water Treatment. International Biodeterioration & Biodegradation 89(2014), 74-81.

Choi, J. and Liu, Y., 2014. Biodegradation of Oil Sands Process Affected Water in Sequencing Batch Reactors and Microbial Community Analysis by High-Throughput Pyrosequencing. International Biodeterioration & Biodegradation 92, 79-85.

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.

De Gusseme, B., Pycke, B., Hennebel, T., Marcoen, A., Vlaeminck, S.E., Noppe, H., Boon, N. and Verstraete, W., 2009. Biological Removal of 17 Alpha-Ethinylestradiol by a Nitrifier Enrichment Culture in a Membrane Bioreactor. Water research 43(9), 2493-2503.

Deni, J. and Penninckx, M.J., 1999. Nitrification and Autotrophic Nitrifying Bacteria in a Hydrocarbon-Polluted Soil. Applied and environmental microbiology 65(9), 4008-4008.

Fallah, N., Bonakdarpour, B., Nasernejad, B. and Moghadam, M.A., 2010. Long-Term Operation of Submerged Membrane Bioreactor (Mbr) for the Treatment of Synthetic Wastewater Containing Styrene as Volatile Organic Compound (Voc): Effect of Hydraulic Retention Time (Hrt). Journal of hazardous materials 178(1), 718-724.

Federation, W.E. and Association, A.P.H., 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA): Washington, DC, USA.

Ford, D.L., Churchwell, R.L. and Kachtick, J.W., 1980. Comprehensive Analysis of Nitrification of Chemical Processing Wastewaters. Journal (Water Pollution Control Federation), 2726-2746.

Frank, R.A., Sanderson, H., Kavanagh, R., Burnison, B.K., Headley, J.V. and Solomon, K.R., 2009. Use of a (Quantitative) Structure–Activity Relationship [(Q) Sar] Model to Predict the Toxicity of Naphthenic Acids. Journal of Toxicology and Environmental Health, Part A 73(4), 319-329.

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? The Science of the total environment 408(23), 5997-6010.

Han, X., MacKinnon, M.D. and Martin, J.W., 2009. Estimating the in Situ Biodegradation of Naphthenic Acids in Oil Sands Process Waters by Hplc/Hrms. Chemosphere 76(1), 63-70.

Han, X., 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.

Headley, J. and McMartin, D., 2004. A Review of the Occurrence and Fate of Naphthenic Acids in Aquatic Environments. Journal of Environmental Science and Health, Part A 39(8), 1989-2010.

Headley, J.V., Peru, K.M., Armstrong, S.A., Han, X., Martin, J.W., Mapolelo, M.M., Smith, D.F., Rogers, R.P. and Marshall, A.G., 2009. Aquatic Plant‐Derived Changes in Oil Sands Naphthenic Acid Signatures Determined by Low‐, High‐and Ultra High‐Resolution Mass Spectrometry. Rapid Communications in Mass Spectrometry 23(4), 515-522.

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.

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.
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.
Hwang, G., Dong, T., Islam, M.S., Sheng, Z., Pérez-Estrada, L.a., Liu, Y. and Gamal El-Din, M., 2013. The Impacts of Ozonation on Oil Sands Process-Affected Water Biodegradability and Biofilm Formation Characteristics in Bioreactors. Bioresource technology 130C, 269-277.
Islam, M.S., Moreira, J., Chelme-Ayala, P. and El-Din, M.G., 2014. Prediction of Naphthenic Acid Species Degradation by Kinetic and Surrogate Models During the Ozonation of Oil Sands Process-Affected Water. Science of the total environment 493, 282-290.
Khor, S.L., Sun, D.D., Liu, Y. and Leckie, J.O., 2007. Biofouling Development and Rejection Enhancement in Long Srt Mf Membrane Bioreactor. Process Biochemistry 42(12), 1641-1648.
Lin, H., 2012. Membrane Bioreactors for Industrial Wastewater Treatment : A Critical Review. Critical Reviews in Environmental Science and Technology 42(7), 677-740.
Loy, A., Schulz, C., Lücker, S., Stoecker, K., Baranyi, C., Lehner, A., Wagner, M., Lu, S. and Scho, A., 2005. Microarray for Environmental Monitoring of the Betaproteobacterial Order 16s Rrna Gene-Based Oligonucleotide Microarray for Environmental Monitoring of the Betaproteobacterial Order "Rhodocyclales". Applied and environmental microbiology 71(3), 1373-1373.
Ma, J., Wang, Z., Yang, Y., Mei, X. and Wu, Z., 2013. Correlating Microbial Community Structure and Composition with Aeration Intensity in Submerged Membrane Bioreactors by 454 High-Throughput Pyrosequencing. Water research 47(2), 859-869.
MacKinnon, M., Meer, T.V. and Verbeek, A., 1993. Assessment of Biological Impact from a Wet Landscape Option for the Reclamation of Fine Tails from Oil Sands. Baddaloo, E.G., Ramamoorthy, S. and Moore, J.W. (eds), Alberta Environment, Environmental Protection, Edmonton.
Martin, J.W., Barri, T., Han, X., 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.
Meng, F., Shi, B., Yang, F. and Zhang, H., 2007. Effect of Hydraulic Retention Time on Membrane Fouling and Biomass Characteristics in Submerged Membrane Bioreactors. Bioprocess and Biosystems Engineering 30, 359-367.
Metcalf, E., Eddy, H., Tchobanoglous, G., Burton, F. and Stensel, H.D., 2003. Wastewater Engineering: Treatment and Reuse, McGraw-Hill, New York.
Oliveros, J.C., 2007. Venny. An Interactive Tool for Comparing Lists with Venn Diagrams.
Pérez-Pantoja, D., Donoso, R., Agulló, L., Córdova, M., Seeger, M., Pieper, D.H. and González, B., 2012. Genomic Analysis of the Potential for Aromatic Compounds Biodegradation in Burkholderiales. Environmental microbiology 14(5), 1091-1117.
Qin, J., Oo, M., Tao, G. and Kekre, K., 2007. Feasibility Study on Petrochemical Wastewater Treatment and Reuse Using Submerged Mbr. Journal of Membrane Science 293(1-2), 161-166.
Qu, X., Gao, W., Han, M., Chen, A. and Liao, B., 2013. Effect of Hydraulic Retention Time on Sludge Properties, Cake Layer Structure, and Membrane Fouling in a Thermophilic

Submerged Aerobic Membrane Bioreactor. Separation science and technology 48(10), 1529-1536.
Quagraine, E., Peterson, H. and Headley, J., 2005. In Situ Bioremediation of Naphthenic Acids Contaminated Tailing Pond Waters in the Athabasca Oil Sands Region—Demonstrated Field Studies and Plausible Options: A Review. Journal of Environmental Science and Health 40(3), 685-722.
Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J. and Chu, K.-H., 2009. Biodegradation Potential of Wastewater Micropollutants by Ammonia-Oxidizing Bacteria. Chemosphere 77(8), 1084-1089.
Röling, W.F., Milner, M.G., Jones, D.M., Lee, K., Daniel, F., Swannell, R.J. and Head, I.M., 2002. Robust Hydrocarbon Degradation and Dynamics of Bacterial Communities During Nutrient-Enhanced Oil Spill Bioremediation. Applied and environmental microbiology 68(11), 5537-5548.
Rowland, S.J., Scarlett, A.G., Jones, D., West, C.E. and Frank, R.A., 2011. Diamonds in the Rough: Identification of Individual Naphthenic Acids in Oil Sands Process Water. Environmental science & technology 45(7), 3154-3159.
Scott, A.C., Mackinnon, M.D. and Fedorak, P.M., 2005. Naphthenic Acids in Athabasca Oil Sands Tailings Waters Are Less Biodegradable Than Commercial Naphthenic Acids. Environmental science & technology 39(21), 8388-8394.
Shariati, S.R.P., Bonakdarpour, B., Zare, N. and Ashtiani, F.Z., 2011. The Effect of Hydraulic Retention Time on the Performance and Fouling Characteristics of Membrane Sequencing Batch Reactors Used for the Treatment of Synthetic Petroleum Refinery Wastewater. Bioresource technology 102(17), 7692-7699.
Sipma, J., Osuna, B., Collado, N., Monclús, H., Ferrero, G., Comas, J. and Rodriguez-Roda, I., 2010. Comparison of Removal of Pharmaceuticals in Mbr and Activated Sludge Systems. Desalination 250(2), 653-659.
Tran, N.H., Urase, T. and Kusakabe, O., 2009. The Characteristics of Enriched Nitrifier Culture in the Degradation of Selected Pharmaceutically Active Compounds. Journal of hazardous materials 171(1), 1051-1057.
Van den Tweel, W., Smits, J. and De Bont, J., 1988. Catabolism of Dl-A-Phenylhydracrylic, Phenylacetic and 3-and 4-Hydroxyphenylacetic Acid Via Homogentisic Acid in a Flavobacterium Sp. Archives of microbiology 149(3), 207-213.
Verstraete, W. and Philips, S., 1998. Nitrification-Denitrification Processes and Technologies in New Contexts. Environmental Pollution 102(1), 717-726.
Viero, A.F. and Sant'Anna, G.L., 2008. Is Hydraulic Retention Time an Essential Parameter for Mbr Performance? Journal of hazardous materials 150(1), 185-186.
Wang, X., Chen, M., Xiao, J., Hao, L., Crowley, D.E., Zhang, Z., Yu, J., Huang, N., Huo, M. and Wu, J., 2015. Genome Sequence Analysis of the Naphthenic Acid Degrading and Metal Resistant Bacterium Cupriavidus Gilardii Cr3. PloS one 10(8), e0132881.
Wen, G., Ma, J., Zhang, L. and Yu, G., 2010. Integrated Membrane Operations in Various Industrial Sectors: Case Studies, pp. 195-209, Elsevier B.V.
Whitby, C., 2010. Microbial Naphthenic Acid Degradation. Advances in applied microbiology 70, 93-125.
Xue, J., Zhang, Y., Liu, Y. and El-Din, M.G., 2016. Treatment of Oil Sands Process-Affected Water (Ospw) Using a Membrane Bioreactor with a Submerged Flat-Sheet Ceramic Microfiltration Membrane. Water research 88, 1-11.

Yang, S.-F., Tay, J.-H. and Liu, Y., 2004. Inhibition of Free Ammonia to the Formation of Aerobic Granules. Biochemical Engineering Journal 17(1), 41-48.
Yergeau, E., Lawrence, J.R., Sanschagrin, S., Waiser, M.J., Korber, D.R. and Greer, C.W., 2012. Next-Generation Sequencing of Microbial Communities in the Athabasca River and Its Tributaries in Relation to Oil Sands Mining Activities. Applied and environmental microbiology 78(21), 7626-7637.
Zhang, Y., McPhedran, K.N. and El-Din, M.G., 2015. Pseudomonads Biodegradation of Aromatic Compounds in Oil Sands Process-Affected Water. Science of the total environment 521, 59-67.

5 Effect of mild-dose ozone pretreatment on subsequent MBR performance for OSPW treatment under the optimized operating condition[6]

5.1 Introduction

Through a 442-day continuous optimization of operating conditions of the MBRs, an operating condition with a supplemented NH_4-N concentration of 25 mg/L in the feed water and an HRT of 12 h was eventually determined as the optimal state among all the six examined conditions. Chapter 4 covers detailed information on optimization of operating conditions of the anoxic-aerobic MBR for raw OSPW treatment. The optimization of the MBR for ozonated OSPW treatment is covered in a separate report first-co-authored by the other collaborator of the project. In this chapter, the NA degradation efficiencies and microbial communities of the two MBRs under the optimal operating condition are compared, and thus the effect of ozone pretreatment is evaluated. Hence, the aims of this chapter are: A) to evaluate the effect of ozonation pretreatment on MBR NA degradation; and (B) to elucidate the effect of ozonation pretreatment on MBR microbial community characteristics. Detailed information on methodology is available in previous chapters. The effect of ozone pretreatment on membrane fouling development is covered in Chapter 6.

5.2 Results and discussion

5.2.1 Degradation of OSPW NAs by the ozone pretreatment

Figure 5-1 displays the concentrations profiles of classical NAs of raw OSPW (Figure 5-1 A) and ozonated OSPW (Figure 5-1 B). The low-dose (utilized dose 30 mg O_3/L) ozonation treatment successfully removed over 50% of total classical NAs from the raw OSPW. To better

[6] This chapter will be submitted to WEFTEC 2016 89th Technical Exhibition and Conference.

illustrate how ozonation affect the degradation of NAs with different molecular structures, Figure 5-2 shows the $NA_{\Sigma ni}/NA_{\Sigma n0}$ ratio for each *n* and the $NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$ ratio for each |*Z*|. The subscript i indicates ozonated OSPW, whereas the subscript 0 referes to raw OSPW.

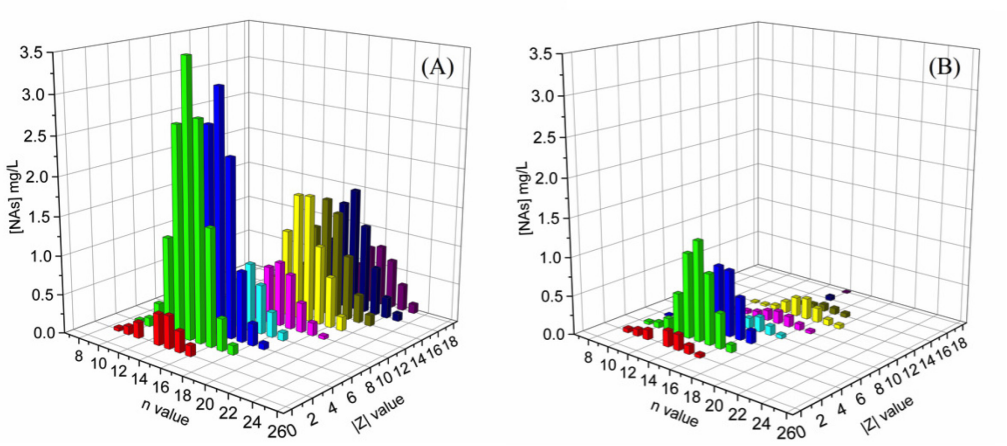


Figure 5-1. Classical NAs concentrations profiles of (A) the raw OSPW and (B) the ozonated OSPW.

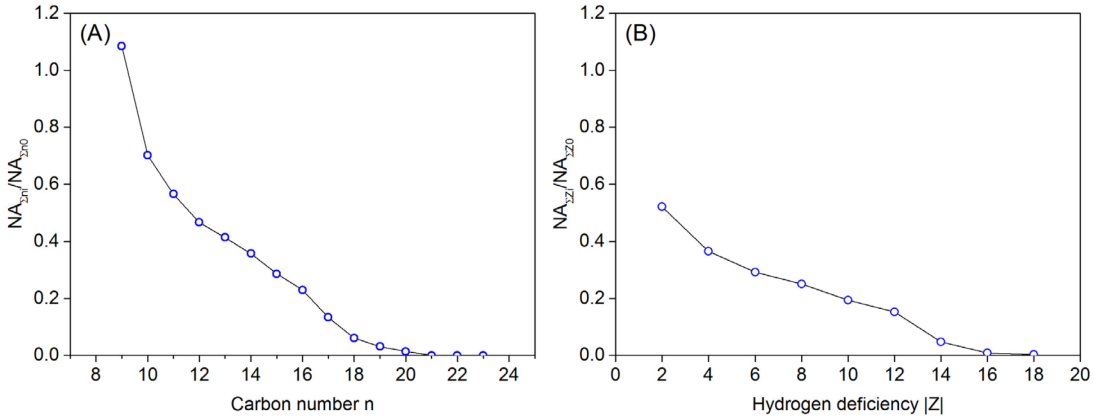


Figure 5-2. The relative residual abundances of classical NAs with (A) different carbon numbers ($NA_{\Sigma ni}/NA_{\Sigma n0}$) and (B) different hydrogen deficiencies ($NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$) after ozonation pretreatment.

(Note: $NA_{\Sigma ni}/NA_{\Sigma n0}$ indicates ratio of the concentration of NAs with *n* of the ozonated OSPW (i) to the concentration of NAs with *n* of the raw OSPW (0).)

According to Figure 5-1and Figure 5-2, ozonation effectively reduced concentrations of all detected classical NAs of raw OSPW except those with C9 (*n* = 9). NAs with higher carbon

numbers and higher cyclicity were more effectively removed. Both NAs with carbon number of 20 – 22 and ring number of 8 – 9 were almost completely removed by ozonation. The efficient removal of NAs with higher *n* and |*Z*| confirms ozone's capability of decomposing NAs with longer chains, more alkyl branches, and higher cyclicity, which are arguably the most bio-persistent fractions of OSPW NAs (Martin et al. 2010, Pérez-Estrada et al. 2011). Additionally, the higher remaining abundances of NAs with lower *n* and fewer rings might be due to conversion from NAs with more complexity (higher *n* and |*Z*|) into simpler NAs.

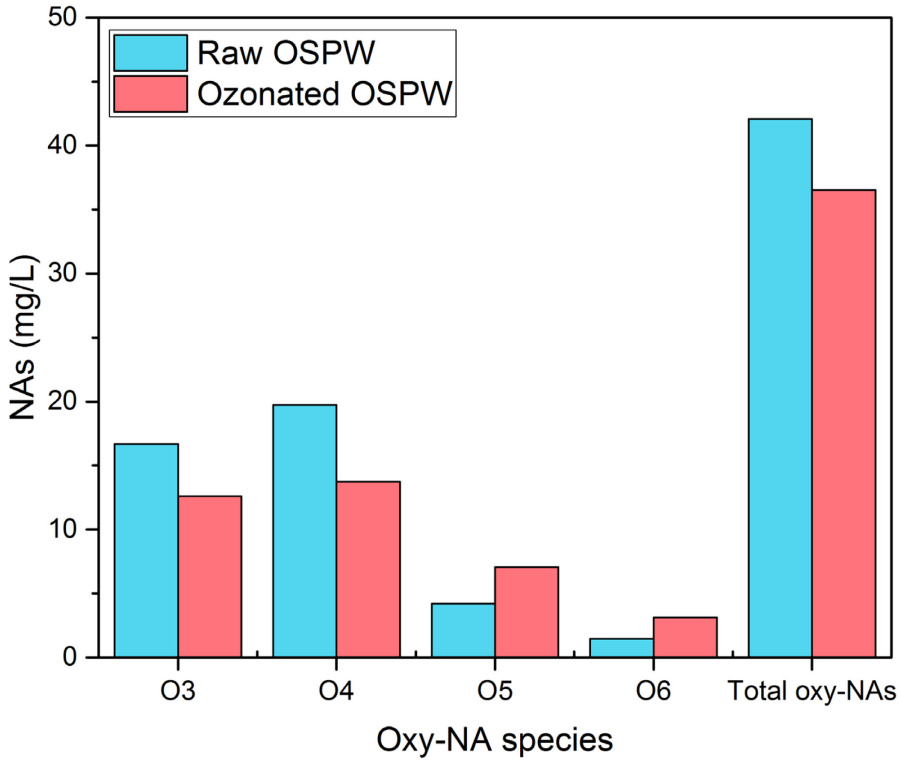


Figure 5-3. Oxy-NAs concentrations of raw and ozonated OSPW.

Changes of oxy-NAs during ozonation are charted in Figure 5-3. Substantial removal of O3 and O4 species could be observed. In contrast, O5 and O6 species are found to be increased after the ozonation treatment, indicating that those two groups of oxy-NAs could be ozonation products of other organic compounds. The increased O5 and O6 oxy-NAs concentration does not necessarily

mean that the low-dose ozonation is unable to degrade them; instead, it could be due to the fact that the creation of those NAs was faster than they were decomposed. Generation of oxy-NAs during ozonation process was reported in some previous studies (Pereira et al. 2013, Sun et al. 2014). According to Wang et al. (2013a), oxy-NAs contains more –OH groups in their molecules than classical NAs do, making it conceivable that oxy-NAs are less hydrophobic. Unlike the statement made previously that removal of oxy-NAs was not feasible using ozonation alone (Islam 2014), the low-dose ozonation pretreatment in this study achieved a total oxy-NAs (O3 – 6) removal rate of 22.3%. However, subsequent biological treatment was still needed due to the large residual fraction of oxy-NAs after ozonation.

The results presented above indicate that the low-dose ozone pretreatment of OSPW substantially degraded classical NAs with more complex molecular structures, which are more recalcitrant to biodegradation. In addition, ozonation effectively degraded oxy-NAs.

5.2.2 Biodegradation of NAs by the MBRs

The degradation efficiencies of classical NAs and oxy-NAs by raw OSPW MBR, ozonated OSPW MBR and the combination of ozone and MBR are tabulated in Table 5-1.

Table 5-1. Removal rates of NAs with different oxygen atoms by raw OSPW MBR (r.MBR), ozonated OSPW MBR (o.MBR) and ozone + MBR hybridization.

NAs groups	Ox	Removal rate (%)		
		r.MBR	o.MBR	Ozone + MBR
Classical NAs	O2	37.6	49.7	94.0
Oxy-NAs	O3	0.1	34.4	50.4
	O4	39.2	35.6	55.2
	O5	35.0	35.2	-8.8
	O6	57.1	38.8	-30.6
	O3-6	23.9	35.4	43.9

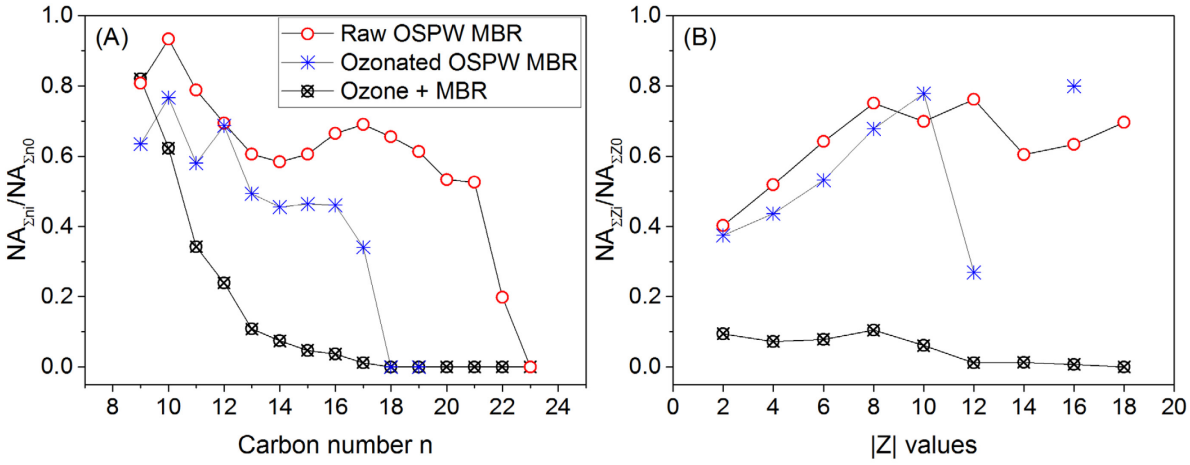


Figure 5-4. The normalized abundances of residual classical NAs with (A) different carbon numbers ($NA_{\Sigma ni}/NA_{\Sigma n0}$) and (B) different cyclicity $NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$ after r.MBR treatmen, o.MBR treatment and the combined process of ozone and MBR.

5.2.2.1 Classical NA degradation

Under the operating condition of 25 mg NH_4-N/L and 12-h HRT, the anoxic-aerobic MBR alone removed 37.6% and 49.7% of the total classical NAs from raw and ozonated OSPWs, respectively. This result indicates the remarkable enhancement effect of the mild-dose ozone pretreatment on subsequent MBR biodegradation. The combined process of ozone and MBR removed 94.0% of the total classical NAs from raw OSPW (Table 5-1). The half-life of classical NAs in the raw OSPW MBR is computed to be ~17.6 h, which is substantially shorter than the estimate of 44 – 240 days in a previous study (Han et al. 2008) and even negligible compared with the suggested ~13-year *in situ* half-life of NAs in tailing ponds (Han et al. 2009). And the low-dose ozone pretreatment further enhanced the overall removal of classical NAs, giving a half-life of ~12.1 hours.

To assess the biodegradation efficiency of NAs with different molecular carbon number (n) and hydrogen deficiency (Z), $NA_{\Sigma ni}/NA_{\Sigma n0}$ vs. n value and $NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$ vs. $|Z|$ value are charted in

Figure 5-4 A and B, respectively. $NA_{\Sigma ni}$ stands for the total concentration of NAs with the same *n* value of the permeate sample from process i (i.e., r.MBR, o.MBR or ozone + MBR); and $NA_{\Sigma n0}$ denotes the total concentration of NAs with the same *n* value of the untreated raw/ozonated OSPW. A higher $NA_{\Sigma ni}/NA_{\Sigma n0}$ ratio means a poorer NA degradation performance. Similarly, the meaning of $NA_{\Sigma |Z|i}/NA_{\Sigma |Z|0}$ could perceived. To further understand how ozone pretreatment, molecular carbon number (*n*), and hydrogen deficiency (*Z*) affected the degradation of classical NAs, Figure 5-5 and Figure 5-6 are generated based on the NAs concentrations profiles. Figure 5-5 A – I illustrate NA_{ni}/NA_{n0} ratios for each |Z| for different processes. NA_{ni} denotes the concentration of NAs with a certain *n* value in the permeated sample from the process i; and NA_{n0} denotes the concentration of NAs with a certain *n* value in the untreated raw/ozonated OSPW. Similarly, Figure 5-6 A to O are for $NA_{|Z|i}/NA_{|Z|0}$ ratios for each *n*.

As is shown in Figure 5-4 A, ozone pretreatment considerably improved the MBR's degradation efficiency of NAs with most *n* values, except *n* = 12. In Figure 5-5, the improvement effect of ozone pretreatment on biodegradation could be observed in charts A – H (*Z* = -2 to -16). The majority removed by the MBRs were NAs with C12 – 23, which is consistent with what has been found during the startup phase (Xue et al. 2016). Examination on Figure 5-5 indicates that NAs with more carbon numbers were generally better degraded within each *Z* series. The apparently negative correlation between carbon number and biodegradation rate is interesting compared with some previous studies conducted by Han et al. (2008) and Wang et al. (2013b) who concluded minor effect of carbon number (*n*) on biodegradation of NAs. NAs with higher molecular weights tend to accumulate on surfaces of activated sludge flocs and biofilm due to their higher non-polarity and stronger hydrophobicity as opposed to smaller organic molecules (Klamerth et al. 2015, Pourrezaei 2013, Zubot et al. 2012). Bacteria with hydrophilic surfaces

may excrete bio-surfactant and EPS to overcome limitations of bioavailability of hydrophobic compounds (Peltola 2008). And the enrichment of those large molecule NAs on bacterial cells might lead to better biodegradation (Parsons and Govers 1990).

Regarding the relatively high residual concentrations of NAs with smaller *n* values after biological treatment, it might be a result of the creation of smaller organic molecules from decomposition of organics with higher molecular weights. Some previous studies have reported similar results (Huang et al. 2015, Islam et al. 2014, Xue et al. 2016).

When it comes to NAs of different degrees of cyclization, the positive impact of ozone pretreatment on subsequent biodegradation was generally limited to species with a ring number < 5 ($|Z| < 10$) (Figure 5-4 B and Figure 5-6). For |Z| of 2 to 8, a negative correlation between ring numbers and biodegradation efficiency is demonstrated (e.g., linear with a $R^2 = 0.9993$ for the raw OSPW MBR) in Figure 5-4 B. Thus, the impact of cyclicity on NA biodegradation is stronger than that of carbon number *n*, which is in agreement with previously claimed conclusions (Han et al. 2008). NAs with more rings (i.e. 4 – 9 rings) were generally more recalcitrant to biodegradation within each *n* series, indicating a great consistence with previous studies (Clemente and Fedorak 2005, Huang et al. 2015, Hwang et al. 2013, Martin et al. 2010). Nevertheless, the raw OSPW MBR demonstrated a removal rate > 20% for NAs with $|Z| > 8$. In contrast, Wang et al. (2013b) observed unchanged concentrations of NAs with $|Z| > 8$ after 28 days of incubation.

It is therefore clear that the mild-dose ozone pretreatment remarkably enhanced subsequent MBR performance in terms of classical NA removal rate (49.7% vs. 37.6%), confirming the previously reported results (Martin et al. 2010, Shi et al. 2015). Moreover, based on Table 5-1, Figure 5-4,

Figure 5-5 and Figure 5-6, it is apparent that the combination of ozone + MBR completely or almost eliminated NAs with C17 – 23 and |Z| of 12 – 18, signaling the competence of this combined process for OSPW treatment.

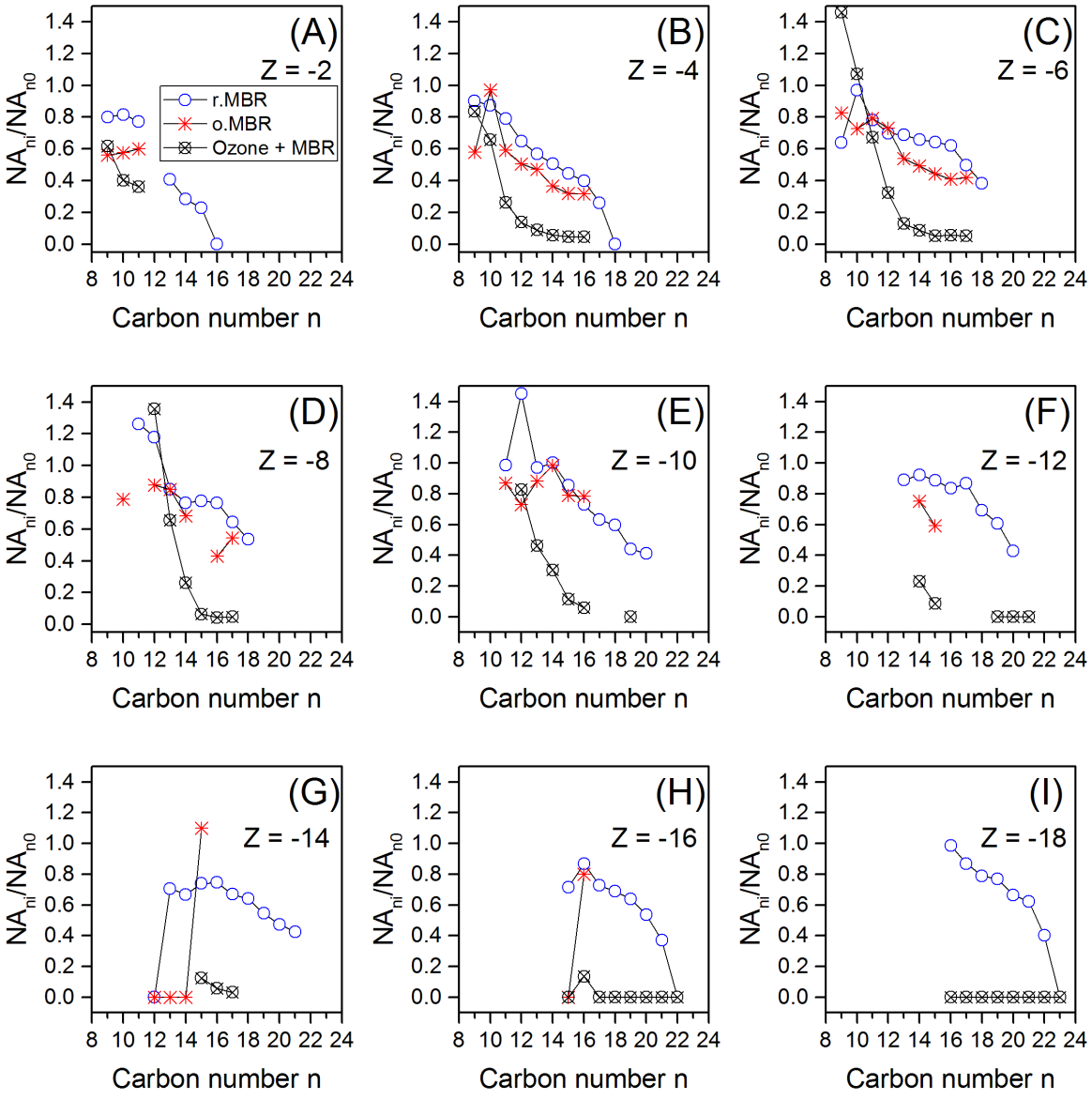


Figure 5-5. The relative remaining abundance of NAs with different carbon numbers (NA_{ni}/NA_{n0}) under each |Z| value.

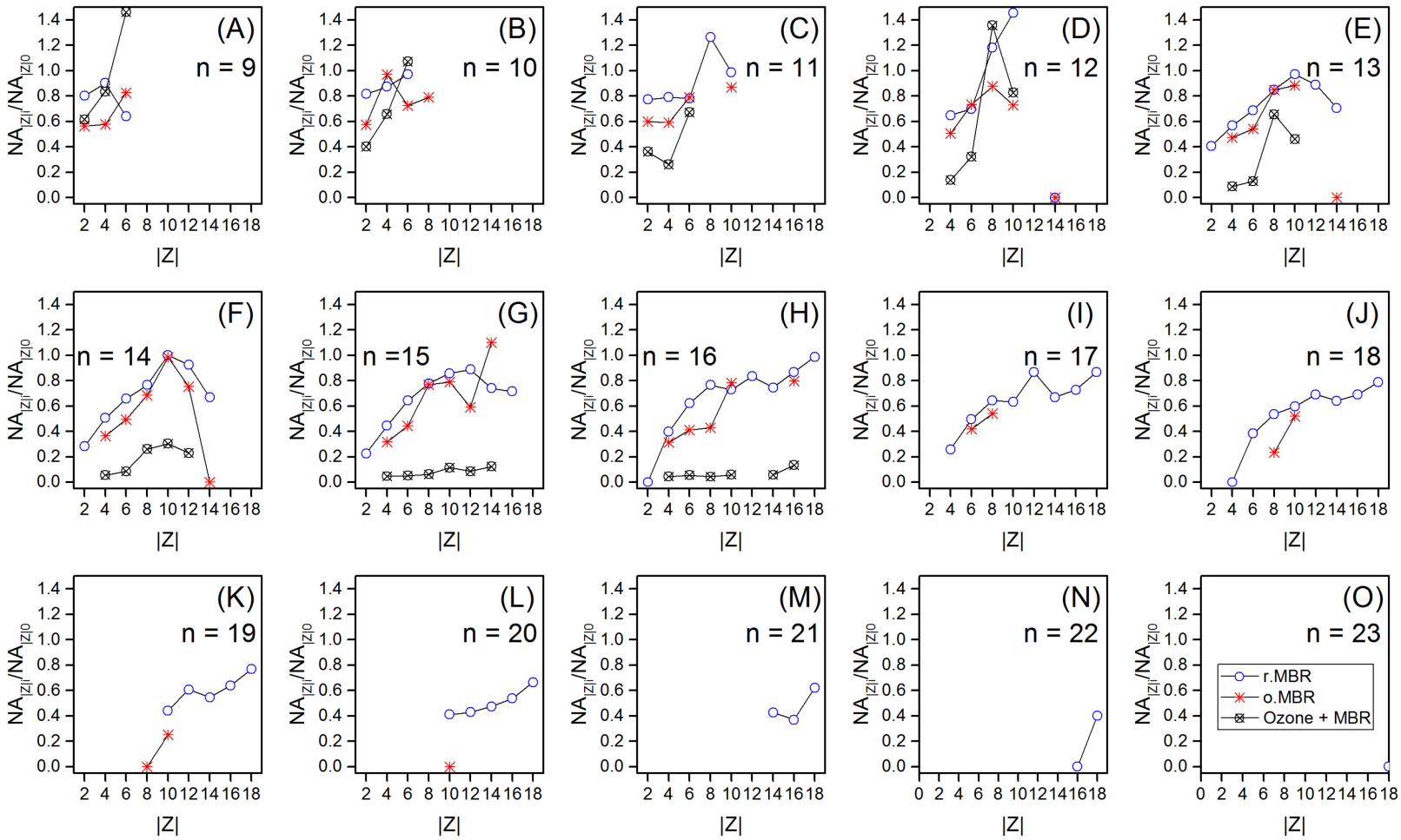


Figure 5-6 The relative remaining abundance of NAs with different carbon numbers (NA_{ni}/NA_{n0}) under each carbon number *n*.

5.2.2.2 Oxy-NA degradation

Regarding degradation of oxy-NAs, the ozone pretreatment made a remarkable improvement on overall biodegradation of the total oxy-NAs (35.4% vs. 23.9%). The enhanced O3 oxy-NA degradation in the ozonated OSPW MBR was impressive compared with its raw OSPW counterpart (34.4% vs. 0.1%, Table 5-1) taken into account that O3 oxy-NAs were reported to be quite recalcitrant to microorganisms (Headley et al. 2009). In fact, a concentration change of O3 or O4 oxy-NAs was detected neither in Han et al. (2008)'s 98-day incubation nor in Brown et al. (2013)'s 84-day biological treatment system. The considerable removal of O3 oxy-NAs in the ozonated OSPW MBR evidenced the positive influence of the mild-dose ozone pretreatment on subsequent MBR performance. With respect to the origin of oxy-NAs, it has been suggested that they are intermediates during biodegradation of classical NAs (Grewer et al. 2010, Han et al. 2009), among which O3 and O4 oxy-NAs are probably hydroxylated NAs (Han et al. 2008) and dicarboxylic acids (Headley et al. 2009), respectively.

Compared with the performance of MBR alone for raw OSPW treatment, the combined process of ozone and MBR demonstrated quite distinct oxy-NA degradation efficacy. The hybrid process removed more than 50% of O3 and O4; meanwhile generating more O5 and O6 species instead of degrading them. Nevertheless, the hybrid process still removed 43.9% of total oxy-NAs owing to the absolute dominance of O3 and O4 species among oxy-NAs in untreated raw OSPW (Figure 5-3). The mild-dose ozonation pretreatment preferrably decreased more O3 oxy-NAs with higher carbon numbers, potentially lessening the bio-persistence of O3 oxy-NAs (Figure 5-2). The increased O5 and O6 oxy-NAs after the ozone + MBR process might be a result of conversion of oxy-NAs with fewer oxygen numbers and even classical NAs.

5.2.3 Microbial communities

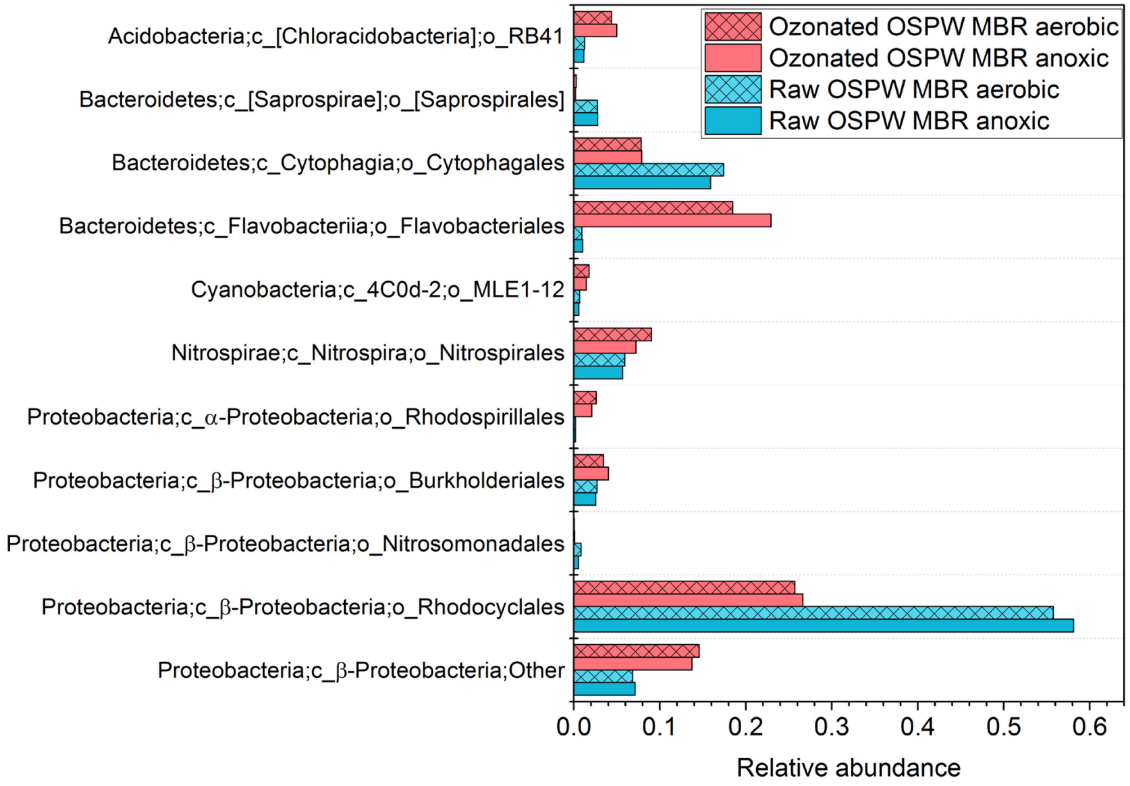


Figure 5-7. The 10 dominating taxonomic orders in the two MBRs under the optimized operating condition.

The 10 most abundant taxonomic orders in the anoxic and aerobic compartments of both the MBRs are displayed in Figure 5-7. Most of the microorganisms were commonly present in the two MBRs though their abundances differed. The abundances of an individual order in the anoxic and aerobic tanks of the same MBR were highly similar compared with the huge difference between the two MBRs. The phyla of *Proteobacteria*, *Nitrospirae*, and *Bacteroidetes* were dominating the communities. *Rhodocyclales*, *Cytophagales*, [*Saprospirales*] and *Nitrosomonadales* were more abundant in the MBR treating raw OSPW; whereas, the MBR treating ozonated OSPW harbored more other *β-Proteobacterial* orders, *Flavobacteriales*, *Nitrospirales*, *Rhodospirillales*, and *Burkholderiales*. Since ozonation dramatically change the

NAs composition in OSPW, it is therefore not unexpected to see changes in abundances of microorganisms that might be specialized in degrading different NAs species.

Regarding the potential functions of those dominating microorganisms detected, most of them have been documented to be involved in degradation of recalcitrant hydrocarbons. The dominance of *β-Proteobacteria* has been reported as NAs and aromatic hydrocarbon degraders in oil sands tailings ponds and Athabasca river sediments (Yergeau et al. 2012). *Rhodocyclales* and *Flavobacteriale* have been documented to be responsible for both of denitrification and degradation of recalcitrant hydrocarbons (Cao et al. 2009, Loy et al. 2005, Van den Tweel et al. 1988, Whitby 2010). In addition, *Cytophagales*, *Burkholderiales* and *Nitrosomonadales* has been linked to degradation of bio-persistent hydrocarbons (Bell et al. 2013a, Pérez‐Pantoja et al. 2012, Röling et al. 2002). In Chapter 4, *Rhodocyclales* and *Cytophagales* were found positively correlated to degradation of the majority fraction of OSPW NAs (C12 – 23). The substantially reduced large molecular weight NAs in the ozonated OSPW and lessened *Rhodocyclales* and *Cytophagales* in the MBR for ozonated OSPW treatment further evidenced the positive correlation between those two orders and degradation of NAs with higher carbon numbers. The remarkably higher abundances of *Flavobacteriales* in the ozonated OSPW MBR imply that those two groups of bacteria may potentially be correlated with degradation of NAs with low carbon numbers, which were the major NAs in ozonated OSPW.

To investigate the bacterial diversities of the two MBRs and the correlation of their microbial communities, Table 5-2 and Figure 5-8 are presented. It is not surprising to see the similar values of the two tanks in the same MBR given the continuous internal recirculation between the anoxic and aerobic tanks in the MBR. The lower Chao1, Shannon, Observed species and PD_whole_tree index values of raw OSPW MBR indicate the lower bacterial richness of the

microbial communities of that MBR. The higher bacterial richness in the ozonated OSPW MBR tanks might be a result of reduced toxicity of ozonated OSPW compared with raw OSPW (Gamal El-Din et al. 2011, Scott et al. 2008, Shi et al. 2015).

Table 5-2. Alpha diversity indices (3% cutoff) of the two MBRs under the optimized operating condition.

Sample	**Parameters** (N = 9934 sequences/sample)			
	Chao1	**Shannon**	**Observed species**	**PD_whole_tree**
r.MBR anoxic	422.4	4.35	319.7	17.77
r.MBR aerobic	456.5	4.35	338.3	18.47
o.MBR anoxic	495.5	5.50	438.9	20.49
o.MBR aerobic	607.3	5.69	495.7	23.61

a. Chao1: The species richness estimator, the total number of OTUs estimated in the community.
b. Shannon: An index characterizing the species diversity in a community. A higher value indicates higher diversity.
c. Observed species: The count of the unique OTUs in the sample.
d. PD whole tree: Faith's Phylogenetic Diversity based on the phylogenetic tree. It adds up all the branch lengths as a measure of diversity.

With respect to the correlation between the two MBRs' microbial communities, the two compartments of the same MBR showed an excellent similarity by sharing a great fraction of OTUs (Figure 5-8 A) and clustering along the PC1 axis (Figure 5-8 B). It is also noticed that 23.7% of the total OTUs were commonly found in all four tanks of the two MBRs. Taking into account the dominating microorganisms in Figure 5-7, it is suggested that the majority of the microbial richness stemmed from quantitatively rare microbial species. The discrepancy between the two MBRs in Figure 5-8 B clearly reveals the impact of ozonation on the microbial community structure of the MBR. It is therefore concluded that ozonation pretreatment remarkably affected the microbial community structure of the subsequent MBR by shifting relative abundances of the dominating species and encouraging more rare microbial species to enrich the community.

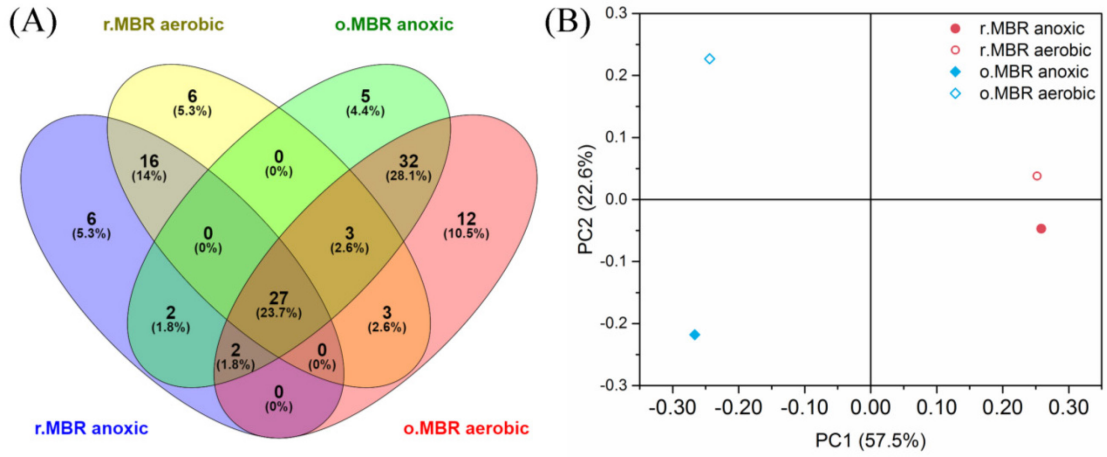


Figure 5-8. Correlation of microbial communities of the two MBRs under the optimized operating condition: (A) Venn diagram based on OTUs with a relative abundance of ≥ 0.1% in each sample; and (B) PCoA chart based on unweighted unifrac rarefaction.

5.3 Conclusions

The MBRs treating raw and ozonated OSPWs are compared for their OSPW NA degradation performance and microbial community structures. The mild-dose ozone pretreatment preferably degraded NAs with higher carbon numbers and more rings, consequentially increasing the bio-treatability of OSPW. The enhancement effect of ozone pretreatment on OSPW NA degradation was remarkable (49.7% vs. 37.6% of classical NA removal; 35.4% vs. 23.9% of oxy-NA removal). Removal rates of 94.0% and 43.9% were achieved by the combination of ozone and MBR process for total classical NAs and total oxy-NAs within 12 h. The MBR process demonstrated excellent efficiency on degrading NAs with high carbon numbers (e.g., C12 – 23) and high cyclization degrees (e.g., 4 – 9 rings) that are traditionally considered the most recalcitrant to microbes.

Illumina MiSeq sequencing analysis showed that ozonation also affected the MBR microbial community structures by shifting the relative abundances of the dominating microorganisms and

encouraging availability of numerically rare bacterial species. Dominating microorganisms, such as *Rhodocyclales, Nitrospirales, Burkholderiales, and Cytophagales*, were commonly found in the two MBRs. The outstanding OSPW NA (both classical and oxidized) degradation performance of the combination of ozone pretreatment and MBR suggests that this combined process is a promising candidate for future OSPW treatment on an industrial scale.

References

Bell, T.H., Yergeau, E., Maynard, C., Juck, D., Whyte, L.G. and Greer, C.W., 2013. Predictable bacterial composition and hydrocarbon degradation in Arctic soils following diesel and nutrient disturbance. The ISME journal 7(6), 1200-1210.

Brown, L.D., Pérez-Estrada, L., Wang, N., El-Din, M.G., Martin, J.W., Fedorak, P.M. and Ulrich, A.C., 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. Chemosphere.

Cao, B., Nagarajan, K. and Loh, K.-C., 2009. Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. Applied microbiology and biotechnology 85(2), 207-228.

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.

Gamal El-Din, M., Fu, H., Wang, N., Chelme-Ayala, P., Pérez-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. The Science of the total environment 409(23), 5119-5125.

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? The Science of the total environment 408(23), 5997-6010.

Han, X., MacKinnon, M.D. and Martin, J.W., 2009. Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS. Chemosphere 76(1), 63-70.

Han, X., 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.

Headley, J.V., Peru, K.M., Armstrong, S.A., Han, X., Martin, J.W., Mapolelo, M.M., Smith, D.F., Rogers, R.P. and Marshall, A.G., 2009. Aquatic plant‐derived changes in oil sands naphthenic acid signatures determined by low‐, high‐and ultra high‐resolution mass spectrometry. Rapid Communications in Mass Spectrometry 23(4), 515-522.

Huang, C., Shi, Y., El-Din, M.G. and Liu, Y., 2015. Treatment of oil sands process-affected water (OSPW) using ozonation combined with integrated fixed-film activated sludge (IFAS). Water research 85, 167-176.

Hwang, G., Dong, T., Islam, M.S., Sheng, Z., Pérez-Estrada, L.a., Liu, Y. and Gamal El-Din, M., 2013. The impacts of ozonation on oil sands process-affected water biodegradability and biofilm formation characteristics in bioreactors. Bioresource technology 130C, 269-277.

Islam, M.S., 2014. Combined Adsorption and Biodegradation Processes for Oil Sands Process-Affected Water Treatment. Doctoral, University of Alberta.
Islam, M.S., Moreira, J., Chelme-Ayala, P. and El-Din, M.G., 2014. Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Science of the total environment 493, 282-290.
Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and El-Din, M.G., 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. Science of the total environment 506, 66-75.
Loy, A., Schulz, C., Lücker, S., Stoecker, K., Baranyi, C., Lehner, A., Wagner, M., Lu, S. and Scho, A., 2005. Microarray for Environmental Monitoring of the Betaproteobacterial Order 16S rRNA Gene-Based Oligonucleotide Microarray for Environmental Monitoring of the Betaproteobacterial Order "Rhodocyclales". Applied and environmental microbiology 71(3), 1373-1373.
Martin, J.W., Barri, T., Han, X., 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.
Parsons, J. and Govers, H., 1990. Quantitative structure-activity relationships for biodegradation. Ecotoxicology and environmental safety 19(2), 212-227.
Peltola, R., 2008. Bioavailability aspects of hydrophobic contaminant degradation in soils.
Pereira, A.S., Islam, S., El-din, M.G. and Martin, J.W., 2013. Ozonation degrades all detectable organic compound classes in oil sands process-affected water ; an application of high-performance liquid chromatography / obitrap mass spectrometry. Rapid communications in mass spectrometry : RCM (27), 1-10.
Pérez-Estrada, L.A., Han, X., Drzewicz, P., Gamal El-Din, M., Fedorak, P.M. and Martin, J.W., 2011. Structure–reactivity of naphthenic acids in the ozonation process. Environmental science & technology 45(17), 7431-7437.
Pérez-Pantoja, D., Donoso, R., Agulló, L., Córdova, M., Seeger, M., Pieper, D.H. and González, B., 2012. Genomic analysis of the potential for aromatic compounds biodegradation in Burkholderiales. Environmental microbiology 14(5), 1091-1117.
Pourrezaei, P., 2013. Physico-chemical Processes for Oil Sands Process-affected Water Treatment, University of Alberta.
Röling, W.F., Milner, M.G., Jones, D.M., Lee, K., Daniel, F., Swannell, R.J. and Head, I.M., 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Applied and environmental microbiology 68(11), 5537-5548.
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.
Shi, Y., Huang, C., Rocha, K.C., El-Din, M.G. and Liu, Y., 2015. Treatment of oil sands process-affected water using moving bed biofilm reactors: with and without ozone pretreatment. Bioresource technology.
Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M. and Hagen, M., 2014. Advanced Analytical Mass Spectrometric Techniques and Bioassays to Characterize Untreated and Ozonated Oil

Sands Process-Affected Water. Environmental science & technology 48(19), 11090-11099.
Van den Tweel, W., Smits, J. and De Bont, J., 1988. Catabolism of dl-α-phenylhydracrylic, phenylacetic and 3-and 4-hydroxyphenylacetic acid via homogentisic acid in a Flavobacterium sp. Archives of microbiology 149(3), 207-213.
Wang, B., Wan, Y., Gao, Y., Yang, M. and Hu, J., 2013a. Determination and characterization of oxy-naphthenic acids in oilfield wastewater. Environmental science & technology 47(16), 9545-9554.
Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Garcia-Garcia, E., Pun, J., Martin, J.W., Belosevic, M. and Gamal El-Din, M., 2013b. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. Environmental science & technology 47(12), 6518-6526.
Whitby, C., 2010. Microbial naphthenic Acid degradation. Advances in applied microbiology 70, 93-125.
Xue, J., Zhang, Y., Liu, Y. and El-Din, M.G., 2016. Treatment of oil sands process-affected water (OSPW) using a membrane bioreactor with a submerged flat-sheet ceramic microfiltration membrane. Water research 88(1 January 2016), 1-11.
Yergeau, E., Lawrence, J.R., Sanschagrin, S., Waiser, M.J., Korber, D.R. and Greer, C.W., 2012. Next-generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities. Applied and environmental microbiology 78(21), 7626-7637.
Zubot, W., MacKinnon, M.D., Chelme-Ayala, P., Smith, D.W. and Gamal El-Din, M., 2012. Petroleum coke adsorption as a water management option for oil sands process-affected water. The Science of the total environment 427-428, 364-372.

6 Effect of mild-dose ozone pretreatment and operating conditions on membrane fouling behavior of an anoxic-aerobic membrane bioreactor for oil sands process-affected water (OSPW) treatment[7]

6.1 Introduction

To extract bitumen from the oil sands deposit in Northern Alberta, Canada, a large amount of water (2 – 4.5 barrels of water for a barrel of crude oil) is needed by the modified Clark Hot Water Extraction Process (Allen 2008b, Shell 2009). The consumed water during this extraction process is named oil sands process-affected water (OSPW), which is a complex mixture of suspended solids, salts, inorganic compounds, dissolved organic compounds (primarily composed of naphthenic acids, NAs), and trace metals (Allen 2008a, Kim et al. 2012, Scarlett et al. 2013, Wang et al. 2013a). According to a zero-discharge policy, oil producers are currently holding OSPW in huge surface tailings ponds. By the year of 2013, a net cumulative footprint of ~220 km^2 had been reportedly occupied by oil sands tailings ponds (containing ~976 million m^3 of fluid tailings) in the region (The Government of Alberta 2015). The enormous volumes of OSPW stored in the region is raising a wide public concern because of its impacts on the regional environment, including soil, water bodies, and wild life. The Alberta Government recently announced the *Tailings Management Framework*, further strengthening environmental protections in the oil sands region by restricting the accumulated amount of tailings water as well as pushing oil sands producers to invest in technology to treat tailings water (The Government of Alberta, 2015).

[7] This chapter has been submitted: Jinkai Xue, Yanyan Zhang, Yang Liu, Mohamed Gamal El-Din, 2016. Effects of ozone pretreatment and operating conditions on membrane fouling behaviors of an anoxic-aerobic membrane bioreactor for oil sands process-affected water (OSPW) treatment. Submitted to: Journal of Membrane Science.

As an efficient technology for municipal and industrial wastewater treatment, MBR has been gaining more popularity in recent years owing to its benefits over conventional activate sludge process, including smaller footprint, enriched biomass, reduced sludge production, and improved permeate quality (Le-Clech et al. 2006, Stephenson et al. 2000). In fact, the effectiveness of MBR in the treatment of OSPW was also demonstrated in our study with a NA removal of 37.6% from raw OSPW within 12 h (Chapter 4 and 5). However, wider application of MBRs is constrained by its major disadvantage- membrane fouling. Membrane fouling is a collective concept of a variety of phenomena that are caused by accumulation of rejected constituents (e.g., substrate components, cells, cell debris and microbial metabolites) in the retentate on the membrane surface or inside the membrane pores, and lead to a reduced permeate flux at a given trans-membrane pressure (TMP), or conversely an increased TMP at a constant permeate flux (Chang et al. 2002, Judd and Judd 2011). Consequences caused by membrane fouling include deteriorated system performance, declined permeate flux, high energy consumption, more frequent membrane cleaning and membrane replacement, and consequently higher cost (Chang et al. 2002, Gander et al. 2000, Meng et al. 2009). To develop strategies for membrane fouling control purpose, extensive studies have been made to better understand the mechanisms involved in membrane fouling development.

Typically, there are four basic mechanistic models used to describe membrane fouling, which are cake filtration, complete blocking, intermediate blocking, and standard blocking (Bolton et al. 2006, Kim et al. 2011b). In reality, fouling phenomenon involved during membrane filtration is usually more complex than a single fouling mechanism. A combination of different mechanisms may better describe the real fouling progress. The factors that affect membrane fouling could be categorized into four groups, including membrane materials (surface charges, hydrophobicity,

etc.), biomass properties (particle size, sludge viscosity, extracellular polymeric substances (EPS) production, etc.), feed water quality, and operating conditions (solid retention time (SRT), hydraulic retention time (HRT), permeate flux, food to microorganism ratio (F/M), etc.) (Le-Clech et al. 2006).

According to Meng et al. (2009), the membrane fouling behaviour of a given MBR is directly influenced by sludge characteristics and hydrodynamic conditions. Regarding effect of feed water quality and operating conditions, such as SRT, HRT, and F/M, they indirectly affect membrane fouling by altering sludge characteristics (Bouhabila et al. 2001, Han et al. 2005, Huang et al. 2001, Meng et al. 2009). Compared with the deposition of microbial cells, microbial metabolites (e.g., extracellular polymeric substances, EPS) released into the liquid matrix are considered as the major determinant of biofouling in MBRs. The soluble fraction of EPS is termed soluble microbial products (SMP). Both bound EPS and SMP are positively correlated to membrane fouling (Jarusutthirak and Amy 2006, Lin et al. 2009, Wang et al. 2009). It is suggested that HRT and organic loading rate (OLR) are the main operating factors affecting the generation of bound EPS through determining biomass growth and decay (Meng et al. 2009). HRT in fact governs F/M ratio and biomass concentration, and thus affects membrane fouling. It was reported that lower HRTs tend to result in increased membrane fouling owing to the increased membrane flux (Chae et al. 2006, Meng et al. 2009, Meng et al. 2007).

In addition to changing operating conditions for fouling control purpose, some pretreatment processes for feed water have proven their effectiveness on fouling mitigation, such as pH adjustment, coagulation, adsorption (e.g., powdered activated carbon and polymers), pre-oxidation etc. (Le-Clech et al. 2006, Zhou and Smith 2002). Despite the effectiveness of those methods, they have disadvantages of being practically complicated or introducing chemical

residuals to the water. For instance, coagulation may require pH adjustment first to maximize its performance, making the process complicated, and inevitably inducing addition of chemicals (Zhou and Smith 2002). It should also be noted that improper doses of polymer addition might lead to serious membrane fouling (Zhou and Smith 2002). Adsorption is not challenge-free, either. Saturated powdered activated carbon (PAC) needs to be regularly replaced with virgin PAC to maintain its fouling control performance (Le-Clech et al. 2006). As a powerful oxidizing agent, ozone is capable of oxidizing a wide spectrum of recalcitrant organic compounds without inducing foreign chemicals. However, to our best knowledge, there is limited published research on the effect of ozonation pretreatment on membrane fouling of MBRs,

The objectives of this chapter are: 1) to investigate the effect of ozonation pretreatment on membrane fouling behaviours of the anoxic-aerobic MBR treating OSPW; and 2) to examine how different HRTs affect membrane fouling in the two systems.

6.2 Materials and methods

6.2.1 Source waters and supplemental substances

OSPW used in this study was from the tailings ponds in Athabasca region. Only supernatants from the barrels were collected for analyses and treatment purpose. Water quality parameters are summarized in Table 6-1. Prior to use, the OSPW was stored in 200-litre polyethylene barrels in dark at 4 °C. Ozonation of raw OSPW was performed with an AGSO 30 Effizon ozone generator (WEDECO AG Water Technology, Herford, Germany). After 10-minute stabilization, a stable ozone concentration can be achieved in the feed gas. A ceramic fine bubble gas diffuser was installed at the bottom of a 200-litre reactor, where the feed gas was added into the liquid phase of raw OSPW. Two identical ozone monitors (HC-500, PCI-WEDECO) were adopted to monitor

ozone concentrations in the feed gas and off-gas lines continuously throughout the ozonation process in order to calculate utilized ozone dose. A utilized ozone dose of 30 mg/L was used in this study. At the end, the reactor was purged with nitrogen for 10 minutes to strip residual ozone from the reactor. Ozonated OSPW was also preserved in a 200-litre polyethylene barrel in dark at 4 °C prior to use. Sodium acetate (CH_3COONa) was supplied as an external carbon source at a constant organic loading rate (OLR) of 125 g COD/($m^3 \cdot d$) by continuously injecting a prepared stock solution in the system with a syringe pump at a pre-set rate throughout the whole study to facilitate microbial growth in the system. A mixture stock solution of KNO_3, NH_4Cl, and NaH_2PO_4 was also injected into the system with the same syringe pump to ensure the sufficiency of nitrogen and phosphorus. In addition, modified Bushnell-Haas medium (BHM) (Clemente et al. 2004) was supplemented in the system to provide essential trace metals. Detailed information on system start-up is available in our previously published report (Xue et al. 2016).

Table 6-1. Characteristics of raw and ozonated OSPWs.

Parameter	Unit	Raw OSPW	Ozonated OSPW
Temperature	°C	23±1	23±1
pH	--	8.8±0.1	8.6±0.2
COD	mg/L	199±19	185±12
NO_3-N	mg/L	1.1±0.6	1.1±0.5
NH_4-N	mg/L	5.5±1.3	4.5±1.1
Classical NAs	mg/L	48.9±4.6	10.7±1.3
TSS	mg/L	34.0±1. 5	38.9±0.2

6.2.2 Experimental setup and operating conditions

Two identical anoxic-aerobic membrane bioreactors have been performed in parallel for raw and ozonated OSPW treatment, respectively. The MBR configuration is depicted in Figure 2-1 in Chapter 2. The MBRs consisted of a feed tank, an anoxic tank, an aerobic tank equipped with an immersed membrane module, and a permeate tank. To maintain the homogeneity of the feed

water, a mixer was equipped in the feed tank. To ensure the suspension of mixed liquor, a stirrer blade was applied in the anoxic compartment. A flat sheet ceramic microfiltration membrane (Meidensha Corporation, Japan) with a pore size of 0.1 μm and an effective area of 128 cm^2 was submerged in the aerobic tank. Aeration was constantly performed to maintain suspension of mixed liquor in the aerobic tank as well as an aerobic dissolved oxygen (DO) level. DO concentrations in the anoxic tank and the aerobic tank were maintained at a level of < 0.3 mg/L and > 4.0 mg/L, respectively. A recirculation with a ratio of 2 was conducted from the aerobic tank to the anoxic tank through overflow. The system automatically switched to a 30-second backwashing after every 9.5 minutes of membrane filtration. Two flowrate meters and a pressure sensor were wired to a Yokogawa Daqstation DX1000 (Yokogawa Electric, Japan) recorder to continuously monitor the permeate flowrate, backwash flowrate and trans-membrane pressure (TMP). Based on the manufacturer's instructions, chemical cleaning (soaking the membrane module in 0.1% HClO solution for 1 h followed by tap water rinsing) was applied once TMP exceeded -35 kPa. Mixed liquor in the MBR was wasted through weekly sampling (30 mL from each tank for solids content measurements).

The system automatically switched to a 30-second backwashing after every 9.5 minutes of membrane filtration, making up a 10-minute working phase. The permeate pump was set at a constant speed to maintain a constant filtration flowrate of 2.58 mL/min during an 9.5 min filtration mode; while the backwashing pump was set to give a constant flowrate of 5.16 mL/min during a 0.5 min backwashing mode. Different HRTs were achieved through adjusting the relaxation duration between two working phases. The whole operation could be divided into 4 stages on the basis of HRTs as depicted in Table 6-2.

Table 6-2. Operating conditions and NA degradation rates at different stages.

Stage	Period	HRT	Real flux during filtration	Calculated SRT
	day	h	LMH [L/(m^2·h)]	day
I	1-630	48	12.1	280
II	630-680	72		
III	680-712	12		
IV	712-742	24		

6.2.3 Analytical methods

6.2.3.1 Fouling rate calculation and fouling model fittings

Total membrane filtration resistance was calculated using the following equation:

$$R_t = \frac{TMP}{\mu J}$$

where J is the membrane permeate flux (m^3/m^2/s), TMP is transmembrane pressure (Pa),μ is dynamic viscosity (Pa·s) of water, and R_t is the total membrane filtration resistance (m^{-1}).

Fouling rate was computed with the equation given below:

$$\Delta R = \frac{R_{t1} - R_{t0}}{\Delta t}$$

where Δt is filtration time (h) between the membrane filtration resistance value R_{t1} and R_{t0}.

The fouling models for constant flow membrane filtration were used in this study. Detailed information on those models are available elsewhere (Bolton et al. 2006, Kim et al. 2011b).

6.2.3.2 Sludge liquor characterization

To measure the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentration (an indicator of microbial growth in the MBR), a 30 mL sludge sample was collected weekly from the middle depth of the anoxic and aerobic tank of each MBR by using a disposable syringe. Standard Method was followed for MLSS and MLVSS

measurement. Because of the recirculation between the anoxic and aerobic tanks, average values of the concentrations in the two tanks of each MBR were calculated for MLSS and MLVSS.EPS was extracted with a method derived from Liu and Fang (2002)'s approach. 10 mL of sludge sample was collected from each tank of the two MBRs for EPS extraction. 60 μL of formaldehyde (36.5%) was added into the sample that was then left in a 4 °C fridge for 1 h. The sample was then kept in a 4 °C fridge for 3 h after 4 mL of 1 N NaOH was added in. Centrifugation at 13000 G at 4 °C for 20 min was conducted. The supernatant was filtered through a 0.45 μm syringe filter. As for EPS on membrane surfaces, 1/2 of the fouling layer on one side of a fouled membrane was carefully scraped off with a plastic spatula and collected in a 50 mL centrifuge tube. The obtained fouling layer material was then dissolved in 10 mL of demineralized water for subsequent EPS extraction procedures. A Thermo Scientific Micro BCA™ Protein Assay Kit was used for quantification of protein in the processed samples. The manufacturer's instructions were followed. The phenol-sulfuric acid method was used for polysaccharides measurement (DuBois et al. 1956).

In addition, the floc size distribution of sludge liquor was determined with a Malvern Mastersizer 3000 instrument (Worcestershire, UK) with a detection range of 100 nm to 3 mm. Each sample was measured in pentaplicate and an average value is reported in this article.

6.2.3.3 Membrane fouling layer characterization

a. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) was performed to characterize the deposited microbial cells and EPS on fouled and chemically cleaned membrane surfaces. The membrane specimens (1 cm × 0.5 cm) were stained with SYTO 9 (Molecular Probes, USA) and Concanavalin A (Molecular Probes, USA). SYTO 9 (excitation wavelength (ex) = 488 nm;

emission wavelength (em) = 522/32 nm) and ConA conjugated with Texas Red (ex = 568 nm; em = 605/32 nm) are used to stain bacterial cells and EPS, respectively. And then the stained membrane specimens were imaged with a confocal laser scanning microscope (Zeiss LSM 710, Carl Zeiss Micro Imaging GmbH, Germany), which was equipped with a spectral detector that had a PMT (photon multiplier tube) array and optical grating elements to monitoring stained cells. Imaris 8.1 by Bitplane was used to process the raw CLSM images. Relative areal abundance of each stained component on each membrane specimen was measured with ImageJ 1.46r.

b. Scanning electron microscopy (SEM) - Energy Dispersive X-ray (EDX) spectroscopy

At the end of the operation, the fouled membranes were broken into pieces for autopsy analyses. Two pieces (~1 cm L × 0.5 cm W) with intact cake-layer on the surface were selected for scanning electron microscopic (SEM) analysis, one of which was chemically cleaned by soaking in 0.1% HClO solution for 1 h and then rinsing with running demineralized water. Thereafter the fouled and the chemically cleaned membrane specimens were fixed by submerging them in a mixture of 2.5% glutaraldehyde and 1% osmium tetraoxide in PBS. The fixed specimens were then dehydrated with serial concentrations of ethanol (50, 70, 90 and 100%, v/v) for 10 minutes each. A Bal-Tec CPD 030 critical point dryer and an Edwards S150B sputter coater were used to dry the samples and coat them with a thin layer of gold. A Zeiss Sigma 300 VP-FESEM coupled with a Bruker EDX spectroscopy was used to obtain SEM images of the specimen surfaces at the voltage of 15 kV.

6.2.3.4 Illumina MiSeq sequencing

Detailed information on the materials and methods used for DNA extraction is available elsewhere (Xue et al. 2016). DNA extraction from cake layers on membrane surfaces was done

with duplication. For each replicate, 1/4 of the fouling layer on one side of a fouled membrane (double-sided) was meticulously scraped off using a spatula for DNA extraction. A PowerSoil® DNA Isolation Kit from Mo-Bio Laboratories, Inc. (CA, USA) was used to extract DNA from collected sludge and cake layer samples according to the manufacturer's manual. Extracted DNA was eluted to a final volume of 100 μL and stored at -20 °C. DNA samples were diluted to a concentration of ~20 ng/μL and a volume of ~30 μL, and then sent to a commercial laboratory (Research and Testing Laboratory, Texas, US) for Illumina MiSeq sequencing. Samples were sequenced using Illumina MiSeq sequencing instruments and reagents according to the manufacturer's guidelines. Primers used for the amplification of 16S rRNA V1-V2 hypervariable regions prior to MiSeq sequencing were 28F (GAGTTTGATCNTGGCTCAG) and 388R (TGCTGCCTCCCGTAGGAGT). Raw sequence data were processed with the Quantitative Insights Into Microbial Ecology (QIIME, http://qiime.org) software package with default settings (Caporaso et al. 2010). Operational taxonomic units (OTUs) were picked through using open-reference clustering algorithm. A similarity of 97% was used for sequence clustering. Both α diversity (diversity within a sample) and β diversity (diversity among a group of samples) were performed based on the OTU table generated. Parameters including Chao1, Shannon, observed species, and phylogenetic diversity whole tree (PD_whole_tree) were computed to measure α diversity. And β diversity was conducted through building weighted and unweighted UniFrac distance matrices on the basis of a phylogenetic tree. Principal coordinate analysis (PCoA) was performed to examine the correlations among microbial communities from different samples. A Venn chart was generated using an online tool (Oliveros 2007).

6.2.3.5 Statistical analysis

The Pearson product-moment correlation coefficient (PMCC, r_p) was used to estimate linear correlations. The coefficient r_p is a numerical value within the range of -1 and 1 that expresses the strength of the linear relationship between two parameters. An r_p closer to 1 indicates a strong positive correlation while an r_p of 0 signalizes that there is no relationship. Values closer to -1 indicate a strong negative relationship between the two variables. Correlations at a 95% confidence interval ($P < 0.05$) are regarded statistically significant.

6.3 Results

6.3.1 Effect of the mild ozonation pretreatment on OSPW water quality

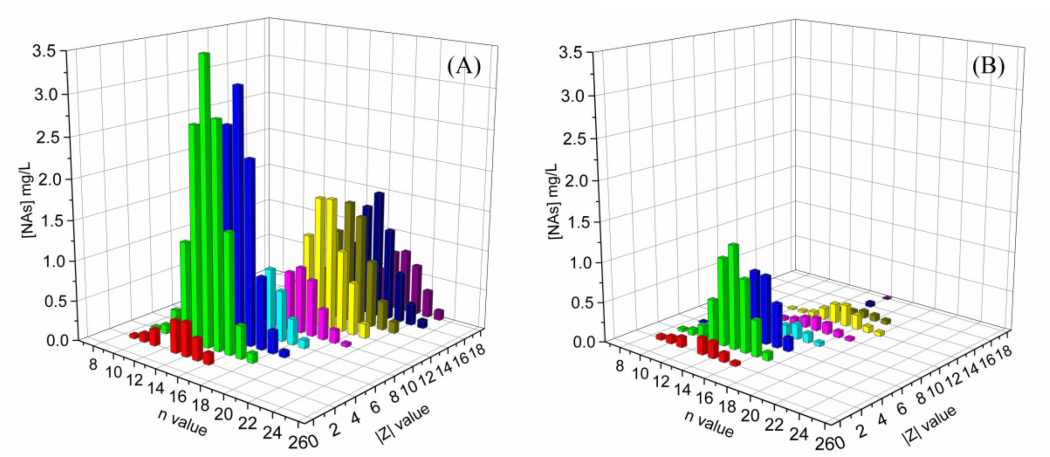


Figure 6-1. Classical NAs concentrations profiles of (A) raw OSPW and (B) ozonated OSPW.

Figure 6-1 displays the concentrations profiles of classical NAs of raw OSPW (A) and ozonated OSPW (B). The low-dose (utilized dose 30 mg O_3/L) ozonation treatment successfully removed over 50% of total classical NAs from the raw OSPW. To better illustrate how ozonation affect the degradation of NAs with different molecular structures, Figure 6-2 is presented, which shows the $NA_{\Sigma ni}/NA_{\Sigma n0}$ ratio for each *n* and the $NA_{\Sigma|Z|i}/NA_{\Sigma|Z|0}$ ratio for each |Z|. The subscript i

indicates ozonated OSPW, whereas the subscript 0 referes to raw OSPW. According to Figure 6-2 A and B, ozonation effectively reduced concentrations of all detected classical NAs of raw OSPW except those with C9 (n = 9). NAs with higher carbon numbers and higher cyclicity were more effectively removed. Both NAs with C20 – 22 and 8 – 9 rings were almost completely removed by ozonation. Unlike the remarkable decrease in NAs concentrations, the ozonation pretreatment did not reduce the TSS concentration of the raw OSPW (Table 6-1).

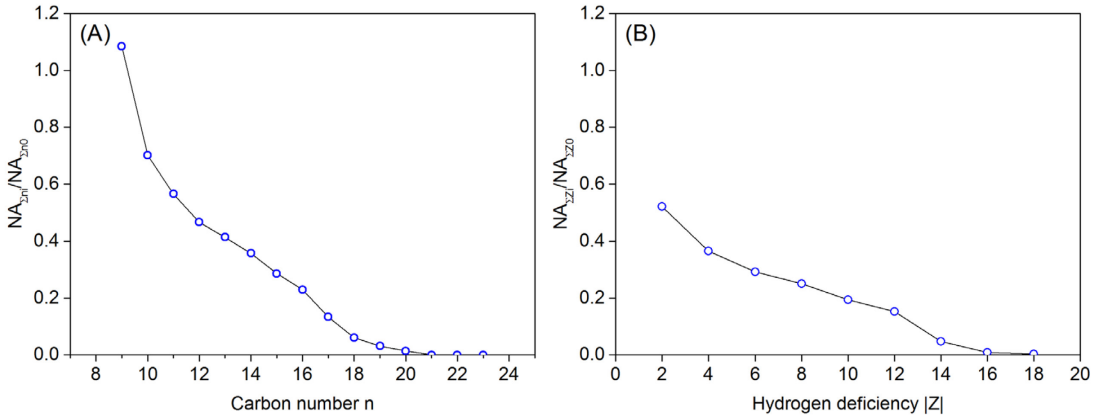


Figure 6-2. The normalized abundances of classical NAs with (A) different carbon numbers ($NA_{\Sigma ni}/NA_{\Sigma n0}$) and (B) different hydrogen deficiencies ($NA_{\Sigma |Z|i}/NA_{\Sigma |Z|0}$) after ozonation pretreatment.

6.3.2 Overall treatment performance and membrane fouling development of the two MBRs

HRTs of 48, 72, 12 and 24 h were applied chronologically for MBR operation as shown in Table 6-2. The total classical NA degradation rates for 48-h, 72-h, 12-h, and 24-h HRTs were 33.7%, 26.8%, 37.6%, and 36.0%, respectively, in the raw OSPW MBR; whereas the ozonated OSPW MBR achieved NA degradation rates of 37.0%, 39.6%, 50.0%, and 32.6%, respectively. The HRT of 12 h was considered as the optimal operation condition based on the system's NA degradation performance and efficiency. The hybrid process of ozonation pretreatment and MBR biological treatment attained a NA removal rate of 94.0% with an HRT of 12 h.

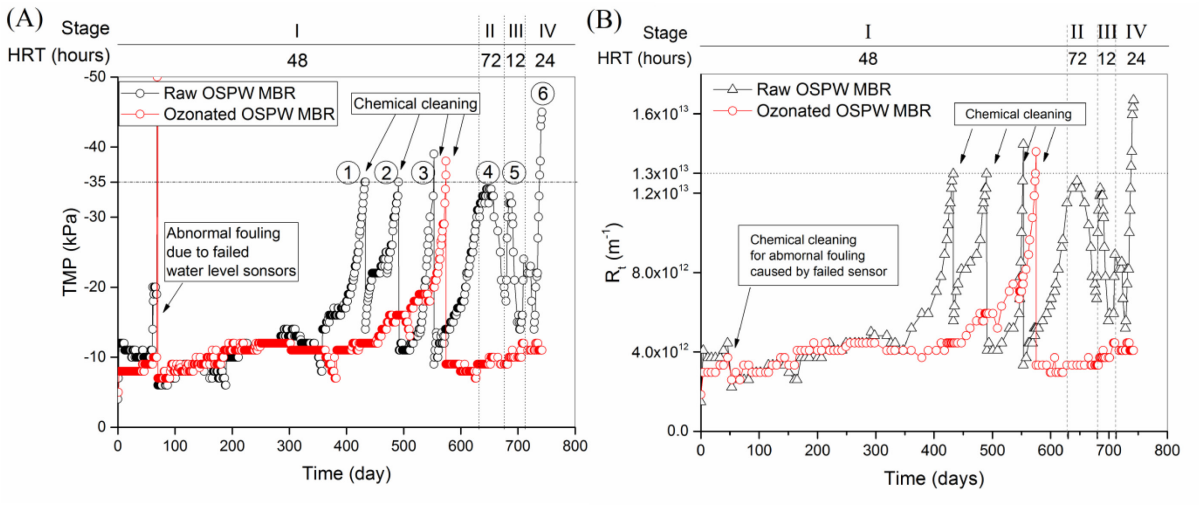


Figure 6-3. (A) TMP over time and (B) R_t over time of the two MBRs.

Since different HRTs were achieved through changing the relaxation length between two working phases, a constant membrane filtration flux of 10.4 LMH ($L/m^2/h$) was maintained for filtration throughout the whole operation. Thus, TMP changes were directly affected by membrane fouling. Figure 6-3 A is presented for TMP changes over time of the two MBRs. To better demonstrate membrane fouling development, the changes of R_t over time is plotted for the MBR treating raw OSPW and ozonated OSPW in Figure 6-3 B. The initial TMPs in the raw OSPW MBR and ozonated OSPW MBR were -4 kPa and -5 kPa, respectively. The two MBRs witnessed a rapid rise of TMP within one week right after the inoculation, which was caused by conditioning fouling due to initial pore blockage and adsorption of solutes. Both of the MBRs had an abnormal TMP on around Day 70 of the operation due to failure of level sensors in the anoxic tank which was fouled by inoculum MFT bitumen. A chemical cleaning process was performed to restore the membrane permeability to a TMP of ~ -6 kPa in each MBR on Day 70. Thereafter, a slow accretion in TMP was observed from Day 70 to 347 in the raw OSPW MBR and from Day 70 to 447 in the ozonated OSPW MBR, respectively, before a steep rise occurred in each MBR.

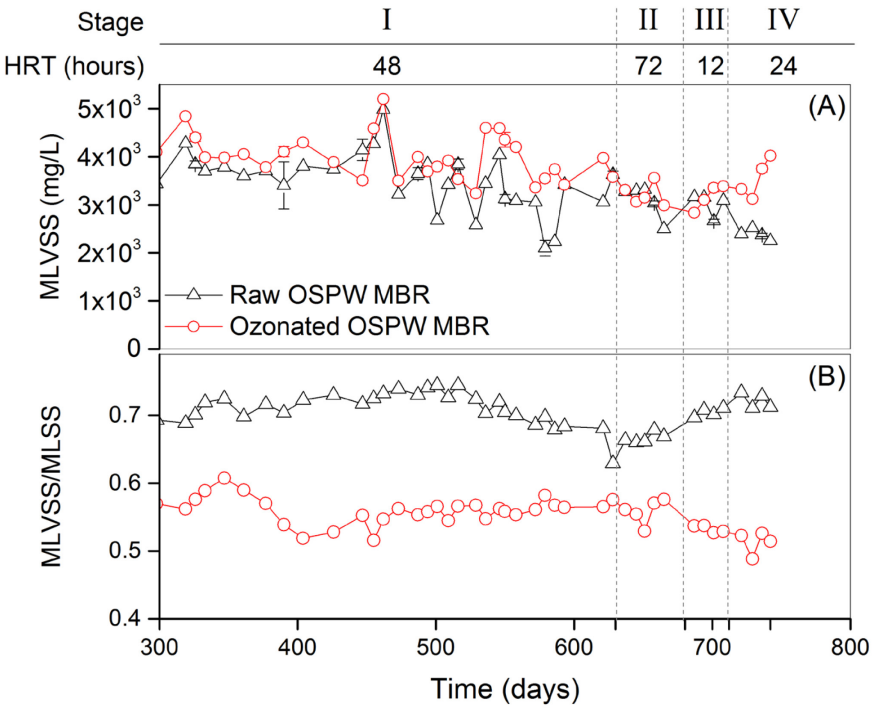


Figure 6-4. MLVSS and MLVSS/MLSS ratio over time of the two MBRs.

The whole 742 days of continuous operation witnessed severe fouling (|TMP| ≥ 35 kPa, Rt ≥ 1.3×10^{13} m^{-1}) for 4 times in the raw OSPW MBR, which were on Day 433, 490, 553, and 737. In contrast, the ozonated OSPW MBR only experienced severe fouling once, which was on Day 575. Interestingly, a spontaneous decline of TMP was noticed after a period of slow TMP growth in each MBR. Once dropped to a certain level, the TMP quickly return to its originally expected path. From Day 358 to 433 (75 days), TMP in the raw OSPW MBR climbed from -14 kPa to -35 kPa (the critical TMP requiring a chemical cleaning). Compared to the raw OSPW MBR, it took the TMP in the ozonated OSPW MBR 121 days (Day 452 to 573) to grow from -12 kPa to -35 kPa. Although it took the raw OSPW MBR 433 days to reach the first severe membrane fouling, it had been severely fouled more frequently ever since the first fouling as is shown in Figure 6-3 A. In addition, the TMP in the raw OSPW MBR was found to be fluctuating vigorously with

high peaks close to the critical line over Stage III, and IV. In contrast, the TMP in the ozonated OSPW MBR was maintained at a slow accruing status after the chemical cleaning on Day 575. In order to explore the role of ozonation in anti-fouling performance, the sludge and cake layer samples in both MBRs were characterized in the following context.

6.3.3 Sludge liquor characterization of the two MBRs

6.3.3.1 Biomass concentrations

Figure 6-4 is made to illustrate biomass concentration (MLVSS) and the MLVSS/MLSS ratio over the period of Day 300 to 742 in each MBR. MLVSS concentration over time prior to Day 300 is reported elsewhere (Xue et al. 2016, Zhang et al. 2016). As internal recirculation was performed between the aerobic and anoxic tanks, the reported biomass values are calculated by averaging concentrations in the two tanks of each MBR. Along the operation time, MLVSS fluctuated around ~3304 mg/L in the raw OSPW MBR, and ~3776 mg/L in the ozonated OSPW MBR (Figure 6-4). However, the difference between the two MBRs' MLVSS concentrations over time was insignificant. MLVSS/MLSS ratio is used as an indicator of the fraction of viable sludge in a biological system. Based on the typical cell composition, the VSS fraction of the total suspended solids is approximately 0.85 (Metcalf et al. 2003). According to von Sperling (2007), this ratio could be as low as 0.60 under certain circumstances. It is found that the MLVSS/MLSS ratios in the raw OSPW MBR and the ozonated OSPW MBR were ~0.63 and ~0.55, respectively, notwithstanding some minor fluctuations over time. T-test showed the MLVSS/MLSS of the ozonated OSPW MBR was significantly lower than that of the raw OSPW MBR ($P = 3.0 \times 10^{-15} < 0.05$), indicating a higher amount of inert solids in the ozonated OSPW MBR. Although low MLVSS/MLSS ratios are expected to result in accumulation of inert compounds in the system (Judd and Judd 2011), this might not be the case in this study based on

the relatively stable MLVSS/MLSS curves in Figure 6-4, which is consistent with what has been found in a previous study (Huang et al. 2001).

6.3.3.2 Total EPS and SMP concentrations

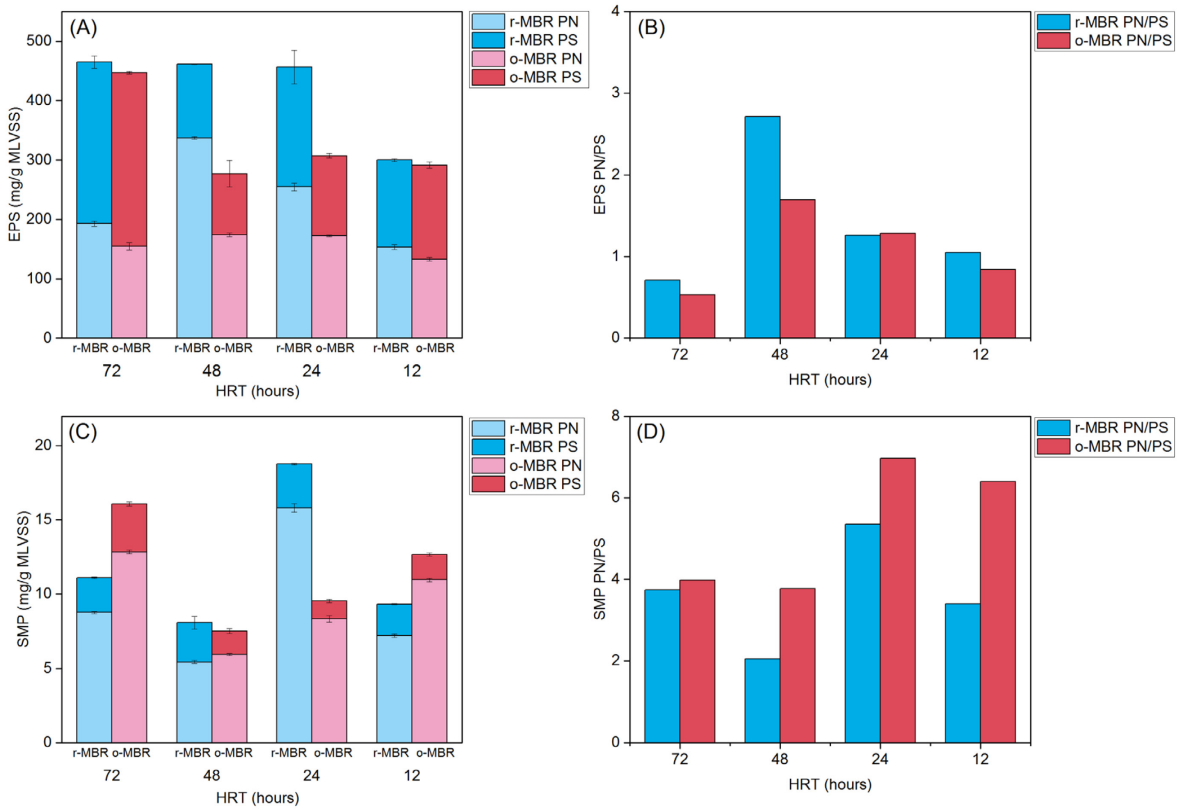


Figure 6-5. EPS and SMP measurement of the two MBRs at different HRTs: (A) EPS concentrations; (B) EPS PN/PS; (C) SMP concentrations; and (D) SMP PN/PS.
(Note: r.MBR refers to raw OSPW MBR; o.MBR refers to ozonated OSPW MBR; PN stands for protein; and PS denotes polysaccharides.)

Total EPS and SMP concentrations were measured for mixed liquor from each MBR at each stage (Figure 6-5). Over the whole operation period, significantly ($P = 0.03 < 0.05$) higher total EPS (protein + polysaccharides) concentrations were observed in the raw OSPW MBR than the ozonated OSPW MBR. As opposed to the different total EPS concentrations, the difference in SMP (protein + polysaccharides) concentrations of the two MBRs over time was not significant

(P = 0.46 > 0.05). Protein to polysaccharides ratio (PN/PS) is used to characterize EPS composition. The raw OSPW MBR constantly showed higher EPS PN/PS than the ozonated OSPW MBR; whereas latter demonstrated higher SMP PN/PS over the four stages than the former.

6.3.3.3 Particle size distributions (PSD)

At the end of the operation, PSD of mixed liquor in each MBR was characterized and summarized in Table 6-3.

Table 6-3. PSD of sludge liquor in the two MBRs at Stage IV (HRT = 24 h).

Sample	D_{10}	D_{50}	D_{90}	Mode
	μm	μm	μm	μm
Raw OSPW MBR aerobic tank	36.5±0.14	86.5±0.38	171.0±2.15	92.0±0.10
Ozonated OSPW MBR aerobic tank	25.4±0.18	66.5±0.65	142.0±2.93	71.3±0.07

Note: D_x indicates the diameter where x percent of the distribution has a smaller particle size and (100-x) percent has a larger particle size.

6.3.4 Membrane fouling layer characterization of the two MBRs

6.3.4.1 Total EPS densities

The density of total EPS on membrane surface upon an occurrence of severe membrane fouling was measured and depicted in Figure 6-6 A. It should be noted that the ozonated OSPW MBR membrane was measured of EPS density on Day 742 the last day of the operation though it had not exceeded the critical TMP of -35 kPa. A comparison of PN/PS at each MBR's respective first occurrence of severe membrane fouling indicates that the ratio of the raw OSPW MBR was apparently higher than that of the ozonated OSPW MBR (1.7 vs. 0.7) (Figure 6-6 B). On the last day (Day 742) of the operation, the raw OSPW MBR membrane was severely fouled while the other's membrane was not. Their PN/PS ratios were 2.7 and 2.0, respectively (Figure 6-6 B).

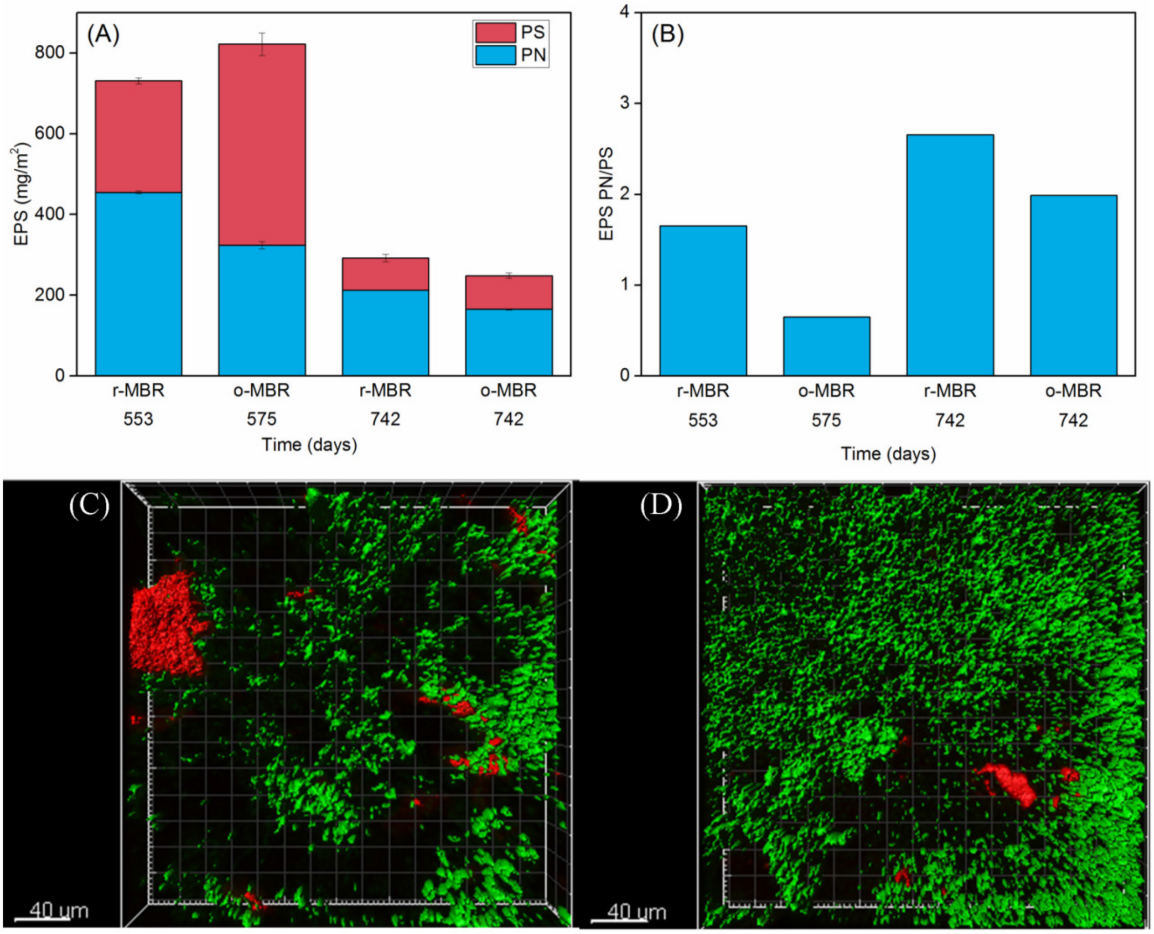


Figure 6-6. EPS measurement and CLSM images of surfaces of fouled membrane specimens obtained at the end of the operation: (A) EPS densities on fouled membrane surfaces of the two MBRs; (B) EPS PN/PS ratios of fouled membranes surfaces of the two MBRs; (C) CLSM image of severely fouled (TMP of -45 kPa) raw OSPW MBR membrane, and (D) CLSM image of fouled ozonated (TMP of -11 kPa) OSPW MBR membrane.

Table 6-4. EPS and live cell areas on membrane surfaces at Stage IV (HRT = 24 h).

MBR	Membrane condition	EPS (%)	Live cells (%)
Raw OSPW MBR	Severely fouled	2.9%	15.4%
Ozonated OSPW MBR	Fouled	0.8%	31.4%

6.3.4.2 CLSM images

Figure 6-6 C and D are presented for the CLSM images of fouled membrane surfaces of the two MBRs at the end of the operation. Relative areas (in percentage of the whole imaged region) of

the two stained component on each membrane specimen are tabulated in Table 6-4. Through scrutinizing Figure 6-6 C, D, and Table 6-4, it is apparent that the amount of EPS on the raw OSPW MBR membrane surface was triple as much as that on the ozonated OSPW MBR membrane surface did (2.9% vs. 0.8%); whereas much more bacterial cells were attached on the ozonated OSPW MBR membrane compared with the raw OSPW MBR membrane (31.4% vs. 15.4%). The CLSM images suggest that the biological fouling layers from the two MBRs differed substantially in terms of cake layer composition and density.

6.3.4.3 SEM images and EDX spectra

Figure 6-7 illustrates the surfaces of pristine membrane, the severely fouled and chemically cleaned raw OSPW MBR membrane, and the fouled and chemically cleaned ozonated OSPW MBR membrane. The membranes in both of the MBRs were covered by fouling deposits (Figure 6-7 C and E). The fouling layer on the raw OSPW MBR membrane in Figure 6-7 C looks denser than that in Figure 6-7 E, which is in accordance with the higher TMP (R_t) observed for raw OSPW MBR at the end of the operation. Curved and rod- shaped bacteria could be recognized in both Figure 6-7 C and E. More curved-shaped bacteria were observed in fouled membrane when treating raw OSPW compared with ozonated OSPW (Figure 6-7 C and E). Figure 6-7 D and F clearly show the chemically irreversible fraction of membrane fouling. Figure 6-7 D indicates that some pores were clogged by fine colloids and microbial metabolites. In contrast, Figure 6-7 F shows an embedment mechanism occurred between the membrane material and foulants.

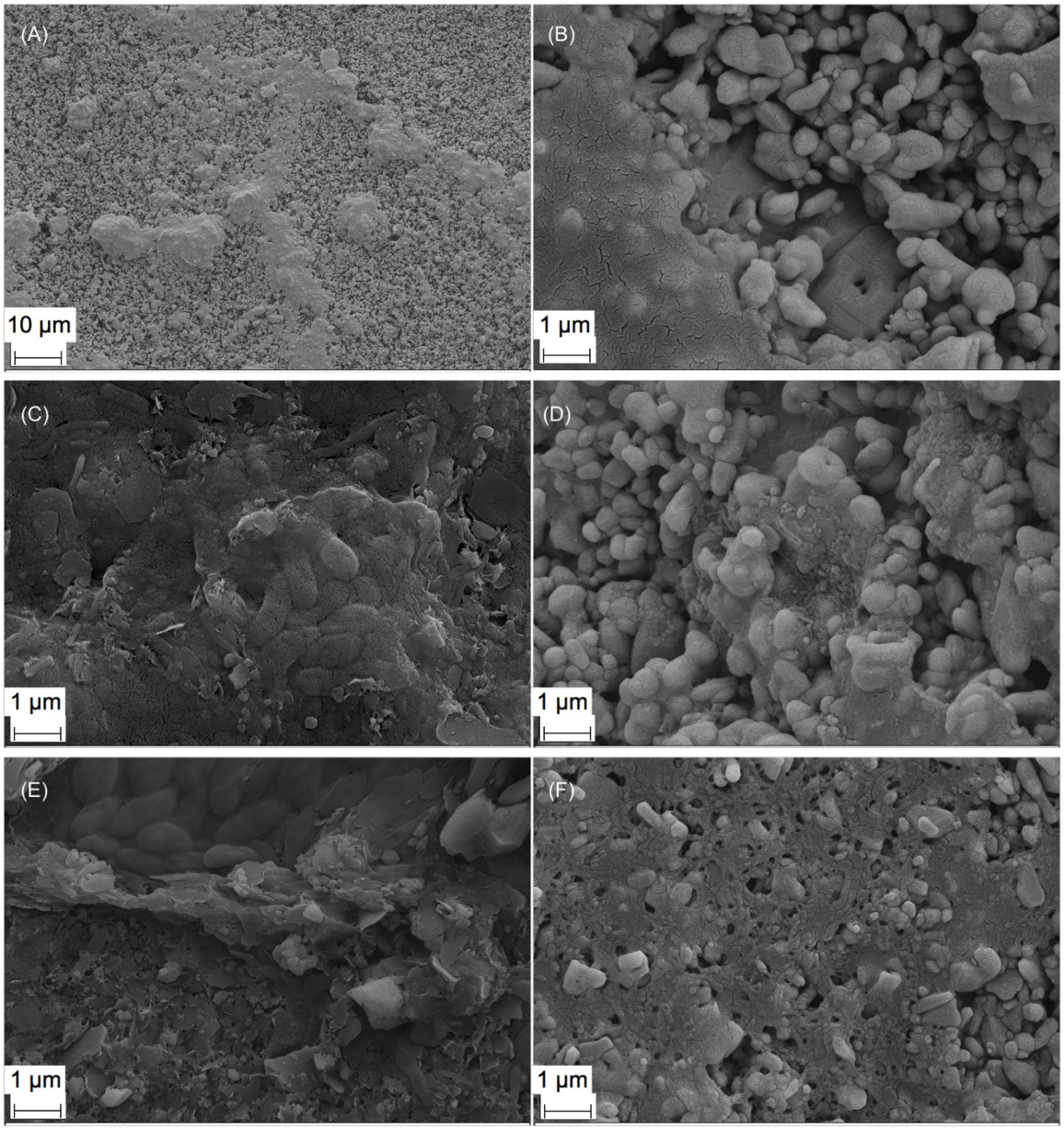


Figure 6-7. SEM images of surfaces of membrane specimens obtained at the end of the operation: (A) Pristine membrane by 1 KX magnification, (B) Pristine membrane by 10 KX magnification, (C) Severely fouled Raw OSPW MBR membrane by 10 KX magnification, (D) Chemically cleaned Raw OSPW MBR membrane by 10 KX magnification, (E) Fouled Ozonated OSPW MBR membrane by 10 KX magnification, and (F) Chemically cleaned Ozonated OSPW MBR membrane by 10 KX magnification.

EDX analysis of the membrane surfaces (Table 6-5) revealed that compositions of organic (C, O) and inorganic (Si, Al, Na, Fe, Mg, K, Cl, etc.) foulants. Intensive signal of Si was detected on both of the fouled membranes, which might come from SiO_2 in OSPW. It is found that the

membrane from the raw OSPW MBR was covered with foulants with higher C, N, Fe and Ca densities than that of the ozonated OSPW MBR.

Table 6-5. EDX analysis results: compositions of the membrane fouling layers.

Elements	Relative abundance of each element (wt. %)				
	Pristine membrane	Raw OSPW MBR membrane		Ozonated OSPW MBR membrane	
		Severely fouled	Chemically cleaned	Fouled	Chemically cleaned
O	20.4	30.1	31.6	41.7	28.5
C	32.6	30.1	27.1	26.1	21.1
Al	47.0	8.0	44.2	7.4	50.4
Si	-	16.8	-	17.8	-
Na	-	1.2	-	2.4	-
Fe	-	6.9	-	2.2	-
Cl	-	0.5	-	1.2	-
K	-	1.3	-	0.7	-
Mg	-	0.5	-	0.6	-
Ca	-	0.6	-	-	-
N	-	4.0	-	-	-

6.3.5 Microbial community characterization

Figure 6-8 is created to illustrate the microbial structure characterization results of four samples collected at Stage IV, including the raw OSPW MBR aerobic sludge, raw OSPW MBR cake layer, ozonated OSPW MBR aerobic sludge, and ozonated OSPW MBR cake layer.

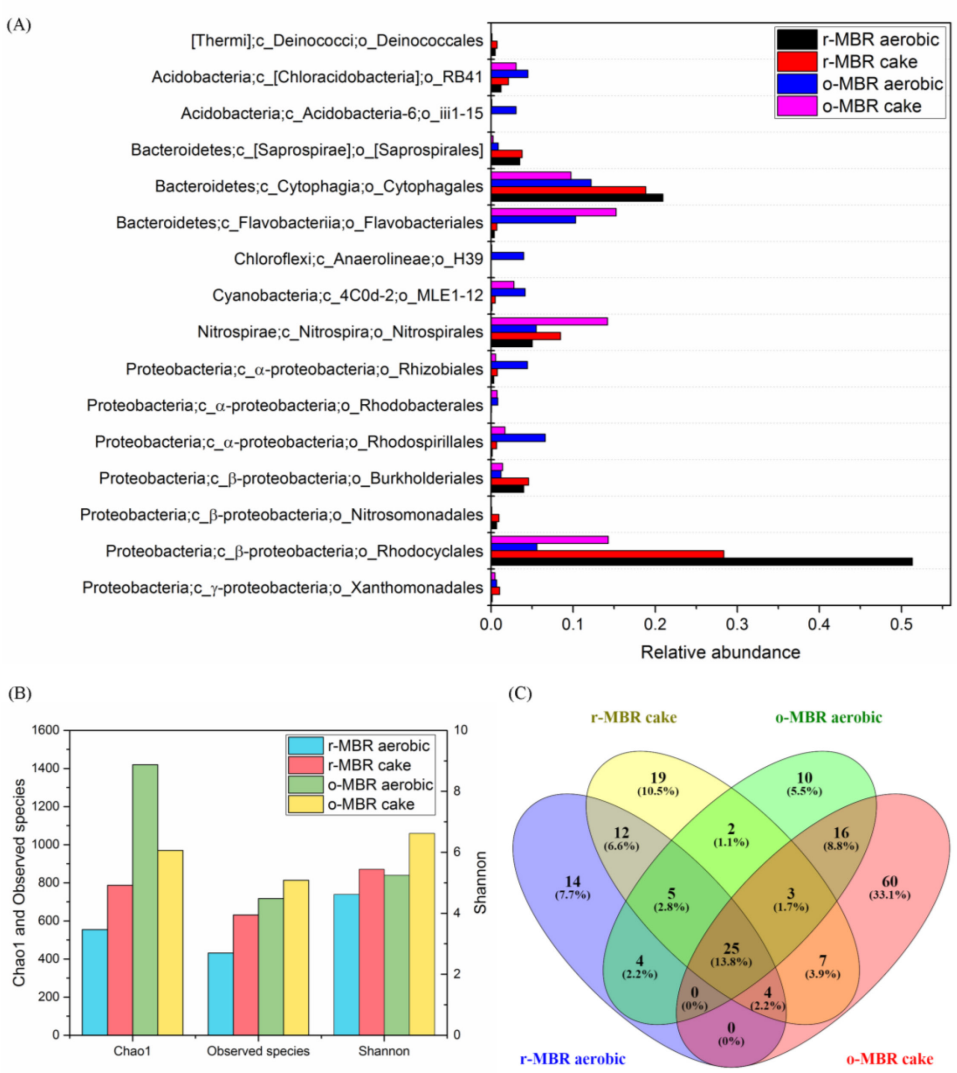


Figure 6-8. Microbial community structure characterization: (A) Bar chart of the 10 top orders in aerobic sludge samples and corresponding cake layer samples; (B) α diversity indices (N = 13555 sequences/sample); and (C) Venn diagram of OTUs with a relative abundance ≥ 0.1% in each sample.

(Note: r.MBR refers to raw OSPW MBR; and o.MBR refers to ozonated OSPW MBR. Square brackets indicate taxonomic changes recommended by Greengenes.)

Figure 6-8 A reveals that most of the main taxonomic orders were commonly founded in all the four samples though variations in relative abundance were observed in different samples. The phyla of *Proteobacteria*， *Bacteroidetes* and *Nitrospirae* were arguably the three dominating groups in all the samples analyzed. Among the subdivisions of *Proteobacteria*, *β-Proteobacteria*

was dominating. Some microorganisms were more abundant in the raw OSPW MBR, such as *Rhodocyclales*, *Cytophagales* and *Burkholderiales*; whereas, some others were found more in the ozonated OSPW MBR, including *Nitrospirales*, *Flavobacteriales*, *Rhodospirillales* and so on. Although all the major taxonomic orders were commonly found in all of the four samples, the microbial communities in the ozonated OSPW MBR showed a higher species richness than those in the raw OSPW MBR (Figure 6-8 B). And for each MBR, the fouling layer demonstrated higher bacterial diversity than the sludge sample. Similarly, a notable or even substantial fraction of the total observed OTUs was exclusively found in each of the four communities though a considerable common fraction (13.8%) was observed (Figure 6-8 C).

6.4 Discussion

The membrane fouling pattern observed in this study was similar to the previously described three-stage fouling pattern (Meng et al. 2009), which consists of an initial short-term rapid increase in TMP, a long-term slow TMP growth, and a TMP jump, chronologically. The spontaneous declines during long term slow TMP growth are considered a result of the fouling control measures used in this study, including bubbling, backwashing and relaxation. The slow fouling development in the MBRs should be attributed to the long SRT, air bubbling, backwashing, relaxation and the great fouling resistance of the ceramic membrane itself (Le-Clech et al. 2006, Wu et al. 2008). The reverse direction force created by backwashing or surface shear force created by aeration reduces and slower the formation of concentration polarization of particulates, and destructs deposition of foulants on membrane, thus contributing to better fouling control (Gao et al. 2011b).

Table 6-6. Error of fit and model coefficients for the raw OSPW MBR fouling during the period of Day 70 to 390 (t_0 = Day 70).

Model	Model fit error, SSR	R^2	Coefficients
Cake complete	2.00	0.962	$K_c = 6.284 \times 10^{-2}$ d/m^2; $K_b = 2.785 \times 10^{-11}$ d^{-1}
Cake intermediate	2.00	0.962	$K_c = 6.284 \times 10^{-2}$ d/m^2; $K_i = 8.651 \times 10^{-12}$ m^{-1}
Cake standard	2.00	0.962	$K_c = 6.284 \times 10^{-2}$ d/m^2; $K_s = 0$ m^{-1}
Complete standard	11.32	0.784	$K_b = 8.078 \times 10^{-6}$ d^{-1}; $K_s = 9.12 \times 10^{-3}$ m^{-1}
Intermediate standard	6.14	0.883	$K_i = 1.152 \times 10^{-2}$ m^{-1}; $K_s = 0$ m^{-1}
Cake filtration	2.00	0.962	$K_c = 6.284 \times 10^{-2}$ d/m^2

Table 6-7. Error of fit and model coefficients for the raw OSPW MBR fouling during the period of Day 397 to 433 (t_0 = Day 397).

Model	Model fit error, SSR	R^2	Coefficients
Cake complete	0.03	0.991	$K_c = 0$ d/m^2; $K_b = 1.445 \times 10^{-2}$ d^{-1}
Cake intermediate	0.14	0.963	$K_c = 4.723 \times 10^{-2}$ d/m^2; $K_i = 4.894 \times 10^{-2}$ m^{-1}
Cake standard	0.09	0.978	$K_c = 0$ d/m^2; $K_s = 5.686 \times 10^{-2}$ m^{-1}
Complete standard	0.03	0.991	$K_b = 1.445 \times 10^{-2}$ d^{-1}; $K_s = 0$ m^{-1}
Intermediate standard	0.09	0.978	$K_i = 6.989 \times 10^{-5}$ m^{-1}; $K_s = 5.673 \times 10^{-2}$ m^{-1}
Complete	0.03	0.991	$K_b = 1.445 \times 10^{-2}$ d^{-1}

Table 6-8. Error of fit and model coefficients for the ozonated OSPW MBR fouling during the period of Day 70 to 280 (t_0 = Day 70).

Model	Model fit error, SSR	R^2	Coefficients
Cake complete	1.36	0.884	$K_c = 4.601 \times 10^{-2}$ d/m^2; $K_b = 3.561 \times 10^{-10}$ d^{-1}
Cake intermediate	2.09	0.821	$K_c = 4.579 \times 10^{-2}$ d/m^2; $K_i = 7.102 \times 10^{-10}$ m^{-1}
Cake standard	1.36	0.884	$K_c = 4.589 \times 10^{-2}$ d/m^2; $K_s = 0$ m^{-1}
Complete standard	2.32	0.802	$K_b = 1.383 \times 10^{-11}$ d^{-1}; $K_s = 9.00 \times 10^{-3}$ m^{-1}
Intermediate standard	2.09	0.821	$K_i = 1.081 \times 10^{-2}$ m^{-1}; $K_s = 0$ m^{-1}
Cake filtration	1.36	0.884	$K_c = 4.589 \times 10^{-2}$ d/m^2

Table 6-9. Error of fit and model coefficients for the ozonated OSPW MBR fouling during the period of Day 552 to 575 (t_0 = Day 552).

Model	Model fit error, SSR	R^2	Coefficients
Cake complete	0.11	0.952	$K_c = 0$ d/m^2; $K_b = 1.927 \times 10^{-2}$ d^{-1}
Cake intermediate	0.20	0.918	$K_c = 0$ d/m^2; $K_i = 1.258 \times 10^{-2}$ m^{-1}
Cake standard	0.16	0.933	$K_c = 0$ d/m^2; $K_s = 8.612 \times 10^{-2}$ m^{-1}
Complete standard	0.11	0.952	$K_b = 1.933 \times 10^{-2}$ d^{-1}; $K_s = 0$ m^{-1}
Intermediate standard	0.16	0.933	$K_i = 4.863 \times 10^{-6}$ m^{-1}; $K_s = 8.599 \times 10^{-2}$ m^{-1}
Complete	0.11	0.952	$K_b = 1.927 \times 10^{-2}$ d^{-1}

6.4.1 Effect of ozonation pretreatment on membrane fouling

6.4.1.1 TMP change

The mild ozonation pretreatment not only substantially enhanced the total NA removal efficiency, but also considerably promoted the MBR's anti-fouling performance. The efficient

removals of NAs with higher *n* and |Z| confirm ozone's capability of decomposing NAs with long chains, more branches, and higher cyclicity, which are arguably the most bio-persistent fractions of OSPW NAs (Martin et al. 2010). Additionally, the higher remaining abundances of NAs with lower *n* and fewer rings in OSPW after ozone pretreatment might be due to conversion of NAs with more complexity (higher *n* and |Z|) into simpler NAs. In addition, Ozone could preferably degrade hydrophobic dissolve organic matters (DOM) in water (Song et al. 2010, Van Geluwe et al. 2011), thus weaken the hydrophobic interaction between the liquid phase organic substances and the membrane surface layer and alleviate membrane fouling.

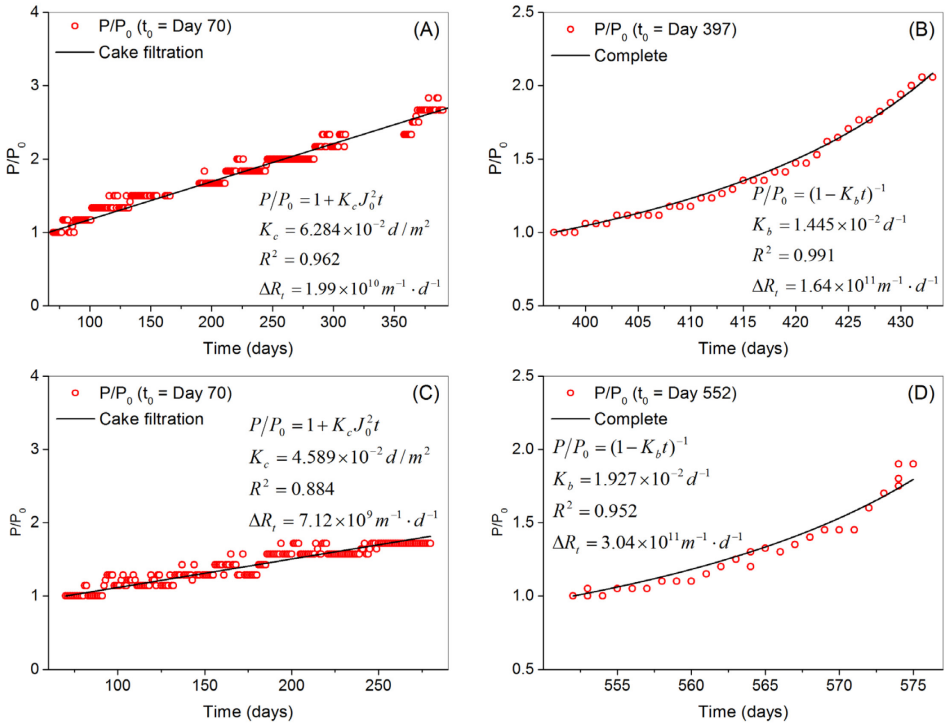


Figure 6-9. P/P_0 vs. time fitted with the optimal fouling model for: (A) the raw OSPW MBR from Day 70 to 390; (B) the raw OSPW MBR from Day 397 to 433; (C) the ozonated OSPW MBR from Day 70 to 280; and (D) the ozonated OSPW MBR from Day 552 to 575.

Besides, the different TMP patterns in the two MBRs after chemical cleaning imply that the membrane fouling in the raw OSPW MBR was less reversible even though chemical cleaning

was performed. To investigate the fouling mechanisms involved in the fouling phenomena of the MBRs during the operation, the TMP data of the MBRs were fitted with the five constant-flow combined fouling models. Those data points during a TMP decline phase (e.g. those points of the raw OSPW MBR during Day 167-188) were omitted before the fitting calculation. The fittings results are completely presented in Table 6-6 to Table 6-9, in which a lower SSR and a higher R^2 indicate a better fit. The optimal fitting model for each investigated period of each MBR turned out to be a single mechanism model. As is shown in Figure 6-9, the dominant mechanisms in the raw OSPW MBR in Day 70-390 and Day 397-433 were cake filtration and complete blocking, respectively. And similarly, the dominant mechanisms in the ozonated OSPW MBR in Day 70-280 and Day 552 to 575 were also cake filtration and complete blocking, respectively. Although the long-term slow fouling phase in both of the two MBRs was dominated by cake filtration mechanism, the raw OSPW MBR exhibited a slightly higher K_c (6.284×10^{-2} d/m^2 vs. 4.589×10^{-2} d/m^2) and ΔR_t (1.99×10^{10} m$^{-1}\cdot$d^{-1} vs. 7.12×10^{10} m$^{-1}\cdot$d^{-1}) over its ozonated OSPW counterpart. Contrarily, the ozonated OSPW MBR showed a higher complete blocking coefficient K_b (1.927×10^{-2} d^{-1} vs. 1.445×10^{-2} d^{-1}) and fouling rate ΔR_t (3.04×10^{11} m$^{-1}\cdot$d^{-1} vs. 1.64×10^{11} m$^{-1}\cdot$d^{-1}) during the TMP jump phase. The lower cake filtration coefficient K_c and fouling rate ΔR_t explained the longer elapsed time in the ozonated OSPW MBR before it started its first TMP jump. Therefore, it is apparent that the mild ozonation pretreatment of OSPW effectively improved the anoxic-aerobic MBR's anti-fouling performance by prolonging the long-term slow TMP growth phase and reducing the frequency of TMP jump occurrence notwithstanding the higher complete blocking coefficient K_b and fouling rate during the TMP jump.

6.4.1.2 Sludge properties

The insignificant difference between MLVSS concentrations of the two MBRs and the significantly higher MLVSS/MLSS ratio of the raw OSPW MBR over the whole operation period reveals that the high MLSS concentration within a certain range does not guarantee a stronger membrane fouling propensity, which is consistent with previous studies (Meng et al. 2007, Meng et al. 2005). Instead, MLVSS/MLSS ratio seemed positively related to membrane fouling tendency.

Regarding the effect of sludge EPS on membrane fouling, total EPS concentration was positively related to the higher fouling propensity, which has been reported by previous researchers The sludge EPS PN/PS in the raw OSPW MBR was higher compared with that in the ozonated OSPW MBR, suggesting that the mild ozonation pretreatment effectively led to a lower sludge PN/PS of total EPS which might consequentially result in a reduced membrane fouling tendency. In contrast, the correlation between SMP or SMP composition and membrane fouling propensity was vague based on the results in this study. Therefore, it is suggested that total mixed liquor EPS rather than SMP was positively related to the membrane fouling tendency in the MBRs.

Some researchers reported that smaller sludge particles sizes result in severer membrane fouling (Fallah et al. 2010, Meng et al. 2007, Meng et al. 2006). However, this was not the case in the current study as the MBR with a higher sludge particle size had severer fouling. Since the membrane pore size in the MBRs was 0.1 μm, it is not surprising to see that the small sludge particle size (D_{10} = 25.4±0.18 μm >> 0.01 μm) in the ozonated OSPW MBR did not result in worse fouling. Regarding the correlation between sludge PSD and EPS, the raw OSPW MBR at HRT of 24 hours showed higher PSD and higher sludge EPS concentration, as compared to the ozonated MBR, which is in agreement with Meng et al. (2007) and Pan et al. (2010).

6.4.1.3 Membrane fouling layer characteristics

With respect to the densities of EPS on fouled membrane surfaces, the higher abundance of EPS protein and EPS PN/PS on the raw OSPW MBR fouled membrane surface suggested that the EPS protein played an important role in fouling layer forming on the membrane surface, which is in accordance with previous studies (Meng et al. 2009, Meng et al. 2007).

Owing to their carboxylate, phosphate and amine functional groups, EPS could enhance the cell deposition onto solid surface through hydrophobic interaction, which facilitate aggregation of microbial cells and adhesion of bacteria onto solid surface (Le-Clech et al. 2006, Sheng et al. 2010). According to previous research, the ratio of PN/PS affects microbial aggregates' hydrophobicity and surface charge (Deng et al. 2015). Hence, it is likely that the higher hydrophobicity caused by the higher PN/PS resulted in the severer fouling formation in the raw OSPW MBR.

Through EDX analysis, more C and N detected on the raw OSPW MBR membrane disclosed higher density of organic foulants (e.g. proteins) attached on the membrane specimen, which is in accordance with the higher EPS density measured on the raw OSPW MBR membrane. Moreover, higher abundance of Fe and Ca in the cake layer of the raw OSPW MBR membrane implied that those elements were playing a role in membrane fouling formation. Ionizable groups (e.g. COO^-) contained in biopolymers might be precipitated by cations such as Ca^{2+}, Fe^{3+}, and Mg^{2+} (Lin et al. 2009). Divalent cations are able to act as bridges between carboxylic groups of two adjacent organic molecules (e.g., NOM), thus increasing the attractive forces between them (Shao et al. 2011). Some researchers showed that soluble organic compounds (e.g. carbohydrates and humics) bound in metal complexes are more impactful than themselves alone (Drews 2010). Thus, membrane fouling would be aggravated by absorption of metal clusters and metal ions

onto flocs and biopolymers through charge neutralization (Seidel and Elimelech 2002). The organic foulants coupled with inorganic precipitation enhance the formation of chemically irreversible fouling (Wang et al. 2008, You et al. 2006). Considering the alkaline pH in the MBRs, the aeration and CO_2 generated by microorganisms could affect the super-saturation of carbonates in the mixed liquor, consequently increasing the tendency of metallic carbonate (e.g. $CaCO_3$, $MgCO_3$, and $Fe_2(CO_3)_3$) scaling on membrane. Additionally, the huge amount of detected Si element on the fouled membranes indicated that clay minerals also contributed to membrane fouling in the two MBRs. Thus, reduced membrane fouling tendency could be expected if clay minerals are removed through a certain pretreatment prior to the MBR process (Kim et al. 2012).

6.4.1.4 Microbial community characteristics

As for the microbial community structure analyses, *α-Proteobacterial, β-Proteobacterial,* and *γ-Proteobacterial* species were correlated with membrane fouling in previous studies (Gao et al. 2013, Ma et al. 2013a, Ma et al. 2013b). In this study, severer membrane fouling observed in the raw OSPW MBR may be attributed to its higher abundances of *β-Proteobacterial* species than the ozonated OSPW MBR (Figure 6-8 A). Although the ozonated OSPW MBR showed higher population of *α-Proteobacteria* than the raw OSPW MBR, the abundances of *α-Proteobacteria* in both of the two MBRs were too low to make any difference in determining fouling severity. The substantially higher abundances of *Rhodocyclales*, *Cytophagales*, *Burkholderiales*, and [*Saprospirales*] in the raw OSPW MBR imply that those microorganisms might be correlated to the higher membrane fouling propensity. Species of *Rhodocyclales* (e.g., *Thauera* and *Dechloromonas*) (Allen et al. 2004, Salinero et al. 2009) and *Cytophagales* (Reichenbach 1991) are reported to be EPS producers. In fact, the relative abundance of *Dechloromonas* in the MBR

treating raw OSPW was remarkably higher (27.1% vs. 0.09% in sludge, and 10.6% vs. 0.5% on membrane surface) than that in the MBR treating ozonated OSPW. Additionally, some genera (e.g., *Leptothrix* and *Sphaerotilus*) within the order of *Burkholderiales* are filamentous bacteria capable of forming biofilm through excreting EPS (Hwang et al. 2012a, Wagner et al. 1994, Weissbrodt et al. 2012), suggesting that higher abundance of *Burholderiales* in raw OSPW MBR may also contribute to the server membrane fouling.

The higher microbial diversity of the cake layer community compared with the suspended biomass in each MBR was in consistency with previous studies (Briones and Raskin 2003). In addition, it is suggested that the bacterial richness was mainly a result of rare species that might not directly affect either the membrane fouling development or the MBR biodegradation performance. However, those minor players are expected to contribute to the stability and flexibility of the system against perturbations in a long term (Briones and Raskin 2003, Molina-Muñoz et al. 2009).

Thus, the mild ozonation pretreatment not only substantially enhanced the total classical NA removal efficiency, but also considerably promoted the MBR's anti-fouling performance. Ozone is capable of decomposing large organic molecules into smaller products, resulting in a membrane cake layer with more loose fragments that can be easily flushed away by backwashing and shear force (Van Geluwe et al. 2011). Ozone could preferably degrade hydrophobic natural organic matters (NOM) into smaller molecules with more hydrophilic function groups, such as carboxylic group (Song et al. 2010, Van Geluwe et al. 2011). Through weakening the hydrophobic interaction between the liquid phase organic substances and the membrane surface layer, the adsorption of NOM on membrane surface could be mitigated. The increased

hydrophilic function groups (i.e., COO^-) after ozonation confront relatively strong electrostatic repulsion from the negatively charged membrane surface layer.

Furthermore, ozonation remarkably reshaped the microbial community structure of the anoxic-aerobic MBR by changing relative abundances of the dominating species and encouraging rarer microbial species. Therefore, the ozone pretreatment effectively alleviated membrane fouling development through decomposing organic matters in feed water and suppressing the growth of microorganisms known for EPS excretion and biofilm formation. By facilitating the appearance of rare microbial species, the ozone pretreatment potentially enhanced the stability of the MBR performance against operational perturbations. In addition, the mild ozonation pretreatment substantially enhanced the MBR's OSPW NA degradation efficiency. The high NA degradation efficiency indicates that the combined process of ozonation and MBR treatment is a promising method for OSPW remediation on a larger scale.

6.4.2 Effect of HRT on membrane fouling

To evaluate the fouling behaviors at different HRTs, the sharp rises of TMP in the raw OSPW MBR are analyzed by fitting their P/P_0 vs. time data (Figure 6-10) and computing their average ΔR_t (Table 6-10). It is found that the fouling mechanisms in the raw OSPW MBR at HRTs of 48 h, 24 h, and 12 h were complete blocking, cake complete, and cake filtration, respectively, suggesting that HRT played a role in determining the dominant mechanism during membrane fouling development, especially the TMP jump period.

Table 6-10. TMP jump phase fouling rates of the raw OSPW MBR at different HRTs.

HRT (h)	72	48	24	12
ΔRt ($m^{-1} \cdot d^{-1}$)	3.20×10^{10} (Peak 4)	1.64×10^{11} (Peak 1*) 1.97×10^{11} (Peak 2) 2.63×10^{11} (Peak 3)	6.80×10^{11} (Peak 5)	1.20×10^{12} (Peak 6)

* Peaks are marked in Figure 6-3 A.

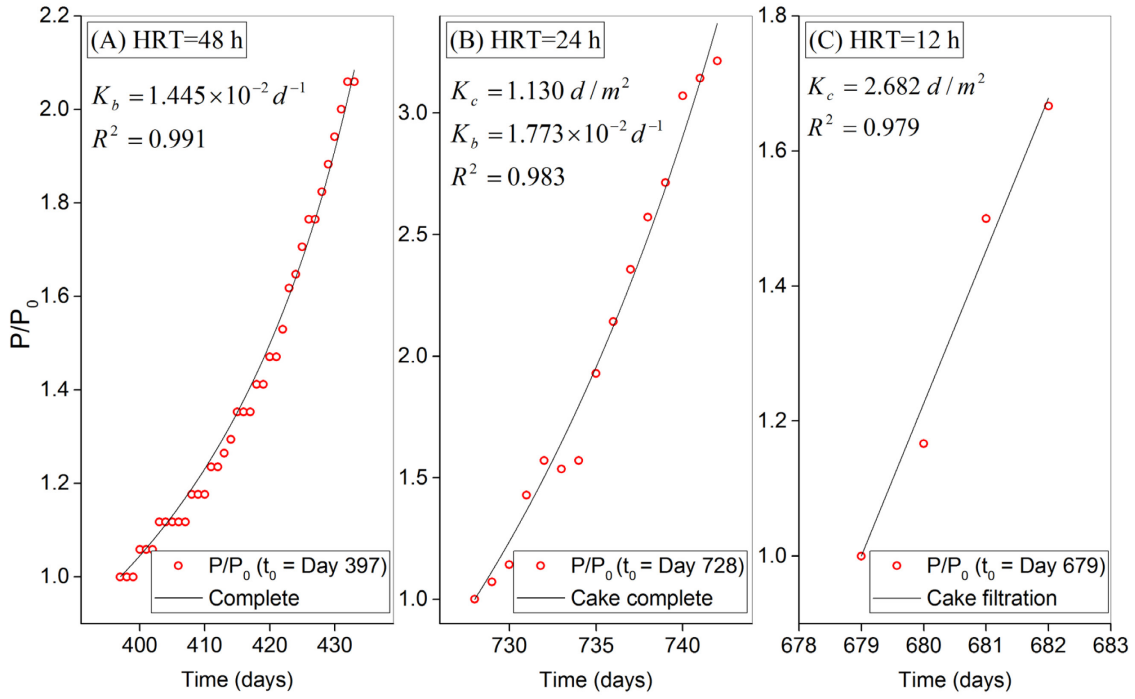


Figure 6-10. P/P_0 vs. time fitted with the optimal model for the sharp TMP rise phase in the raw OSPW MBR at: (A) HRT = 48 h; (B) HRT = 24 h; and (C) HRT = 12 h.

Since longer HRTs were attained through adjusting the relaxation length in this study, a longer HRT means a longer relaxation phase between two working phases. A long enough relaxation phase allowed air scour-caused shear force fully disturbed the fluffy cake layer formation on the membrane surface over the long-term slow fouling development, making it hard for the cake filtration mechanism to dominate the TMP jump period (Figure 6-10 A). Instead, the mixed

liquor suspended particles quickly sealed off the pore entrances of the cake layer (which could be considered as a membrane) formed during the long-term slow growth phase, and thus changed the inner structure of the cake layer by reducing its porosity and increasing its consolidatedness. A TMP jump therefore took place as local flux exceeded the membrane critical flux (Qu et al. 2013). As the relaxation between filtration phases was shortened, this cake layer demolition by air scouring inevitably diminished, making it reasonable to observe the co-dominance of cake filtration and complete blocking during the TMP jump (Figure 6-10 B). At HRT of 12 h, there was no relaxation phase to substantially repress the continuous formation of cake layer. Thus, it is not surprising to observe the absolute dominance of cake filtration mechanism during the TMP jump at the HRT of 12 h (Figure 6-10 C). Moreover, statistical analysis indicates that the MBR HRT length had a significant negative effect on membrane fouling rate with a PMCC r_p of -0.9374 ($P = 0.006 < 0.05$). Hence, it is suggested that HRT is negatively correlated with membrane fouling rate, which is in accordance with previous literature (Chang et al. 2002, Meng et al. 2009, Shariati et al. 2011).

It has been reported by some researchers that significant increase in EPS of sludge liquor was detected at decreased HRTs (Qu et al. 2013). However, this negative correlation between EPS/SMP and HRT was not observed in this study, which might be due to the constant external carbon source loading rate throughout the whole operation. In fact, the relationship between HRT and EPS/SMP might be more complex than expected (Fallah et al. 2010, Shariati et al. 2011).

6.5 Conclusions

To conclude this report, the anoxic-aerobic MBR system demonstrated excellent OSPW NA degradation efficiency and outstanding anti-fouling performance. The mild ozonation pretreatment of OSPW substantially enhanced not only the OSPW NA degradation but also the system's fouling control. Ozonation remarkably reshaped the microbial community structure of the anoxic-aerobic MBR by changing relative abundances of the dominating species and encouraging more rare microbial species. The substantially higher abundances of *Rhodocyclales*, *Cytophagales*, *Burkholderiales*, and [*Saprospirales*] in the raw OSPW MBR imply that those microorganisms might be related to the higher membrane fouling propensity. Through comparing the two MBRs, it is suggested that ozonation pretreatment of OSPW resulted in significantly lower EPS production and EPS PN/PS ratio, which brought about the better membrane fouling control in the ozonated OSPW MBR. Scrutiny on fouling behaviors at different HRTs of the raw OSPW MBR indicates that HRT is a factor that determines the dominating fouling mechanism during the sharp TMP rise phase. The excellent OSPW NA removal efficiency and great anti-fouling performance signal that the combined process of mild ozonation and anoxic-aerobic MBR is a promising choice for OSPW treatment on a large scale in the future.

References

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.

Allen, M.S., Welch, K.T., Prebyl, B.S., Baker, D.C., Meyers, A.J. and Sayler, G.S., 2004. Analysis and glycosyl composition of the exopolysaccharide isolated from the floc-forming wastewater bacterium Thauera sp. MZ1T. Environmental microbiology 6(8), 780-790.

Bolton, G., LaCasse, D. and Kuriyel, R., 2006. Combined models of membrane fouling: Development and application to microfiltration and ultrafiltration of biological fluids. Journal of Membrane Science 277, 75-84.

Bouhabila, E.H., Aïm, R.B. and Buisson, H., 2001. Fouling characterisation in membrane bioreactors. Separation and purification Technology 22, 123-132.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K. and Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods 7(5), 335-336.

Chae, S.-R., Ahn, Y.-T., Kang, S.-T. and Shin, H.-S., 2006. Mitigated membranefoulinginaverticalsubmergedmembranebioreactor (VSMBR). Journal of Membrane Science 280(1-2), 572-581.

Chang, I.-s., Clech, P.L., Jefferson, B. and Judd, S., 2002. Membrane Fouling in Membrane Bioreactors for Wastewater Treatment. JOURNAL OF ENVIRONMENTAL ENGINEERING (November), 1018-1029.

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.

Deng, L., Guo, W., Ngo, H.H., Zuthi, M.F.R., Zhang, J., Liang, S., Li, J., Wang, J. and Zhang, X., 2015. Membrane fouling reduction and improvement of sludge characteristics by bioflocculant addition in submerged membrane bioreactor. Separation and purification Technology 156, 450-458.

Drews, A., 2010. Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures. Journal of Membrane Science 363(1-2), 1-28.

DuBois, M., Gilles, K.a., Hamilton, J.K., Rebers, P.a. and Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28(3), 350-356.

Fallah, N., Bonakdarpour, B., Nasernejad, B. and Moghadam, M.A., 2010. Long-term operation of submerged membrane bioreactor (MBR) for the treatment of synthetic wastewater containing styrene as volatile organic compound (VOC): Effect of hydraulic retention time (HRT). Journal of hazardous materials 178(1), 718-724.

Gander, M., Jefferson, B. and Judd, S., 2000. Aerobic MBRs for domestic wastewater treatment: a review with cost considerations. Separation and purification Technology 18(2), 119-130.

Gao, D.-W., Wen, Z.-D., Li, B. and Liang, H., 2013. Membrane fouling related to microbial community and extracellular polymeric substances at different temperatures. Bioresource technology.

Han, S.-S., Bae, T.-H., Jang, G.-G. and Tak, T.-M., 2005. Influence of sludge retention time on membrane fouling and bioactivities in membrane bioreactor system. Process Biochemistry 40(7), 2393-2400.

Huang, X., Gui, P. and Qian, Y., 2001. Effect of sludge retention time on microbial behaviour in a submerged membrane bioreactor. Process Biochemistry 36(10), 1001-1006.

Hwang, G., Kang, S., El-Din, M.G. and Liu, Y., 2012. Impact of an extracellular polymeric substance (EPS) precoating on the initial adhesion of Burkholderia cepacia and Pseudomonas aeruginosa. Biofouling 28(6), 525-538.

Judd, S. and Judd, C., 2011. The MBR Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, Elsevier, Oxford.

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.
Kim, E.-S., Liu, Y. and Gamal El-Din, M., 2012. Evaluation of membrane fouling for in-line filtration of oil sands process-affected water: the effects of pretreatment conditions. Environmental science & technology 46(5), 2877-2884.
Kim, M., Yoo, G. and Yoo, C., 2011. Development of combined fouling model in a membrane bioreactor. Asia - Pacific Journal of Chemical Engineering 6(3), 423-432.
Le-Clech, P., Chen, V. and Fane, T.a.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. Journal of Membrane Science 284(1-2), 17-53.
Lin, H.J., Xie, K., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N. and Liao, B.Q., 2009. Sludge properties and their effects on membrane fouling in submerged anaerobic membrane bioreactors (SAnMBRs). Water research 43(15), 3827-3837.
Liu, H. and Fang, H.H.P., 2002. Extraction of extracellular polymeric substances (EPS) of sludges. Journal of biotechnology 95(3), 249-256.
Ma, J., Wang, Z., Yang, Y., Mei, X. and Wu, Z., 2013a. Correlating microbial community structure and composition with aeration intensity in submerged membrane bioreactors by 454 high-throughput pyrosequencing. Water research 47(2), 859-869.
Ma, Z., Wen, X., Zhao, F., Xia, Y., Huang, X., Waite, D. and Guan, J., 2013b. Effect of temperature variation on membrane fouling and microbial community structure in membrane bioreactor. Bioresource technology.
Martin, J.W., Barri, T., Han, X., 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.
Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S. and Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water research 43(6), 1489-1512.
Meng, F., Shi, B., Yang, F. and Zhang, H., 2007. Effect of hydraulic retention time on membrane fouling and biomass characteristics in submerged membrane bioreactors. Bioprocess and Biosystems Engineering 30, 359-367.
Meng, F., Zhang, H., Li, Y., Zhang, X. and Yang, F., 2005. Application of fractal permeation model to investigate membrane fouling in membrane bioreactor. Journal of Membrane Science 262(1), 107-116.
Meng, F., Zhang, H., Yang, F., Zhang, S., Li, Y. and Zhang, X., 2006. Identification of activated sludge properties affecting membrane fouling in submerged membrane bioreactors. Separation and purification Technology 51(1), 95-103.
Metcalf, E., Eddy, H., Tchobanoglous, G., Burton, F. and Stensel, H.D., 2003. Wastewater Engineering: Treatment and Reuse, McGraw-Hill, New York.
Oliveros, J.C., 2007. VENNY. An interactive tool for comparing lists with Venn Diagrams.
Pan, J.R., Su, Y.-C., Huang, C. and Lee, H.-C., 2010. Effect of sludge characteristics on membrane fouling in membrane bioreactors. Journal of Membrane Science 349(1), 287-294.
Qu, X., Gao, W., Han, M., Chen, A. and Liao, B., 2013. Effect of Hydraulic Retention Time on Sludge Properties, Cake Layer Structure, and Membrane Fouling in a Thermophilic

Submerged Aerobic Membrane Bioreactor. Separation science and technology 48(10), 1529-1536.
Reichenbach, H., 1991. DWORKIN^ The myxobacteria. Starr KP, Stolp H, Trper HG, et a1. The Prokaryotes 4, 3.
Salinero, K.K., Keller, K., Feil, W.S., Feil, H., Trong, S., Di Bartolo, G. and Lapidus, A., 2009. Metabolic analysis of the soil microbe Dechloromonas aromatica str. RCB: indications of a surprisingly complex life-style and cryptic anaerobic pathways for aromatic degradation. BMC genomics 10(1), 1.
Scarlett, a.G., Reinardy, H.C., Henry, T.B., West, C.E., Frank, R.a., Hewitt, L.M. and Rowland, S.J., 2013. Acute toxicity of aromatic and non-aromatic fractions of naphthenic acids extracted from oil sands process-affected water to larval zebrafish. Chemosphere.
Seidel, A. and Elimelech, M., 2002. Coupling between chemical and physical interactions in natural organic matter (NOM) fouling of nanofiltration membranes: implications for fouling control. Journal of Membrane Science 203(1), 245-255.
Shao, J., Hou, J. and Song, H., 2011. Comparison of humic acid rejection and flux decline during filtration with negatively charged and uncharged ultrafiltration membranes. Water research 45(2), 473-482.
Shariati, S.R.P., Bonakdarpour, B., Zare, N. and Ashtiani, F.Z., 2011. The effect of hydraulic retention time on the performance and fouling characteristics of membrane sequencing batch reactors used for the treatment of synthetic petroleum refinery wastewater. Bioresource technology 102(17), 7692-7699.
Shell, 2009. Royal Dutch Shell PLC Sustainability Report.
Sheng, G.-P., Yu, H.-Q. and Li, X.-Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnology advances 28(6), 882-894.
Song, Y., Dong, B., Gao, N. and Xia, S., 2010. Huangpu River water treatment by microfiltration with ozone pretreatment. Desalination 250(1), 71-75.
Stephenson, T., Judd, S., Jefferson, B., Brindle, K. and Association, I.W., 2000. Membrane bioreactors for wastewater treatment, Citeseer.
The Government of Alberta, 2015. Lower Athabasca Region-Tailings Management Framework for the Mineable Athabasca Oil Sands. The Government of Alberta (ed).
Van Geluwe, S., Braeken, L. and Van der Bruggen, B., 2011. Ozone oxidation for the alleviation of membrane fouling by natural organic matter: A review. Water research 45(12), 3551-3570.
von Sperling, M., 2007. Activated sludge and aerobic biofilm reactors, IWA publishing.
Wagner, M., Amann, R., Kämpfer, P., Assmus, B., Hartmann, A., Hutzler, P., Springer, N. and Schleifer, K.-H., 1994. Identification and in situ detection of gram-negative filamentous bacteria in activated sludge. Systematic and Applied Microbiology 17(3), 405-417.
Wang, B., Wan, Y., Gao, Y., Yang, M. and Hu, J., 2013. Determination and characterization of oxy-naphthenic acids in oilfield wastewater. Environmental science & technology 47(16), 9545-9554.
Wang, Z., Wu, Z., Yin, X. and Tian, L., 2008. Membrane fouling in a submerged membrane bioreactor (MBR) under sub-critical flux operation: membrane foulant and gel layer characterization. Journal of Membrane Science 325(1), 238-244.

Weissbrodt, D.G., Lochmatter, S., Ebrahimi, S., Rossi, P., Maillard, J. and Holliger, C., 2012. Bacterial selection during the formation of early-stage aerobic granules in wastewater treatment systems operated under wash-out dynamics. Front Microbiol 3, 332.

Wu, J., Le-Clech, P., Stuetz, R.M., Fane, A.G. and Chen, V., 2008. Effects of relaxation and backwashing conditions on fouling in membrane bioreactor. Journal of Membrane Science 324(1-2), 26-32.

Xue, J., Zhang, Y., Liu, Y. and El-Din, M.G., 2016. Treatment of oil sands process-affected water (OSPW) using a membrane bioreactor with a submerged flat-sheet ceramic microfiltration membrane. Water research 88, 1-11.

You, H., Huang, C., Pan, J. and Chang, S., 2006. Behavior of membrane scaling during crossflow filtration in the anaerobic MBR system. Separation science and technology 41(7), 1265-1278.

Zhang, Y., Xue, J., Liu, Y. and El-Din, M.G., 2016. Treatment of oil sands process-affected water (OSPW) using membrane bioreactor coupled with ozonation: A comparative study. Chemical Engineering Journal.

Zhou, H. and Smith, D.W., 2002. Advanced technologies in water and wastewater treatment. Journal of Environmental Engineering and Science (1), 247-264.

7 Conclusions and recommendations

7.1 Thesis overview

Fluid waste generated from bitumen extraction, known as oil sands process-affected water (OSPW) is currently stored in vast tailings ponds. By the end of 2015, the total area occupied by tailings ponds was ~176 km^2, holding enough liquid to fill the equivalent of 390,000 Olympic-sized swimming pools. Concerns are widely raised about the impacts of the enormous quantity of OSPW on the regional environment and ecological system. In OSPW, NAs are regarded as the most problematic organic compound groups since they have toxicity to aquatic organisms (Allen 2008a, b). Among all those examined methods for OSPW treatment, biodegradation is generally considered the most cost-effective and practical way (Han et al. 2008, Kannel and Gan 2012, Scott et al. 2005b). However, it is estimated that the OSPW NAs' *in situ* biodegradation half-lives could be as long as ~13 years (Han et al. 2009), making it necessary and demanding to develop engineered biological processes to accelerate the biodegradation of OSPW NAs.

The study in this thesis, focused on the application of an anoxic-aerobic MBR for raw and ozonated OSPW treatment. A > 300-day startup/acclimatization phase took place to gradually acclimatize microorganisms in the MBRs to 100% OSPW environment, revealing the feasibility of the MBR system for OSPW treatment. To better understand the contribution of denitrification and nitrification to degradation of OSPW classical NAs and oxy-NAs, a batch study was performed on the biodegradation of raw and ozonated OSPW under anoxic and nitrifying aerobic conditions. After the MBRs were acclimatized and stabilized, a ~442-day continuous operation for operating condition optimization was conducted, through which the MBRs' performance on raw and ozonated OSPW NA degradation was successfully improved by changing the

supplemented inorganic nitrogen composition and HRT. Impacts of molecular structures of NAs on their biodegradation efficiency were unveiled; and possible correlations between certain microorganisms and biodegradation of NAs with particular molecular features were found. Meanwhile, the fouling behaviors of the two MBRs for raw and ozonated OSPW treatment were closely monitored over the whole operation period. Dominating fouling mechanisms involved during the operation were suggested by performing mathematical model fitting. Compositions of the membrane fouling cake layers were examined using techniques including SEM-EDX and CLSM. Effects of ozone pretreatment on NA biodegradation, microbial community structure, and membrane fouling development were revealed.

7.2 Conclusions

By investigating the performance of the anoxic-aerobic MBRs for raw and ozonated OSPW treatment under different operating conditions, this study unveiled the possibility of MBR treatment of the enormous volume of OSPW generated by the oil sands industry. Additionally, the study disclosed the effects of different supplemented inorganic nitrogen compositions and HRTs on the MBRs' performance on OSPW NA degradation.

The study identified the dominating microorganisms within the MBRs under different operating conditions, some of which showed statistically strong positive correlation with the degradation of NAs with certain molecular structures. This finding may be a good starting point for future studies on the identification of degraders of different NAs. Moreover, the study evidences that the anoxic-aerobic MBR is effective in degrading oxy-NAs. It had been reported that the removal of oxy-NAs was not feasible using either ozonation or biological process alone (Brown et al. 2013, Han et al. 2008, Islam 2014). The study also revealed the impacts of low-dose ozone

on classical NA and oxy-NA degradation, membrane fouling development, and microbial community characteristics of the MBR system.

Overall, the study provided future researchers with insightful information on improving biological systems treating OSPW and similar persistent petroleum industrial wastewaters. The study would contribute to the alleviation of the environmental issues caused by the huge volume of OSPW. Conclusions to each chapter are listed as follows.

7.2.1 Chapter 2: Reactor start-up/sludge acclimatization of the anoxic-aerobic MBR for raw OSPW treatment: a preliminary study.

A. After 361-day of reactor startup/sludge acclimatization operation, the anoxic-aerobic MBR achieved a stabilized NA removal of 24.7% with an HRT of 48 h, indicating the effectiveness of the anoxic-aerobic MBR for OSPW remediation. It has been demonstrated that biotransformation rather than complete mineralization was the dominant biodegradation pathway of OSPW parent NAs in the MBR. Parent NAs with larger molecules were broken down into molecules with fewer carbons. And more degradation was observed for NAs with fewer degrees of cyclization.
B. 454 Pyrosequencing analysis revealed that *β-Proteobacteria* were dominant in sludge samples from the anoxic-aerobic MBR. The presence of hydrocarbon degraders (such as *Rhodocyclales* and *Sphingobacteriales*) in the MBR justifies the biodegradation of organic constituents in OSPW.
C. During the whole reactor start-up/sludge acclimatization phase, no severe membrane fouling was observed as the TMP of the anoxic-aerobic MBR never exceeded the

manufacturer's suggested critical TMP (-35 kPa) for chemical cleaning, suggesting the MBR's excellent anti-fouling performance under the operating conditions.

7.2.2 Chapter 3: Treatment of raw and mildly ozonated OSPWs under decoupled anoxic and aerobic conditions: a comprehensive study.

A. Both of anoxic and aerobic conditions were found effective in degrading OSPW classical and oxidized NAs though the aerobic reactors showed higher effectiveness in removing classical NAs. The mild-dose ozone pretreatment remarkably accelerated the subsequent aerobic biodegradation of classical NAs (35.8% vs. 30.0%) within the first 14 days. Besides, the ozone pretreatment enhanced the NA removal by leaving a considerably lower final residual concentration of total classical NAs (10.4 mg/L vs. 18.8 mg/L under anoxic conditions, and 5.7 mg/L vs. 7.5 mg/L under aerobic conditions). It is suggested that bioconversion rather than mineralization was the major pathway of NA biodegradation.

B. After 33 days of batch operation, microbial species capable of degrading recalcitrant hydrocarbons were detected in great abundance in the reactors. The dominating genus in the raw and ozonated OSPW anoxic reactors was identified as *Thauera,* whereas *Rhodanobacter* and *Pseudomonas* dominated the raw and ozonated OSPW aerobic reactors, respectively. It is suggested that the dominating denitrifiers might be involved in anoxic degradation of NAs, whereas the contribution of nitrifiers to aerobic degradation of NAs needs to be clarified in the future.

C. The combination of ozonation pretreatment and aerobic degradation ensured higher NA removal. Decreased pH in the aerobic reactors might indirectly enhance the biodegradation of NAs with higher carbon numbers through affecting their

hydrophobicity. Further study is needed to confirm the role of pH and hydrophobicity on NA biodegradation. The results obtained from this study may be beneficial to optimization of biological treatment processes (e.g., on-site bioremediation and engineered bioreactor) for OSPW treatment in the future.

7.2.3 Chapter 4 and 5: Optimization of operating conditions of the anoxic-aerobic MBR for raw OSPW treatment, and evaluation of the effect of mild ozonation pretreatment on the subsequent MBR process.

A. The study showed that higher supplemented NH_4-N concentrations or longer HRTs did not guarantee better NA removal. Instead, the operating stage with a supplemented NH_4-N concentration of 25 mg/L and an HRT of 12 h was considered as the optimal among the six examined operating conditions for best overall removal of classical NAs (37.6%) and oxy-NAs (23.9%) from raw OSPW. In addition, it should be noted that different optimal operating conditions might exist for best degradation of different NAs groups.
B. The mild ozonation pretreatment remarkably enhanced the subsequent MBR performance in terms of classical and oxidized NA degradation rates (49.7% vs. 37.6% of classical NA removal; 35.4% vs. 23.9% of oxy-NA removal). The hybridization of low-dose ozone pretreatment and MBR process delivered a removal rate of 94.0% of classical NAs and 43.9% of oxy-NAs (O3 – 6) within an HRT of 12 h, indicating the competency of the combined process for future OSPW treatment in large-scale application.
C. MiSeq sequencing analysis revealed that orders of *Proteobacteria* (i.e., *Rhodocyclales*, *Burkholderiales* and *Nitrosomonadales*), *Bacteroidetes* (i.e., *Cytophagales*, *[Saprospirales]* and *Flavobacteriales*), and *Nitrospirae* (i.e., *Nitrospirales*) were the dominating microbial groups over the whole study duration though their relative

abundances varied as the operating conditions changed. Statistical calculations indicate strong positive correlations between some microbial orders and certain NA groups. Ozone pretreatment not only resulted in different microbial community compositions (e.g., different relative abundances of the dominating microorganisms), but also promoted the MBR's bacterial diversity.

7.2.4 Chapter 6: Membrane fouling behaviors of the anoxic-aerobic MBRs for raw and ozonated OSPW treatment under different operating conditions.

A. The anoxic-aerobic MBRs demonstrated excellent OSPW NA degradation rates and outstanding anti-fouling performance. The mild ozonation pretreatment of OSPW substantially enhanced not only the OSPW NA degradation but also the system's fouling control. Compared with the raw OSPW MBR, the fouling formation of the ozonated OSPW MBR was much slower and more chemically reversible. Through comparing the two MBRs, it is suggested that ozone pretreatment of OSPW resulted in lower growth of bacteria producing EPS and facilitating biofilm formation, thus leading to significantly lower EPS production and EPS PN/PS ratio. This justifies the better membrane fouling control in the ozonated OSPW MBR.
B. Examination on fouling behaviors at different HRTs of the raw OSPW MBR indicates that HRT is a factor that plays a role in determining the dominating fouling mechanism during the sharp TMP jump phase.

Overall, the anoxic-aerobic MBRs demonstrated competent OSPW classical and oxidized NA degradation rates within a relative short HRT (i.e., 12 h). The excellent OSPW NA removal efficiency and great anti-fouling performance signal that the combined process of low-dose

ozonation and anoxic-aerobic MBR is a promising choice for OSPW treatment on a large scale. With respect to membrane fouling that troubles most MBR systems, the MBR operated in this study showed an outstanding anti-fouling performance. This study also provides insightful information on how different inorganic nitrogen concentration and HRT would affect the MBR's performance. Besides, this study reveals the impacts of NAs molecular structure on their biodegradation, and suggests potential biodegradation pathways of OSPW NAs within the MBR. Moreover, the characterization of the microbial communities in this study would benefit future studies on biological treatment of OSPW.

7.3 Recommendations for future work

On the basis of the study covered herein, recommendations for future work are proposed as follows.

A. Optimization of SRT of the MBR system. As SRT is one of the most important parameters affecting MBR performance, particularly membrane fouling, it is essential to make effort to find an SRT that would result in the best contaminant degradation efficiency and lowest fouling propensity.
B. Optimization of the utilized ozone dose for OSPW pretreatment. Although 30 mg O_3/L is comparably low, a lower ozone dose may still exist, which would significantly reduce energy consumption of the ozonation process without apparently compromising the pretreatment performance. The effects of different ozone pretreatment on NA biodegradation, microbial community architecture, and membrane fouling development could be investigated.

C. Confirmation of correlations between certain microorganisms and NAs with particular molecular features. Further effort needs to be made through using model compounds and isolated bacteria cultures to examine and confirm the contribution and specificity of those microbes to the degradation of NAs with particular molecular structural features.

D. Investigation on biodegradation pathways of NAs in the MBRs. As is suggested in the thesis, different microorganisms may be responsible for degradation of NA species with different molecular structures. It is likely that different degradation pathways may be adopted by microorganisms to break down NAs, such as β-oxidation, combined α- and β-oxidation, and aromatization (Han et al. 2008). In addition, different enzymes (e.g., monooxygenase and dioxygenase) must be involved during biodegradation of NAs. It is of interest to broaden to understanding of fundamental mechanisms of OSPW NA degradation in the MBR system. Next generation RNA-seq (RNA sequencing) analysis could be performed to analyze the continually changing cellular transcriptome in the biological system, which would help uncover the genes expressed during biodegradation of OSPW NAs.

E. Post-treatment of MBR permeate using ozonation. Low-dose ozonation has been reported to be capable of degrading more recalcitrant NAs (Martin et al. 2010). It is therefore of interest to explore how low-dose ozonation would affect residual NAs in the MBR permeate.

F. Hybridization of attached growth system and MBR system. In contrast to the rarity of study on MBRs for OSPW treatment, plentiful studies have been published on the effectiveness of biofilm reactors (e.g., with granular activated carbon (GAC), and high-density polyethylene as carriers) in treating OSPW (Hwang et al. 2012a, Islam et al. 2013,

Shi et al. 2015). Biofilms consists of a consortium of diverse microorganisms in an EPS matrix. Microbial community members act synergistically within the EPS matrix, making it possible to degrade recalcitrant substances that could not be degraded by planktonic microorganisms. To combine the merits of MBRs and biofilm systems, a hybrid system integrating MBR and biofilm is proposed.

G. Exploration of alternative external carbon sources. The added sodium acetate is helpful to maintain a relatively high biomass concentration and potentially beneficial to NA biodegradation in the MBRs. However, it is definitely economically appealing if a cheaper external carbon source is used. For instance, passing municipal wastewater that contains sufficient easily biodegradable carbon source and nutrients into the MBR system treating OSPW; or periodically adding wasted municipal wastewater treatment plant activated sludge into the MBR system for OSPW treatment. As such, the potential advantages are obvious. In addition to the reduction of energy and chemical consumption, the MBR may benefit from abundant enzymes from the externally added waste sludge, which may contribute to biodegradation of NAs.

References

Aga, D.S., 2007. Fate of Pharmaceuticals in the Environment and in Water Treatment Systems, CRC Press.

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.

Allen, M.S., Welch, K.T., Prebyl, B.S., Baker, D.C., Meyers, A.J. and Sayler, G.S., 2004. Analysis and Glycosyl Composition of the Exopolysaccharide Isolated from the Floc - Forming Wastewater Bacterium Thauera Sp. Mz1t. Environmental microbiology 6(8), 780-790.

Alpatova, A., Kim, E.-S., Dong, S., Sun, N., Chelme-Ayala, P. and El-Din, M.G., 2014. Treatment of Oil Sands Process-Affected Water with Ceramic Ultrafiltration Membrane: Effects of Operating Conditions on Membrane Performance. Separation and purification Technology 122, 170-182.

Anders, H.-J., Kaetzke, A., KÄMPFER, P., Ludwig, W. and Fuchs, G., 1995. Taxonomic Position of Aromatic-Degrading Denitrifying Pseudomonad Strains K 172 and Kb 740 and Their Description as New Members of the Genera Thauera, as Thauera Aromatica Sp. Nov., and Azoarcus, as Azoarcus Evansii Sp. Nov., Respectively, Members of the Beta Subclass of the Proteobacteria. International journal of systematic bacteriology 45(2), 327-333.

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., 2012. Effectiveness of Ozonation Treatment in Eliminating Toxicity of Oil Sands Process-Affected Water to Chironomus Dilutus. Environmental science & technology 46(1), 486-493.

Anthonisen, A., Loehr, R., Prakasam, T. and Srinath, E., 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. Journal (Water Pollution Control Federation), 835-852.

AWWA, 2002. Nitrification, United States Environmental Protection Agency, Washington DC.

Bell, T.H., Yergeau, E., Maynard, C., Juck, D., Whyte, L.G. and Greer, C.W., 2013a. Predictable Bacterial Composition and Hydrocarbon Degradation in Arctic Soils Following Diesel and Nutrient Disturbance. The ISME journal 7(6), 1200-1210.

Bell, T.H., Yergeau, E., Maynard, C., Juck, D., Whyte, L.G. and Greer, C.W., 2013b. Predictable Bacterial Composition and Hydrocarbon Degradation in Arctic Soils Following Diesel and Nutrient Disturbance. ISME J 7(6), 1200-1210.

Bellona, C., Drewes, J.E., Xu, P. and Amy, G., 2004. Factors Affecting the Rejection of Organic Solutes During Nf/Ro Treatment—a Literature Review. Water research 38(12), 2795-2809.

Biryukova, O.V., Fedorak, P.M. and Quideau, S.A., 2007. Biodegradation of Naphthenic Acids by Rhizosphere Microorganisms. Chemosphere 67(10), 2058-2064.

Bock, E. and Koops, H., 1992. The Genus Nitrobacter and Related Genera. Balows A, Tr€ uper HG, Dworkin M, Harder W, Schleifer KH, editors. The prokaryotes. New York: Springer, 2302-2309.

Bolton, G., LaCasse, D. and Kuriyel, R., 2006. Combined Models of Membrane Fouling: Development and Application to Microfiltration and Ultrafiltration of Biological Fluids. Journal of Membrane Science 277, 75-84.

Bouhabila, E.H., Aïm, R.B. and Buisson, H., 2001. Fouling Characterisation in Membrane Bioreactors. Separation and purification Technology 22, 123-132.

Briones, A. and Raskin, L., 2003. Diversity and Dynamics of Microbial Communities in Engineered Environments and Their Implications for Process Stability. Current Opinion in Biotechnology 14(3), 270-276.

Brown, L.D., Pérez-Estrada, L., Wang, N., El-Din, M.G., Martin, J.W., Fedorak, P.M. and Ulrich, A.C., 2013. Indigenous Microbes Survive in Situ Ozonation Improving Biodegradation of Dissolved Organic Matter in Aged Oil Sands Process-Affected Waters. Chemosphere.

Brown, L.D. and Ulrich, A.C., 2015. Oil Sands Naphthenic Acids: A Review of Properties, Measurement, and Treatment. Chemosphere 127, 276-290.

Calli, B., Mertoglu, B., Inanc, B. and Yenigun, O., 2005. Effects of High Free Ammonia Concentrations on the Performances of Anaerobic Bioreactors. Process Biochemistry 40(3), 1285-1292.

Cao, B., Nagarajan, K. and Loh, K.-C., 2009. Biodegradation of Aromatic Compounds: Current Status and Opportunities for Biomolecular Approaches. Applied microbiology and biotechnology 85(2), 207-228.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K. and Gordon, J.I., 2010. Qiime Allows Analysis of High-Throughput Community Sequencing Data. Nature methods 7(5), 335-336.

CAPP, 2014. The Facts On: Oil Sands, Canadian Association of Petroleum Producers, oilsandstoday.ca.

Cebron, A. and Garnier, J., 2005. Nitrobacter and Nitrospira Genera as Representatives of Nitrite-Oxidizing Bacteria: Detection, Quantification and Growth Along the Lower Seine River (France). Water research 39(20), 4979-4992.

Chae, S.-R., Ahn, Y.-T., Kang, S.-T. and Shin, H.-S., 2006. Mitigated Membranefoulinginaverticalsubmergedmembranebioreactor (Vsmbr). Journal of Membrane Science 280(1-2), 572-581.

Chang, I.-s., Clech, P.L., Jefferson, B. and Judd, S., 2002. Membrane Fouling in Membrane Bioreactors for Wastewater Treatment. JOURNAL OF ENVIRONMENTAL ENGINEERING (November), 1018-1029.

Chang, J.-S., Chang, C.-Y., Chen, A.-C., Erdei, L. and Vigneswaran, S., 2006. Long-Term Operation of Submerged Membrane Bioreactor for the Treatment of High Strength Acrylonitrile-Butadiene-Styrene (Abs) Wastewater: Effect of Hydraulic Retention Time. Desalination 191(1), 45-51.

Choi, J., Hwang, G., Gamal El-Din, M. and Liu, Y., 2014. Effect of Reactor Configuration and Microbial Characteristics on Biofilm Reactors for Oil Sands Process-Affected Water Treatment. International Biodeterioration & Biodegradation 89(2014), 74-81.

Choi, J. and Liu, Y., 2014. Biodegradation of Oil Sands Process Affected Water in Sequencing Batch Reactors and Microbial Community Analysis by High-Throughput Pyrosequencing. International Biodeterioration & Biodegradation 92, 79-85.

Cicek, N., 2003. A Review of Membrane Bioreactors and Their Potential Application in the Treatment of Agricultural Wastewater. CANADIAN BIOSYSTEMS ENGINEERING 45, 37-49.

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.
Das, S., 2014. Microbial Biodegradation and Bioremediation, Elsevier.
De Gusseme, B., Pycke, B., Hennebel, T., Marcoen, A., Vlaeminck, S.E., Noppe, H., Boon, N. and Verstraete, W., 2009. Biological Removal of 17 Alpha-Ethinylestradiol by a Nitrifier Enrichment Culture in a Membrane Bioreactor. Water research 43(9), 2493-2503.
Deng, L., Guo, W., Ngo, H.H., Zuthi, M.F.R., Zhang, J., Liang, S., Li, J., Wang, J. and Zhang, X., 2015. Membrane Fouling Reduction and Improvement of Sludge Characteristics by Bioflocculant Addition in Submerged Membrane Bioreactor. Separation and purification Technology 156, 450-458.
Deni, J. and Penninckx, M.J., 1999. Nitrification and Autotrophic Nitrifying Bacteria in a Hydrocarbon-Polluted Soil. Applied and environmental microbiology 65(9), 4008-4008.
Ding, G.-C., Heuer, H. and Smalla, K., 2012. Dynamics of Bacterial Communities in Two Unpolluted Soils after Spiking with Phenanthrene: Soil Type Specific and Common Responders. Frontiers in microbiology 3.
Dong, T., Zhang, Y., Islam, M.S., Liu, Y. and El-Din, M.G., 2015. The Impact of Various Ozone Pretreatment Doses on the Performance of Endogenous Microbial Communities for the Remediation of Oil Sands Process-Affected Water. International Biodeterioration & Biodegradation 100, 17-28.
Drews, A., 2010. Membrane Fouling in Membrane Bioreactors—Characterisation, Contradictions, Cause and Cures. Journal of Membrane Science 363(1-2), 1-28.
Drury, B., Rosi-Marshall, E. and Kelly, J.J., 2013. Wastewater Treatment Effluent Reduces the Abundance and Diversity of Benthic Bacterial Communities in Urban and Suburban Rivers. Applied and environmental microbiology 79(6), 1897-1905.
DuBois, M., Gilles, K.a., Hamilton, J.K., Rebers, P.a. and Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28(3), 350-356.
Etchebehere, C. and Tiedje, J., 2005. Presence of Two Different Active Nirs Nitrite Reductase Genes in a Denitrifying Thauera Sp. From a High-Nitrate-Removal-Rate Reactor. Applied and environmental microbiology 71(9), 5642-5645.
Fallah, N., Bonakdarpour, B., Nasernejad, B. and Moghadam, M.A., 2010. Long-Term Operation of Submerged Membrane Bioreactor (Mbr) for the Treatment of Synthetic Wastewater Containing Styrene as Volatile Organic Compound (Voc): Effect of Hydraulic Retention Time (Hrt). Journal of hazardous materials 178(1), 718-724.
Federation, W.E. and Association, A.P.H., 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA): Washington, DC, USA.
Ford, D.L., Churchwell, R.L. and Kachtick, J.W., 1980. Comprehensive Analysis of Nitrification of Chemical Processing Wastewaters. Journal (Water Pollution Control Federation), 2726-2746.
Frank, R.A., Sanderson, H., Kavanagh, R., Burnison, B.K., Headley, J.V. and Solomon, K.R., 2009. Use of a (Quantitative) Structure–Activity Relationship [(Q) Sar] Model to Predict

the Toxicity of Naphthenic Acids. Journal of Toxicology and Environmental Health, Part A 73(4), 319-329.
Gamal El-Din, M., Fu, H., Wang, N., Chelme-Ayala, P., Pérez-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. The Science of the total environment 409(23), 5119-5125.
Gander, M., Jefferson, B. and Judd, S., 2000. Aerobic Mbrs for Domestic Wastewater Treatment: A Review with Cost Considerations. Separation and purification Technology 18(2), 119-130.
Gao, D.-w., Fu, Y., Tao, Y., Li, X.-x., Xing, M., Gao, X.-h. and Ren, N.-q., 2011a. Linking Microbial Community Structure to Membrane Biofouling Associated with Varying Dissolved Oxygen Concentrations. Bioresource technology 102(10), 5626-5633.
Gao, D.-W., Wen, Z.-D., Li, B. and Liang, H., 2013. Membrane Fouling Related to Microbial Community and Extracellular Polymeric Substances at Different Temperatures. Bioresource technology.
Gao, W., Liang, H., Ma, J., Han, M., Chen, Z.-l., Han, Z.-s. and Li, G.-b., 2011b. Membrane Fouling Control in Ultrafiltration Technology for Drinking Water Production: A Review. Desalination 272(1), 1-8.
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., 2011. Ozone Treatment Ameliorates Oil Sands Process Water Toxicity to the Mammalian Immune System. Water research 45(18), 5849-5857.
Germirli Babuna, F., Camur, S., Alaton, I.A., Okay, O. and Iskender, G., 2009. The Application of Ozonation for the Detoxification and Biodegradability Improvement of a Textile Auxiliary: Naphtalene Sulphonic Acid. Desalination 249(2), 682-686.
Glaze, W.H., 1987. Drinking-Water Treatment with Ozone. Environmental science & technology 21(3), 224-230.
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? The Science of the total environment 408(23), 5997-6010.
Gunawan, Y., Nemati, M. and Dalai, A., 2014. Biodegradation of a Surrogate Naphthenic Acid under Denitrifying Conditions. Water research 51, 11-24.
Han, S.-S., Bae, T.-H., Jang, G.-G. and Tak, T.-M., 2005. Influence of Sludge Retention Time on Membrane Fouling and Bioactivities in Membrane Bioreactor System. Process Biochemistry 40(7), 2393-2400.
Han, X., MacKinnon, M.D. and Martin, J.W., 2009. Estimating the in Situ Biodegradation of Naphthenic Acids in Oil Sands Process Waters by Hplc/Hrms. Chemosphere 76(1), 63-70.
Han, X., 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.
He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., El-Din, M.G., Giesy, J.P. and Wiseman, S.B., 2012. Toxicity of Untreated and Ozone-Treated Oil Sands Process-Affected Water (Ospw) to Early Life Stages of the Fathead Minnow (Pimephales Promelas). Water research 46(19), 6359-6368.

Headley, J. and McMartin, D., 2004. A Review of the Occurrence and Fate of Naphthenic Acids in Aquatic Environments. Journal of Environmental Science and Health, Part A 39(8), 1989-2010.

Headley, J.V., Peru, K.M., Armstrong, S.A., Han, X., Martin, J.W., Mapolelo, M.M., Smith, D.F., Rogers, R.P. and Marshall, A.G., 2009. Aquatic Plant‐Derived Changes in Oil Sands Naphthenic Acid Signatures Determined by Low‐, High‐and Ultra High‐Resolution Mass Spectrometry. Rapid Communications in Mass Spectrometry 23(4), 515-522.

Headley, J.V., Peru, K.M., McMartin, D.W. and Winkler, M., 2002. Determination of Dissolved Naphthenic Acids in Natural Waters by Using Negative-Ion Electrospray Mass Spectrometry. Journal of AOAC International 85(1), 182-187.

Heider, J., Boll, M., Breese, K., Breinig, S., Ebenau-Jehle, C., Feil, U., Gad'on, N., Laempe, D., Leuthner, B. and Mohamed, M.E.-S., 1998. Differential Induction of Enzymes Involved in Anaerobic Metabolism of Aromatic Compounds in the Denitrifying Bacterium Thauera Aromatica. Archives of microbiology 170(2), 120-131.

Heider, J. and Fuchs, G., 1997. Microbial Anaerobic Aromatic Metabolism. Anaerobe 3(1), 1-22.

Heider, J., Spormann, A.M., Beller, H.R. and Widdel, F., 1999. Anaerobic Bacterial Metabolism of Hydrocarbons. FEMS Microbiology Reviews 22, 459-473.

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., Sato, S. and Fukui, M., 2009. Microbial Community Analysis at Crude Oil-Contaminated Soils Targeting the 16s Ribosomal Rna, Xylm, C23o, and Bcr Genes. Journal of applied microbiology 107(1), 126-135.

Hochstein, L.I. and Tomlinson, G.A., 1988. The Enzymes Associated with Denitrification. Annual Reviews in Microbiology 42(1), 231-261.

Hofs, B., Ogier, J., Vries, D., Beerendonk, E.F. and Cornelissen, E.R., 2011. Comparison of Ceramic and Polymeric Membrane Permeability and Fouling Using Surface Water. Separation and purification Technology 79(3), 365-374.

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.

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.

Huang, C., Shi, Y., El-Din, M.G. and Liu, Y., 2015. Treatment of Oil Sands Process-Affected Water (Ospw) Using Ozonation Combined with Integrated Fixed-Film Activated Sludge (Ifas). Water research 85, 167-176.

Huang, X., Gui, P. and Qian, Y., 2001. Effect of Sludge Retention Time on Microbial Behaviour in a Submerged Membrane Bioreactor. Process Biochemistry 36(10), 1001-1006.

Hwang, G., Dong, T., Islam, M.S., Sheng, Z., Pérez-Estrada, L.a., Liu, Y. and Gamal El-Din, M., 2013. The Impacts of Ozonation on Oil Sands Process-Affected Water Biodegradability and Biofilm Formation Characteristics in Bioreactors. Bioresource technology 130C, 269-277.

Hwang, G., Kang, S., El-Din, M.G. and Liu, Y., 2012a. Impact of an Extracellular Polymeric Substance (Eps) Precoating on the Initial Adhesion of Burkholderia Cepacia and Pseudomonas Aeruginosa. Biofouling 28(6), 525-538.

Hwang, G., Kang, S., El-Din, M.G. and Liu, Y., 2012b. Impact of Conditioning Films on the Initial Adhesion of Burkholderia Cepacia. Colloids and surfaces. B, Biointerfaces 91, 181-188.
Islam, M.S., 2014. Combined Adsorption and Biodegradation Processes for Oil Sands Process-Affected Water Treatment. Doctoral, University of Alberta.
Islam, M.S., Dong, T., Sheng, Z., Zhang, Y., Liu, Y., Gamal El-Din, M. and El-Din, M.G., 2013. Microbial Community Structure and Operational Performance of a Fluidized Bed Biofilm Reactor Treating Oil Sands Process-Affected Water. International Biodeterioration & Biodegradation 91, 111-118.
Islam, M.S., Moreira, J., Chelme-Ayala, P. and El-Din, M.G., 2014. Prediction of Naphthenic Acid Species Degradation by Kinetic and Surrogate Models During the Ozonation of Oil Sands Process-Affected Water. Science of the total environment 493, 282-290.
Jarusutthirak, C. and Amy, G., 2006. Role of Soluble Microbial Products (Smp) in Membrane Fouling and Flux Decline. Environmental science & technology 40(3), 969-974.
Jiang, Y., Ulrich, A.C. and Liu, Y., 2013. Coupling Bioelectricity Generation and Oil Sands Tailings Treatment Using Microbial Fuel Cells. Bioresource technology.
Johnson, R.J., Smith, B.E., Sutton, P.a., McGenity, T.J., Rowland, S.J. and Whitby, C., 2011. Microbial Biodegradation of Aromatic Alkanoic Naphthenic Acids Is Affected by the Degree of Alkyl Side Chain Branching. The ISME journal 5(3), 486-496.
Judd, S. and Judd, C., 2011. The Mbr Book: Principles and Applications of Membrane Bioreactors for Water and Wastewater Treatment, Elsevier, Oxford.
Kanaly, R.A., Harayama, S. and Watanabe, K., 2002. Rhodanobacter Sp. Strain Bpc1 in a Benzo [a] Pyrene-Mineralizing Bacterial Consortium. Applied and environmental microbiology 68(12), 5826-5833.
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.
Kertesz, M. and Kawasaki, A., 2010. Handbook of Hydrocarbon and Lipid Microbiology, pp. 1693-1705, Springer.
Khor, S.L., Sun, D.D., Liu, Y. and Leckie, J.O., 2007. Biofouling Development and Rejection Enhancement in Long Srt Mf Membrane Bioreactor. Process Biochemistry 42(12), 1641-1648.
Kim, D.-J. and Kim, S.-H., 2006. Effect of Nitrite Concentration on the Distribution and Competition of Nitrite-Oxidizing Bacteria in Nitratation Reactor Systems and Their Kinetic Characteristics. Water research 40(5), 887-894.
Kim, E.-S., Liu, Y. and Gamal El-Din, M., 2011a. The Effects of Pretreatment on Nanofiltration and Reverse Osmosis Membrane Filtration for Desalination of Oil Sands Process-Affected Water. Separation and purification Technology 81(3), 418-428.
Kim, E.-S., Liu, Y. and Gamal El-Din, M., 2012. Evaluation of Membrane Fouling for in-Line Filtration of Oil Sands Process-Affected Water: The Effects of Pretreatment Conditions. Environmental science & technology 46(5), 2877-2884.
Kim, M., Yoo, G. and Yoo, C., 2011b. Development of Combined Fouling Model in a Membrane Bioreactor. Asia‐Pacific Journal of Chemical Engineering 6(3), 423-432.
Kim, S.-J. and Kwon, K.K., 2010. Handbook of Hydrocarbon and Lipid Microbiology. Timmis, K.N. (ed), Springer, Berlin, Heidelberg.

Kim, Y.M., Lee, D.S., Park, C., Park, D. and Park, J.M., 2011c. Effects of Free Cyanide on Microbial Communities and Biological Carbon and Nitrogen Removal Performance in the Industrial Activated Sludge Process. Water research 45(3), 1267-1279.
Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and El-Din, M.G., 2015. Effect of Ozonation on the Naphthenic Acids' Speciation and Toxicity of Ph-Dependent Organic Extracts of Oil Sands Process-Affected Water. Science of the total environment 506, 66-75.
Le-Clech, P., Chen, V. and Fane, T.a.G., 2006. Fouling in Membrane Bioreactors Used in Wastewater Treatment. Journal of Membrane Science 284(1-2), 17-53.
Ledakowicz, S., Michniewicz, M., Jagiella, A., Stufka-Olczyk, J. and Martynelis, M., 2006. Elimination of Resin Acids by Advanced Oxidation Processes and Their Impact on Subsequent Biodegradation. Water Research 40(18), 3439-3446.
Lew, B., Tarre, S., Beliavski, M., Dosoretz, C. and Green, M., 2009. Anaerobic Membrane Bioreactor (Anmbr) for Domestic Wastewater Treatment. Desalination 243(1-3), 251-257.
Li, H., Yang, M., Zhang, Y., Yu, T. and Kamagata, Y., 2006. Nitrification Performance and Microbial Community Dynamics in a Submerged Membrane Bioreactor with Complete Sludge Retention. Journal of biotechnology 123(1), 60-70.
Liao, A.B.Q., Bagley, D.M., Kraemer, H.E., Leppard, G.G. and Liss, S.N., 2013. A Review of Biofouling and Its Control in Membrane Separation Bioreactors. Water Environment Research 76(5), 425-436.
Liao, B.-Q., Kraemer, J.T. and Bagley, D.M., 2006. Anaerobic Membrane Bioreactors: Applications and Research Directions.
Lin, H., 2012. Membrane Bioreactors for Industrial Wastewater Treatment : A Critical Review. Critical Reviews in Environmental Science and Technology 42(7), 677-740.
Lin, H.J., Xie, K., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N. and Liao, B.Q., 2009. Sludge Properties and Their Effects on Membrane Fouling in Submerged Anaerobic Membrane Bioreactors (Sanmbrs). Water research 43(15), 3827-3837.
Liu, G., Bakker, G.L., Li, S., Vreeburg, J.H.G., Verberk, J.Q.J.C., Medema, G.J., Liu, W.T. and Van Dijk, J.C., 2014. Pyrosequencing Reveals Bacterial Communities in Unchlorinated Drinking Water Distribution System: An Integral Study of Bulk Water, Suspended Solids, Loose Deposits, and Pipe Wall Biofilm. Environmental science & technology.
Liu, H. and Fang, H.H.P., 2002. Extraction of Extracellular Polymeric Substances (Eps) of Sludges. Journal of biotechnology 95(3), 249-256.
Loy, A., Schulz, C., Lücker, S., Stoecker, K., Baranyi, C., Lehner, A., Wagner, M., Lu, S. and Scho, A., 2005. Microarray for Environmental Monitoring of the Betaproteobacterial Order 16s Rrna Gene-Based Oligonucleotide Microarray for Environmental Monitoring of the Betaproteobacterial Order “Rhodocyclales”. Applied and environmental microbiology 71(3), 1373-1373.
Ma, J., Wang, Z., Yang, Y., Mei, X. and Wu, Z., 2013a. Correlating Microbial Community Structure and Composition with Aeration Intensity in Submerged Membrane Bioreactors by 454 High-Throughput Pyrosequencing. Water research 47(2), 859-869.
Ma, Z., Wen, X., Zhao, F., Xia, Y., Huang, X., Waite, D. and Guan, J., 2013b. Effect of Temperature Variation on Membrane Fouling and Microbial Community Structure in Membrane Bioreactor. Bioresource technology.
MacKinnon, M., Meer, T.V. and Verbeek, A., 1993. Assessment of Biological Impact from a Wet Landscape Option for the Reclamation of Fine Tails from Oil Sands. Baddaloo, E.G.,

Ramamoorthy, S. and Moore, J.W. (eds), Alberta Environment, Environmental Protection, Edmonton.
Martin, J.W., Barri, T., Han, X., 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.
Mechichi, T., Stackebrandt, E., Gad'on, N. and Fuchs, G., 2002. Phylogenetic and Metabolic Diversity of Bacteria Degrading Aromatic Compounds under Denitrifying Conditions, and Description of Thauera Phenylacetica Sp. Nov., Thauera Aminoaromatica Sp. Nov., and Azoarcus Buckelii Sp. Nov. Archives of microbiology 178(1), 26-35.
Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S. and Yang, F., 2009. Recent Advances in Membrane Bioreactors (Mbrs): Membrane Fouling and Membrane Material. Water research 43(6), 1489-1512.
Meng, F., Shi, B., Yang, F. and Zhang, H., 2007. Effect of Hydraulic Retention Time on Membrane Fouling and Biomass Characteristics in Submerged Membrane Bioreactors. Bioprocess and Biosystems Engineering 30, 359-367.
Meng, F., Zhang, H., Li, Y., Zhang, X. and Yang, F., 2005. Application of Fractal Permeation Model to Investigate Membrane Fouling in Membrane Bioreactor. Journal of Membrane Science 262(1), 107-116.
Meng, F., Zhang, H., Yang, F., Zhang, S., Li, Y. and Zhang, X., 2006. Identification of Activated Sludge Properties Affecting Membrane Fouling in Submerged Membrane Bioreactors. Separation and purification Technology 51(1), 95-103.
Metcalf, E., Eddy, H., Tchobanoglous, G., Burton, F. and Stensel, H.D., 2003. Wastewater Engineering: Treatment and Reuse, McGraw-Hill, New York.
Mincer, T.J., Church, M.J., Taylor, L.T., Preston, C., Karl, D.M. and DeLong, E.F., 2007. Quantitative Distribution of Presumptive Archaeal and Bacterial Nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environmental microbiology 9, 1162-1175.
Misiti, T., Tandukar, M., Tezel, U. and Pavlostathis, S.G., 2013a. Inhibition and Biotransformation Potential of Naphthenic Acids under Different Electron Accepting Conditions. Water research 47(1), 406-418.
Misiti, T., Tezel, U. and Pavlostathis, S.G., 2013b. Fate and Effect of Naphthenic Acids on Oil Refinery Activated Sludge Wastewater Treatment Systems. Water research 47(1), 449-460.
Misiti, T.M., Tezel, U., Tandukar, M. and Pavlostathis, S.G., 2013c. Aerobic Biotransformation Potential of a Commercial Mixture of Naphthenic Acids. Water research 47(15), 5520-5534.
Molina-Muñoz, M., Poyatos, J., Sánchez-Peinado, M., Hontoria, E., González-López, J. and Rodelas, B., 2009. Microbial Community Structure and Dynamics in a Pilot-Scale Submerged Membrane Bioreactor Aerobically Treating Domestic Wastewater under Real Operation Conditions. Science of the total environment 407(13), 3994-4003.
Moustafa, A.M., Kim, E.-S., Alpatova, A., Sun, N., Smith, S., Kang, S. and El-Din, M.G., 2014. Impact of Polymeric Membrane Filtration of Oil Sands Process Water on Organic Compounds Quantification. Water Science & Technology 70(5), 771-779.
Oliveros, J.C., 2007. Venny. An Interactive Tool for Comparing Lists with Venn Diagrams.
Oller, I., Malato, S. and Sánchez-Pérez, J., 2011. Combination of Advanced Oxidation Processes and Biological Treatments for Wastewater Decontamination—a Review. Science of the total environment 409(20), 4141-4166.

Oren, A., 2014. The Prokaryotes, pp. 975-998, Springer.
Pan, J.R., Su, Y.-C., Huang, C. and Lee, H.-C., 2010. Effect of Sludge Characteristics on Membrane Fouling in Membrane Bioreactors. Journal of Membrane Science 349(1), 287-294.
Parsons, J. and Govers, H., 1990. Quantitative Structure-Activity Relationships for Biodegradation. Ecotoxicology and environmental safety 19(2), 212-227.
Peltola, R., 2008. Bioavailability Aspects of Hydrophobic Contaminant Degradation in Soils.
Peng, H., Volchek, K., MacKinnon, M., Wong, W.P. and Brown, C.E., 2004. Application of Nanofiltration to Water Management Options for Oil Sands Operation. Desalination 170(2), 137-150.
Pereira, A.S., Islam, S., El-din, M.G. and Martin, J.W., 2013. Ozonation Degrades All Detectable Organic Compound Classes in Oil Sands Process-Affected Water ; an Application of High-Performance Liquid Chromatography / Obitrap Mass Spectrometry. Rapid communications in mass spectrometry : RCM (27), 1-10.
Pérez-Estrada, L.A., Han, X., Drzewicz, P., Gamal El-Din, M., Fedorak, P.M. and Martin, J.W., 2011. Structure–Reactivity of Naphthenic Acids in the Ozonation Process. Environmental science & technology 45(17), 7431-7437.
Pérez‐Pantoja, D., Donoso, R., Agulló, L., Córdova, M., Seeger, M., Pieper, D.H. and González, B., 2012. Genomic Analysis of the Potential for Aromatic Compounds Biodegradation in Burkholderiales. Environmental microbiology 14(5), 1091-1117.
Philippot, L. and Hallin, S., 2005. Finding the Missing Link between Diversity and Activity Using Denitrifying Bacteria as a Model Functional Community. Current opinion in microbiology 8(3), 234-239.
Pourrezaei, P., 2013. Physico-Chemical Processes for Oil Sands Process-Affected Water Treatment, University of Alberta.
Prosser, I., 1989. Autotrophic Nitrification in Bacteria. Adv. Microb. Physiol 20, 125-192.
Qin, J., Oo, M., Tao, G. and Kekre, K., 2007. Feasibility Study on Petrochemical Wastewater Treatment and Reuse Using Submerged Mbr. Journal of Membrane Science 293(1-2), 161-166.
Qu, X., Gao, W., Han, M., Chen, A. and Liao, B., 2013. Effect of Hydraulic Retention Time on Sludge Properties, Cake Layer Structure, and Membrane Fouling in a Thermophilic Submerged Aerobic Membrane Bioreactor. Separation science and technology 48(10), 1529-1536.
Quagraine, E., Peterson, H. and Headley, J., 2005. In Situ Bioremediation of Naphthenic Acids Contaminated Tailing Pond Waters in the Athabasca Oil Sands Region—Demonstrated Field Studies and Plausible Options: A Review. Journal of Environmental Science and Health 40(3), 685-722.
Rahman, K., Rahman, T., Lakshmanaperumalsamy, P., Marchant, R. and Banat, I., 2003. The Potential of Bacterial Isolates for Emulsification with a Range of Hydrocarbons. Acta biotechnologica 23(4), 335-345.
Reichenbach, H., 1991. Dworkin^ the Myxobacteria. Starr KP, Stolp H, Trper HG, et a1. The Prokaryotes 4, 3.
Robles, a., Ruano, M.V., Ribes, J. and Ferrer, J., 2012. Sub-Critical Long-Term Operation of Industrial Scale Hollow-Fibre Membranes in a Submerged Anaerobic Mbr (Hf-Sanmbr) System. Separation and purification Technology 100, 88-96.

Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J. and Chu, K.-H., 2009. Biodegradation Potential of Wastewater Micropollutants by Ammonia-Oxidizing Bacteria. Chemosphere 77(8), 1084-1089.
Röling, W.F., Milner, M.G., Jones, D.M., Lee, K., Daniel, F., Swannell, R.J. and Head, I.M., 2002. Robust Hydrocarbon Degradation and Dynamics of Bacterial Communities During Nutrient-Enhanced Oil Spill Bioremediation. Applied and environmental microbiology 68(11), 5537-5548.
Rowland, S.J., Scarlett, A.G., Jones, D., West, C.E. and Frank, R.A., 2011. Diamonds in the Rough: Identification of Individual Naphthenic Acids in Oil Sands Process Water. Environmental science & technology 45(7), 3154-3159.
Salinero, K.K., Keller, K., Feil, W.S., Feil, H., Trong, S., Di Bartolo, G. and Lapidus, A., 2009. Metabolic Analysis of the Soil Microbe Dechloromonas Aromatica Str. Rcb: Indications of a Surprisingly Complex Life-Style and Cryptic Anaerobic Pathways for Aromatic Degradation. BMC genomics 10(1), 1.
Sayavedra-Soto, L.a., Gvakharia, B., Bottomley, P.J., Arp, D.J. and Dolan, M.E., 2010. Nitrification and Degradation of Halogenated Hydrocarbons--a Tenuous Balance for Ammonia-Oxidizing Bacteria. Applied microbiology and biotechnology 86(2), 435-444.
Scarlett, A.G., Reinardy, H.C., Henry, T.B., West, C.E., Frank, R.a., Hewitt, L.M. and Rowland, S.J., 2013. Acute Toxicity of Aromatic and Non-Aromatic Fractions of Naphthenic Acids Extracted from Oil Sands Process-Affected Water to Larval Zebrafish. Chemosphere.
Schmidt, I., Sliekers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J.G., Jetten, M.S.M. and Strous, M., 2003. New Concepts of Microbial Treatment Processes for the Nitrogen Removal in Wastewater. FEMS Microbiology Reviews 27(4), 481-492.
Schramm, A., de Beer, D., van den Heuvel, J.C., Ottengraf, S. and Amann, R., 1999. Microscale Distribution of Populations and Activities Ofnitrosospira and Nitrospira Spp. Along a Macroscale Gradient in a Nitrifying Bioreactor: Quantification by in Situ Hybridization and the Use of Microsensors. Applied and environmental microbiology 65(8), 3690-3696.
Scott, A.C., MacKinnon, M.D. and Fedorak, P.M., 2005a. Naphthenic Acids in Athabasca Oil Sands Tailings Waters Are Less Biodegradable Than Commercial Naphthenic Acids. Environmental Science and Technology 39(21), 8388-8394.
Scott, A.C., Mackinnon, M.D. and Fedorak, P.M., 2005b. Naphthenic Acids in Athabasca Oil Sands Tailings Waters Are Less Biodegradable Than Commercial Naphthenic Acids. Environmental science & technology 39(21), 8388-8394.
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.
Seidel, A. and Elimelech, M., 2002. Coupling between Chemical and Physical Interactions in Natural Organic Matter (Nom) Fouling of Nanofiltration Membranes: Implications for Fouling Control. Journal of Membrane Science 203(1), 245-255.
Seviour, R.J. and Nielsen, P.H., 2010. Microbial Ecology of Activated Sludge, IWA publishing.
Shao, J., Hou, J. and Song, H., 2011. Comparison of Humic Acid Rejection and Flux Decline During Filtration with Negatively Charged and Uncharged Ultrafiltration Membranes. Water research 45(2), 473-482.
Shariati, S.R.P., Bonakdarpour, B., Zare, N. and Ashtiani, F.Z., 2011. The Effect of Hydraulic Retention Time on the Performance and Fouling Characteristics of Membrane

Sequencing Batch Reactors Used for the Treatment of Synthetic Petroleum Refinery Wastewater. Bioresource technology 102(17), 7692-7699.
Shell, 2009. Royal Dutch Shell Plc Sustainability Report.
Sheng, G.-P., Yu, H.-Q. and Li, X.-Y., 2010. Extracellular Polymeric Substances (Eps) of Microbial Aggregates in Biological Wastewater Treatment Systems: A Review. Biotechnology advances 28(6), 882-894.
Shestakova, N., Korshunova, A., Mikhailova, E., Sokolova, D.S., Tourova, T., Belyaev, S., Poltaraus, A. and Nazina, T., 2011. Characterization of the Aerobic Hydrocarbon-Oxidizing Enrichments from a High-Temperature Petroleum Reservoir by Comparative Analysis of DNA-and Rna-Derived Clone Libraries. Microbiology 80(1), 60-69.
Shi, Y., Huang, C., Rocha, K.C., El-Din, M.G. and Liu, Y., 2015. Treatment of Oil Sands Process-Affected Water Using Moving Bed Biofilm Reactors: With and without Ozone Pretreatment. Bioresource technology.
Singh, S.N., 2012. Microbial Degradation of Xenobiotics, Springer.
Sipma, J., Osuna, B., Collado, N., Monclús, H., Ferrero, G., Comas, J. and Rodriguez-Roda, I., 2010. Comparison of Removal of Pharmaceuticals in Mbr and Activated Sludge Systems. Desalination 250(2), 653-659.
Siripong, S. and Rittmann, B.E., 2007. Diversity Study of Nitrifying Bacteria in Full-Scale Municipal Wastewater Treatment Plants. Water research 41(5), 1110-1120.
Skouteris, G., Hermosilla, D., López, P., Negro, C. and Blanco, Á., 2012. Anaerobic Membrane Bioreactors for Wastewater Treatment: A Review. Chemical Engineering Journal 198-199, 138-148.
Song, Y., Dong, B., Gao, N. and Xia, S., 2010. Huangpu River Water Treatment by Microfiltration with Ozone Pretreatment. Desalination 250(1), 71-75.
Stephenson, T., Judd, S., Jefferson, B., Brindle, K. and Association, I.W., 2000. Membrane Bioreactors for Wastewater Treatment, Citeseer.
Stumm, W. and Morgan, J.J., 2012. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters, John Wiley & Sons.
Suarez, S., Lema, J.M. and Omil, F., 2010. Removal of Pharmaceutical and Personal Care Products (Ppcps) under Nitrifying and Denitrifying Conditions. Water research 44(10), 3214-3224.
Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M. and Hagen, M., 2014. Advanced Analytical Mass Spectrometric Techniques and Bioassays to Characterize Untreated and Ozonated Oil Sands Process-Affected Water. Environmental science & technology 48(19), 11090-11099.
Tewari, P., Singh, R., Batra, V. and Balakrishnan, M., 2012. Field Testing of Polymeric Mesh and Ash-Based Ceramic Membranes in a Membrane Bioreactor (Mbr) for Decentralised Sewage Treatment. Water SA 38(5), 727-730.
The Government of Alberta, 2015. Lower Athabasca Region-Tailings Management Framework for the Mineable Athabasca Oil Sands. The Government of Alberta (ed).
Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M. and Michel, G., 2011. Environmental and Gut Bacteroidetes: The Food Connection. Frontiers in microbiology 2.
Timmis, K.N., McGenity, T., Van Der Meer, J. and De Lorenzo, V., 2010. Handbook of Hydrocarbon and Lipid Microbiology, Springer Berlin/Heidelberg.

Tran, N.H., Urase, T. and Kusakabe, O., 2009. The Characteristics of Enriched Nitrifier Culture in the Degradation of Selected Pharmaceutically Active Compounds. Journal of hazardous materials 171(1), 1051-1057.
Um, Y., 2004. Isolation and Characterization of Polycyclic Aromatic Hydrocarbon-Degrading Microorganisms under Methanogenic Conditions.
Vaiopoulou, E., Misiti, T.M. and Pavlostathis, S.G., 2015. Removal and Toxicity Reduction of Naphthenic Acids by Ozonation and Combined Ozonation-Aerobic Biodegradation. Bioresource technology 179, 339-347.
Van den Tweel, W., Smits, J. and De Bont, J., 1988. Catabolism of Dl-A-Phenylhydracrylic, Phenylacetic and 3-and 4-Hydroxyphenylacetic Acid Via Homogentisic Acid in a Flavobacterium Sp. Archives of microbiology 149(3), 207-213.
Van Geluwe, S., Braeken, L. and Van der Bruggen, B., 2011. Ozone Oxidation for the Alleviation of Membrane Fouling by Natural Organic Matter: A Review. Water research 45(12), 3551-3570.
Verstraete, W. and Philips, S., 1998. Nitrification-Denitrification Processes and Technologies in New Contexts. Environmental Pollution 102(1), 717-726.
Viero, A.F. and Sant'Anna, G.L., 2008. Is Hydraulic Retention Time an Essential Parameter for Mbr Performance? Journal of hazardous materials 150(1), 185-186.
Visvanathan, C., Aim, R.B. and Parameshwaran, K., 2010. Membrane Separation Bioreactors for Wastewater Treatment. Critical Reviews in Environmental Science and Technology 30(1), 1-48.
von Sperling, M., 2007. Activated Sludge and Aerobic Biofilm Reactors, IWA publishing.
Wagner, M., Amann, R., Kämpfer, P., Assmus, B., Hartmann, A., Hutzler, P., Springer, N. and Schleifer, K.-H., 1994. Identification and in Situ Detection of Gram-Negative Filamentous Bacteria in Activated Sludge. Systematic and Applied Microbiology 17(3), 405-417.
Wang, B., Wan, Y., Gao, Y., Yang, M. and Hu, J., 2013a. Determination and Characterization of Oxy-Naphthenic Acids in Oilfield Wastewater. Environmental science & technology 47(16), 9545-9554.
Wang, C., Yediler, A., Lienert, D., Wang, Z. and Kettrup, A., 2003. Ozonation of an Azo Dye C.I. Remazol Black 5 and Toxicological Assessment of Its Oxidation Products. Chemosphere 52(7), 1225-1232.
Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Garcia-Garcia, E., Pun, J., Martin, J.W., Belosevic, M. and Gamal El-Din, M., 2013b. Impact of Ozonation on Naphthenic Acids Speciation and Toxicity of Oil Sands Process-Affected Water to Vibrio Fischeri and Mammalian Immune System. Environmental science & technology 47(12), 6518-6526.
Wang, X., Chen, M., Xiao, J., Hao, L., Crowley, D.E., Zhang, Z., Yu, J., Huang, N., Huo, M. and Wu, J., 2015. Genome Sequence Analysis of the Naphthenic Acid Degrading and Metal Resistant Bacterium Cupriavidus Gilardii Cr3. PloS one 10(8), e0132881.
Wang, Z., Wu, Z. and Tang, S., 2009. Extracellular Polymeric Substances (Eps) Properties and Their Effects on Membrane Fouling in a Submerged Membrane Bioreactor. Water research 43(9), 2504-2512.
Wang, Z., Wu, Z., Yin, X. and Tian, L., 2008. Membrane Fouling in a Submerged Membrane Bioreactor (Mbr) under Sub-Critical Flux Operation: Membrane Foulant and Gel Layer Characterization. Journal of Membrane Science 325(1), 238-244.

Weissbrodt, D.G., Lochmatter, S., Ebrahimi, S., Rossi, P., Maillard, J. and Holliger, C., 2012. Bacterial Selection During the Formation of Early-Stage Aerobic Granules in Wastewater Treatment Systems Operated under Wash-out Dynamics. Front Microbiol 3, 332.

Wells, G.F., Park, H.-D., Yeung, C.-H., Eggleston, B., Francis, C.a. and Criddle, C.S., 2009. Ammonia-Oxidizing Communities in a Highly Aerated Full-Scale Activated Sludge Bioreactor: Betaproteobacterial Dynamics and Low Relative Abundance of Crenarchaea. Environmental microbiology 11(9), 2310-2328.

Wen, G., Ma, J., Zhang, L. and Yu, G., 2010. Integrated Membrane Operations in Various Industrial Sectors: Case Studies, pp. 195-209, Elsevier B.V.

Whitby, C., 2010. Microbial Naphthenic Acid Degradation. Advances in applied microbiology 70, 93-125.

Wu, J., Le-Clech, P., Stuetz, R.M., Fane, A.G. and Chen, V., 2008. Effects of Relaxation and Backwashing Conditions on Fouling in Membrane Bioreactor. Journal of Membrane Science 324(1-2), 26-32.

Xue, J., Zhang, Y., Liu, Y. and El-Din, M.G., 2016. Treatment of Oil Sands Process-Affected Water (Ospw) Using a Membrane Bioreactor with a Submerged Flat-Sheet Ceramic Microfiltration Membrane. Water research 88, 1-11.

Yang, S.-F., Tay, J.-H. and Liu, Y., 2004. Inhibition of Free Ammonia to the Formation of Aerobic Granules. Biochemical Engineering Journal 17(1), 41-48.

Yang, Y., Li, M., Michels, C., Moreira‐Soares, H. and Alvarez, P.J., 2014a. Differential Sensitivity of Nitrifying Bacteria to Silver Nanoparticles in Activated Sludge. Environmental Toxicology and Chemistry 33(10), 2234-2239.

Yang, Y., Quensen, J., Mathieu, J., Wang, Q., Wang, J., Li, M., Tiedje, J.M. and Alvarez, P.J.J., 2014b. Pyrosequencing Reveals Higher Impact of Silver Nanoparticles Than Ag+ on the Microbial Community Structure of Activated Sludge. Water research 48, 317-325.

Yergeau, E., Lawrence, J.R., Sanschagrin, S., Waiser, M.J., Korber, D.R. and Greer, C.W., 2012. Next-Generation Sequencing of Microbial Communities in the Athabasca River and Its Tributaries in Relation to Oil Sands Mining Activities. Applied and environmental microbiology 78(21), 7626-7637.

You, H., Huang, C., Pan, J. and Chang, S., 2006. Behavior of Membrane Scaling During Crossflow Filtration in the Anaerobic Mbr System. Separation science and technology 41(7), 1265-1278.

Zhang, H., Zhai, H., Ji, M., Su, X., Du, Z. and Liu, J., 2015a. Long-Term Effect of Cr (Vi) on Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria in an Activated Sludge System. Desalination and Water Treatment 54(7), 1981-1989.

Zhang, Y., McPhedran, K.N. and El-Din, M.G., 2015b. Pseudomonads Biodegradation of Aromatic Compounds in Oil Sands Process-Affected Water. Science of the total environment 521, 59-67.

Zhang, Y., Xue, J., Liu, Y. and El-Din, M.G., 2016. Treatment of Oil Sands Process-Affected Water (Ospw) Using Membrane Bioreactor Coupled with Ozonation: A Comparative Study. Chemical Engineering Journal 302(October), 485-497.

Zhou, H. and Smith, D.W., 2002. Advanced Technologies in Water and Wastewater Treatment. Journal of Environmental Engineering and Science (1), 247-264.

Zsirai, T., Buzatu, P., Aerts, P. and Judd, S., 2012. Efficacy of Relaxation, Backflushing, Chemical Cleaning and Clogging Removal for an Immersed Hollow Fibre Membrane Bioreactor. Water research 46(14), 4499-4507.

Zubot, W., MacKinnon, M.D., Chelme-Ayala, P., Smith, D.W. and Gamal El-Din, M., 2012. Petroleum Coke Adsorption as a Water Management Option for Oil Sands Process-Affected Water. The Science of the total environment 427-428, 364-372.

Appendix

Table A 1. Pearson Correlation Coefficient between the major orders and removal rates of classical NAs with different *n*.

Major orders over the study duration	Molecular carbon number n														
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Proteobacteria;c_ *β*-Proteobacteria;o_Rhodocyclales	0.122	0.215	0.248	0.726	0.739	0.706	0.714	0.755	0.743	0.428	0.165	-0.138	-0.090	-0.411	-0.570
Proteobacteria;c_ *β*-Proteobacteria;o_ASSO-13	-0.364	0.036	0.290	0.562	0.229	0.135	0.036	0.152	0.143	-0.099	-0.339	-0.724	-0.609	-0.691	-0.946
Proteobacteria;c_ *β*-Proteobacteria;o_Nitrosomonadales	0.544	-0.059	0.207	0.277	-0.128	-0.226	-0.289	-0.183	-0.159	-0.353	-0.561	-0.833	-0.664	-0.680	-0.821
Bacteroidetes;c_Cytophagia;o_Cytophagales	0.670	0.393	-0.057	-0.212	0.197	0.273	0.311	0.174	0.093	0.479	0.755	0.981	0.827	0.857	0.766
Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales	-0.123	-0.072	0.363	-0.090	-0.435	-0.446	-0.445	-0.478	-0.405	-0.280	-0.289	-0.407	-0.629	0.056	0.088
Bacteroidetes;c_[Saprospirae];o_[Saprospirales]	-0.042	-0.192	-0.207	-0.693	-0.697	-0.658	-0.633	-0.743	-0.749	-0.377	-0.075	0.200	0.088	0.497	0.596
Nitrospirae;c_Nitrospira;o_Nitrospirales	-0.340	-0.628	-0.550	-0.880	-0.860	-0.807	-0.738	-0.789	-0.730	-0.634	-0.420	-0.008	0.010	0.171	0.590
Planctomycetes;c_Phycisphaerae;o_Phycisphaerales	-0.795	-0.698	-0.320	-0.353	-0.661	-0.695	-0.668	-0.569	-0.455	-0.776	-0.916	-0.831	-0.634	-0.673	-0.301
Proteobacteria;c_ *β*-proteobacteria;o_Burkholderiales	0.075	-0.041	-0.262	-0.710	-0.575	-0.526	-0.456	-0.569	-0.574	-0.220	0.064	0.426	0.392	0.582	0.730
Proteobacteria;c_ *γ*-Proteobacteria;o_Xanthomonadales	-0.634	-0.438	-0.021	-0.179	-0.554	-0.597	-0.568	-0.488	-0.368	-0.612	-0.787	-0.814	-0.709	-0.558	-0.291
Acidobacteria;c_[Chloracidobacteria];o_RB41	-0.008	-0.141	-0.133	-0.649	-0.666	-0.627	-0.587	-0.695	-0.684	-0.332	-0.067	0.190	0.063	0.500	0.621
Proteobacteria;c_ *α*-proteobacteria;o_Rhizobiales	-0.662	-0.436	-0.477	-0.443	-0.509	-0.532	-0.420	-0.319	-0.205	-0.562	-0.730	-0.457	-0.056	-0.563	-0.097
Cyanobacteria;c_4C0d-2;o_MLE1-12	-0.145	-0.295	0.008	-0.429	-0.582	-0.549	-0.467	-0.505	-0.390	-0.354	-0.335	-0.188	-0.323	0.131	0.470
[Thermi];c_Deinococci;o_Deinococcales	0.205	0.038	-0.096	-0.573	-0.482	-0.434	-0.410	-0.548	-0.583	-0.126	0.203	0.451	0.285	0.693	0.689

Table A 2. Pearson Correlation Coefficient between the major orders and removal rates of classical NAs with different *Z*

Major orders over the study duration	Absolute value of hydrogen deficiency Z									
	0	2	4	6	8	10	12	14	16	18
Proteobacteria;c_ β-Proteobacteria;o_Rhodocyclales		0.445	0.585	0.794	0.895	0.591	0.567	0.208	0.338	0.367
Proteobacteria;c_ β-Proteobacteria;o_ASSO-13		-0.291	-0.076	0.286	0.464	0.188	0.292	-0.384	-0.328	0.057
Proteobacteria;c_ β-Proteobacteria;o_Nitrosomonadales		-0.560	-0.440	-0.061	0.150	-0.053	0.152	-0.614	-0.578	-0.166
Bacteroidetes;c_Cytophagia;o_Cytophagales		0.613	0.530	0.060	-0.291	0.215	-0.105	0.762	0.680	0.355
Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales		-0.543	-0.576	-0.398	-0.376	-0.347	-0.114	-0.350	-0.426	-0.070
Bacteroidetes;c_[Saprospirae];o_[Saprospirales]		-0.419	-0.519	-0.771	-0.920	-0.585	-0.611	-0.132	-0.288	-0.296
Nitrospirae;c_Nitrospira;o_Nitrospirales		-0.524	-0.703	-0.849	-0.786	-0.803	-0.686	-0.411	-0.488	-0.663
Planctomycetes;c_Phycisphaerae;o_Phycisphaerales		-0.784	-0.855	-0.526	-0.162	-0.620	-0.257	-0.897	-0.846	-0.723
Proteobacteria;c_ β-Proteobacteria;o_Burkholderiales		-0.161	-0.346	-0.655	-0.826	-0.408	-0.462	0.044	-0.070	-0.233
Proteobacteria;c_ γ-Proteobacteria;o_Xanthomonadales		-0.722	-0.800	-0.426	-0.124	-0.458	-0.072	-0.787	-0.745	-0.500
Acidobacteria;c_[Chloracidobacteria];o_RB41		-0.383	-0.512	-0.727	-0.865	-0.535	-0.521	-0.116	-0.256	-0.254
Proteobacteria;c_ α-Proteobacteria;o_Rhizobiales		-0.376	-0.613	-0.385	-0.048	-0.332	-0.013	-0.646	-0.517	-0.679
Cyanobacteria;c_4C0d-2;o_MLE1-12		-0.410	-0.605	-0.518	-0.437	-0.485	-0.214	-0.325	-0.357	-0.307
[Thermi];c_Deinococci;o_Deinococcales		-0.162	-0.253	-0.592	-0.845	-0.374	-0.497	0.144	-0.018	-0.068