Application of Ozone and Peroxone Processes for Naphthenic Acids Degradation in Oil Sands Process-Affected Water: Characterization of Water Before and After Treatment

by

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ABSTRACT

Appling ozone (O_3) with high doses (>100 mg/L) to remove naphthenic acids (NAs) from oil sands process-affected water (OSPW); limits its application and feasibility in the OSPW remediation. To decrease the required doses and their associated costs, this study examined the application of ozone (O_3) and peroxone (hydrogen peroxide/ozone; H_2O_2:O_3) processes for the treatment of OSPW using mild oxidant doses (i.e., ozone doses of 30 and 50 mg/L and H_2O_2 doses of 10, 11 and 20 mg/L). The performance of both processes was compared in terms of structure reactivity of NAs, the dominant pathways for removal, the kinetics of individual NA species and variation of compositions and abundance of species before and after treatment.

To attain/ensure better characterization for the contaminants of concern (NAs) in water samples, the initial phase of this research encompassed examining two different analytical methods (Fourier transform infrared (FTIR) spectroscopy and ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS)) with different extraction/pre-treatment methods for samples; liquid-liquid extraction (LLE) and solid-phase extraction (SPE). A correlation between these methods was developed to implement the best techniques available for sample analysis. The results after examining groundwater and OSPW samples showed higher recovery of classical and oxidized NAs or (O_x-NAs) and naphthenic acid fraction compounds (NAFCs) for SPE compared to LLE, regardless the water source and quantification methods (i.e., FTIR and UPLC-TOFMS). However, higher abundance for classical NAs (O_2-NAs) was found in LLE than SPE (e.g., OSPW samples:

(63.1±2.1%) versus (58.5±3.0%)). A strong correlation was observed between the UPLC-TOFMS and FTIR which highlights the possibility of using FTIR and Fluka as a standard with LLE pretreated samples as an affordable substitute to the high resolution techniques (e.g. UPLC-TOFMS).

In the second phase of this research, the structure reactivity and reaction pathways during ozonation and peroxone treatment were investigated. Suppressing the hydroxyl radical (•OH) pathway by adding the scavenger tert-butyl alcohol did significantly reduce the degradation in all treatments, while molecular ozone contribution was 50% and 35% for O_2-NAs and O_x-NAs, respectively. Structure reactivity was observed with a degradation increase for both O_2-NAs and O_x-NAs with the increase of both carbon (n) and hydrogen deficiency (i.e., |-Z| numbers, double bond equivalent (DBE)) for all treatments.

The variations in the compositions of treated water were evaluated using two different high resolution mass spectrometry methods; UPLC-TOFMS and Fourier transform ion cyclotron resonance. Assessing two markers (O_2S:O_3S:O_4S and O_2:O_4 ratios) revealed changes and similarities of the peroxone treated water (i.e., 20 mg/L H_2O_2: 50 mg/L O_3 at 1:2 ratio) to natural waters. Both ratios decreased from 2.7:4.8:2.1 and 3.59 in raw OSPW to 0:1.4:0.5 and 0.7, respectively, becoming close to the reported ratios in natural waters. Although peroxone (1:2) 20+50 (i.e., 20 mg/L H_2O_2: 50 mg/L O_3) and 50 mg/L ozone were the two most effective treatments to degrade O_2-NAs and O_x-NAs (e.g., for O_2-NAs: 86% and 84%, respectively) as well as to reduce the toxicity toward *Vibrio fischeri* (40% and 50%, respectively), the fastest kinetics treatments were observed at peroxone (1:1) 20+30 (i.e., 20 mg/L H_2O_2:

30 mg/L O_3) and 30 mg/L ozone (i.e., reaction rate constant of 0.236 min^{-1} and 0.251 min^{-1}, respectively). The increase of the DBE increased the reaction rate constant, specifically at DBE = 7-9 with similar values at DBE =3-6.

With respect to *in vitro* assays, while the highest production of nitrite (i.e., attributed as the lowest toxicity effects on the goldfish primary kidney macrophages) was observed in peroxone (1:2) 11+30 (i.e., 11 mg/L H_2O_2: 30 mg/L O_3) followed by peroxone (1:3) 10+50 (i.e., 10 mg/L H_2O_2: 50 mg/L O_3), their O_2-NA degradation was the lowest, 47% and 61%, respectively.

The residual toxic effects after different ozone and peroxone processes, suggest that part of OSPW toxicity may be caused by specific compounds of NAs (i.e., similar reduction (50%) was achieved in both toxicity and abundance in O_2 species with carbon 15-26) and/or generated by-products (e.g., O_3S classes at DBE = 4 and $C_9H_{12}O_2$ at DBE = 4). Although by-products were generated, slight enhancement in the biodegradability and the reduction of chemical oxygen demand was achieved in peroxone at 1:2 ratio compared to ozone, suggesting the possibility of using combined OSPW remediation approaches (i.e., peroxone coupled with biological process).

PREFACE

All research completed on this thesis is an original work in which I, Mohamed Meshref, planned, designed and conducted all these experiments as well as the interpretation, the analysis of the data and the preparation of the manuscripts, under full revision and the supervision of Dr. Mohamed Gamal El-Din. Some colleagues also contributed to manuscript edits, sample analysis, or chemical preparation, and some of them were co-authors of the manuscripts submitted for publication. Other analyses were done in other departments of the University of Alberta, as specified below.

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done by me with the help of Mr. Yuan Chen and Ms. Shimiao Dong in Dr. Gamal El-Din's research group. Dr. Singh and Dr. Belosevic contributed to the analysis of toxicity on goldfish primary kidney macrophages.

DEDICATION

This work is dedicated:

- *To my beloved father and the soul of my mother who gave me strength and encouragement, words can never tell how much I am grateful to you for your care and love.*
- *To my beloved wife Nesma, my hero Ahmed and my lovely daughter Salma. You are an endless support, joy and inspiration to me.*
- *To my best sister and brother in the world who are far away but their prayers are always with me.*
- *To my lovely aunt and my parents-in-law. You always pushed me for greater achievements; you were always there for me when I needed you.*

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NOMENCLATURE

OSPW	Oil sands process-affected water
O_3	Ozone
H_2O_2	Hydrogen peroxide
H_2O_2/O_3	Peroxone
GW	Groundwater
AOPs	Advanced oxidation processes
TBA	Tert-butyl alcohol
NAs	naphthenic acids
NAFCs	naphthenic acid fractions compounds
AEF	Acid-extractable organic fractions
DBE	Double bond equivalent
O_2-NAs	Classical naphthenic acids
O_x-NAs	Sum of classical NAs and oxidized NAs
DCM	Dichloromethane
LLE	Liquid-liquid extraction
SPE	Solid-phase extraction
UPLC-TOFMS	Ultra-performance liquid chromatography time-of-flight mass spectrometry
FTICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
H_2SO_4	Sulphuric acid
FTIR	Fourier transform infrared spectrometry
•OH	Hydroxyl radical
TOC	Total organic carbon
DOC	Dissolved organic carbon
BOD_5	5 days biochemical oxygen demand
hr	Hour
min	Minute
k	Pseudo first-order rate constant (min^{-1})
PKMs	Primary kidney macrophages

1 GENERAL INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Background and Motivation

1.1.1 Oil sands process-affected water

Oil sands deposits in northern Alberta are estimated to contain 900 billion barrels of bitumen, with over 169 billion barrels (i.e., around 27000 million m^3) currently considered as economically recoverable for conversion to oil (Brown and Ulrich 2015, ERCB 2012). Although being the third largest oil reserve/deposit known in the world and contributing to the Canada's economic growth, the oil sands industry has an adverse impact on the environment in Alberta. Specifically, the mining operation or the bitumen extraction uses alkaline hot water (i.e., Clark process), resulting in the generation of large volumes of oil sands process-affected water (OSPW) (Allen 2008). For every barrel of bitumen, a corresponding 1.6 barrels of fresh water are required (McQueen et al. 2017). Currently, OSPWs are stored in tailing ponds in order to comply with the zero discharge practice, following the provincial environmental legislation (Brown and Ulrich 2015, Martin 2015).

1.1.2 OSPW toxicity

The release of OSPW into the environment will possess an extreme risk due to its toxicity. The toxicological effects of OSPW to aquatic organisms were initially attributed to the organic acid fraction (i.e., naphthenic acids (NAs)) (Grewer et al. 2010b, Rowland et al. 2011a, Rowland et al. 2011b, Scott et al.

2005). Considered to be one of the main contributors of the acute toxicity of OSPW (Anderson et al. 2012, Garcia-Garcia et al. 2011b, Jones et al. 2013, Lo et al. 2006), NAs have been the focus of many OSPW treatment studies (Huang et al. 2015a, Leshuk et al. 2016, Wang et al. 2016a, Wang et al. 2016b, Xue et al. 2016, Zhang et al. 2016). The significant toxicity of NAs toward bacteria, fish (Dorn et al. 1992) and mammals (Rogers et al. 2002c) among other organisms has been reported (He et al. 2012a). This toxicity has been partially associated to specific species (Peng et al. 2016) or specific chemical structure (Rogers et al. 2002a). Beside the chemical structure, it is worth to note that the NAs toxicity might be influenced by NAs' molecular size and water characteristics such as pH and salinity (Frank et al. 2009). NAs can act as surfactants ((CEATAG) 1998, Headley et al. 2013c) while it has both a hydrophilic end (carboxyl group) and a hydrophobic (non-polar aliphatic) ends (Armstrong et al. 2009). NAs dissociate and become more water-soluble when the pH increases. In contrast, the salinity decreases the solubility of NAs (Peng et al. 2002).

1.1.3 Naphthenic acids and their quantification

The general formula of NAs is designated as $C_nH_{2n+Z}O_x$ where the number of carbons and the number of hydrogens lost are represented by n and Z, respectively, and the double bond equivalent (DBE) can be an alternative for the Z. The NA species differ according to the number of oxygens ($2 \leq x \leq 6$). The classical NAs are denoted by O_2 species at x= 2 and the oxidized NAs are the O_3, O_4, O_5, O_6 species at ($3 \leq x \leq 6$). Furthermore, the heteroatomic NAs (i.e., nitrogen-containing and sulfur-containing species) are labeled as ($C_nH_{2n+Z}NO_x$) and

($C_nH_{2n+z}SO_x$) (Nyakas et al. 2013a). Both classical and oxy-NAs encompass 64% of the acid-extractable fraction (AEF) in OSPW, while the heteroatomic NAs (S–NAs and N) represent 31% (Nyakas et al. 2013a).

To date, there are several procedures, protocols and methods available for the extraction and analysis of NAs and the AEF from OSPW. The extraction protocol initiated and developed by Syncrude Canada (Jivraj et al. 1995, Rogers et al. 2002) is well implemented through the acidification of OSPW with H_2SO_4 to pH 2. The acidification of water samples is implemented to assure the protonation of the carboxylic acids due to the fact that pKa of NAs ranges from 5 to 6 and can be protonated at low pH with >99.99% efficiency (Young et al. 2008). Different solvents such as toluene, hexane and dichloromethane (DCM) can be used to selectively extract NAs (Headley et al. 2013a), though the DCM was mostly used in previous studies to isolate the NAs from OSPW after centrifugation or filtration of the acidified water sample (Headley et al. 2013a, Huang et al. 2015b, Rogers et al. 2002, Scott et al. 2008b, Young et al. 2007a, Young et al. 2008).

In addition to the extraction and sample pretreatment, the analysis of either the AEF (NAs and other compounds) or NAs in negative mode has to be consistent and comparable with different analytical methods and procedures. Ultra-performance liquid chromatography time-of-flight (UPLC-TOFMS) and Fourier transform ion cyclotron resonance (FTICR-MS), analytical methods have been investigated for raw and ozonated OSPWs (Sun et al. 2014), showing good correlations between the two methods for the classical NAs. Alternatively, Fourier transform infrared spectroscopy (FTIR) method has been implemented to measure

AEF after liquid-liquid extraction. Although the FTIR method estimates are not specific to individual NAs and lack the ability to resolve carbon numbers and Z families, FTIR results are still implemented. The FTIR was previously used as surrogate parameter to monitor the efficiency of water treatments, NA degradation, and the OSPW water quality (Gamal El-Din et al. 2011, Islam et al. 2014, Zubot et al. 2012). As well, previous research studied the correlation between AEF and NAs (Han et al. 2009, Martin et al. 2008) using treated water samples (Islam et al. 2014).

1.1.4 OSPW treatment

Though numerous approaches have been effectively examined to detoxify and decontaminate OSPW (Martin et al. 2010a, Quesnel et al. 2015, Wang et al. 2016b), the identification and the elucidation of their removal mechanisms are still warranted. Additionally, identification of all constituents of concern (McQueen et al. 2017) and the relative contributions of all constituents present in OSPW that prompt toxicity toward selected organisms as well as the individual toxicological effect of each component (He et al. 2012a, Jones et al. 2013, Morandi et al. 2015, Rowland et al. 2014) are not known (Grewer et al. 2010a, Jones et al. 2013, Sun et al. 2014a, Thomas et al. 2009, Zhang et al. 2016).

Advanced oxidation processes (AOPs) capability in water remediation has been developed and compared to conventional technologies such as adsorption using activated carbon or biodegradation (Parsons 2004). Ozone (O_3) is one of the most efficient AOPs to attain the mineralization of refractory and toxic compounds (Beltrán 2004, Beltrán et al. 1998). O_3 reactions includes two

pathways: direct (molecular ozone) reactions, while the hydroxyl radical (•OH) route (i.e., indirect pathway) can react unselectively (Hoigne and Bader 1983). The selective molecular ozone pathway can result in low reaction rate constants with the organic compounds (Beltrán 2004, Gottschalk et al. 2010).

Ozonation of OSPW has been reported to partially (He et al. 2010, Martin et al. 2010a) or completely (Scott et al. 2008b) reduce the acute OSPW toxicity as measured by the Microtox bacterial toxicity assay (Garcia-Garcia et al. 2011a) as well as to partially reduce the toxicity toward mice using *in vitro* and *in vivo* assays as reported by Wang et al. (2013a). For commercial NA degradation, Perez-Estrada et al. (2011) reported that the residual NAs after ozonation were less cyclic (Perez-Estrada et al. 2011) and microbial biodegradation was enhanced after ozonation when compared with untreated OSPW (Hwang et al. 2013, Martin et al. 2010b). Though the application of ozonation alone has some limitations as molecular O_3 reactions are selective and limited to aromatic, unsaturated aliphatic pollutants and some functional groups, a combination of peroxone (H_2O_2/O_3) seems to be a suitable alternative to overcome these limitations. The combination of O_3 and H_2O_2 can significantly produce more •OH for the degradation of O_3-resistant compounds (Audenaert 2012, Suty et al. 2004). H_2O_2 is an initiator for O_3 decomposition; however, high H_2O_2 concentration should be avoided as it acts as an inhibitor for the O_3 decomposition (Gottschalk et al. 2010). The reactions of H_2O_2/O_3 chemistry are illustrated in equations 1-3 (Siminiceanu et al. 2012):

$$H_2O_2 \rightarrow H^+ + HO_2^- \quad (1)$$

$$O_3 + HO_2^- \rightarrow \bullet OH + 2 \bullet O_2^- + O_2 \quad (2)$$

$$2O_3 + H_2O_2 \rightarrow 2 \bullet OH + 3O_2 \quad (3)$$

As the oil sands industry is expanding in northern Alberta producing huge amounts of OSPW, potential advancement should be presented in the degradation of OSPW NAs using different AOPs to investigate its potential as viable alternatives and approaches for OSPW remediation. Given the limitations of previous research and due to the lack of comprehensive studies on peroxone applications in OSPW, a significant step forward is taken with this research in determining the feasibility of peroxone for OSPW treatment by minimizing the oxidant doses. The objectives of this research were accomplished through series of phases listed and discussed in the following section.

1.2 Research Scope and Objectives

The OSPW characterization and treatment warrant further research to identify the main contributors of the OSPW toxicity or at least to quantify the compounds of concern then treat them.

The organic acids of OSPW are initially dissolved in OSPW after their partitioning from the oil phase into the aqueous at neutral /or alkaline pH, leaving the natural OSPW pH at 8-8.5 (Young et al. 2008). The acid mixtures in OSPW are very complex and cannot be easily resolved using current chromatography methods (Young et al. 2008).

As the main contributor to the chronic and acute toxicity of OSPW, NAs have been investigated in several studies (Hagen et al. 2012, Hagen et al. 2014, He et al. 2012a, He et al. 2012b, Morandi et al. 2015, Wang et al. 2013, Wiseman et al. 2013). A protocol for extracting NAs from OSPW, using liquid-liquid extraction (LLE) with a specific solvent: water ratio, was previously suggested and developed by Syncrude Canada Ltd. (Jivraj MN 1995, Rogers et al. 2002) and implemented in several studies (Headley et al. 2013a, Huang et al. 2015c, Jivraj MN 1995, Rogers et al. 2002, Scott et al. 2008a, Young et al. 2007b, Young et al. 2008). While the development of the LLE is necessary, to be feasible for large water volumes, it is still limited by the quality of the phase separation as well as compound extraction to waive any step (for instance, the centrifugation before extraction cannot be excluded) (Rogers et al. 2002). Initially, it was reported that NAs accounted around 95% of acidic component in OSPW (Rogers et al. 2002); then, it was suggested that classical and oxidized NAs are <50% of acid extractable organics in OSPW and more than 50% is not accounting for NAs (Grewer et al. 2010).

To compare results of the water samples, the standardization of experimental procedures is crucial by employing the same extraction procedure and encompassing the influence of the selectivity of solvent on extraction (Headley et al. 2013b). In addition, the intended use of the data can be affected by the selectivity of solvent on extraction. For instance, monitoring focused only on O_2 species can be done using hexane, while the full characterization of a sample can be accomplished by using ENV+ solid phase extraction (SPE) to offer less

selectivity in the extraction (Headley et al. 2013b). Therefore, the goal of the first phase of this research was to examine two different analytical methods (FTIR and UPLC-TOFMS) with different extraction methods (LLE and SPE) to analyze O_x-NAs (sum of classical NAs and oxidized NAs) and acid extractable organic fraction (AEF). The overall objective of this phase was to compare the different methods and techniques that could help the researchers to implement the best techniques available for sample analysis and preparation.

The specific objectives for Phase 1 (Chapter 2) were:

i) To show the differences in characterization between UPLC-TOFMS and FTIR using different water samples from different sources in the oil sands region;

ii) To examine the influence of selectivity between the extraction/pre-treatment methods (SPE and LLE);

iii) To explore the similarities and differences between OSPW extract standard and commercial NA standard; and

iv) To assess the differences between UPLC-TOFMS and FTIR as well as between standards using statistical multivariate analysis.

The second phase of the research aimed to examine the peroxone treatment of raw OSPW which might increase the levels of the •OH produced, enhancing the treatment performance. Given the limitations of the peroxone applications in OSPW and the high cost of the ozone doses higher than 100 mg/L, the second phase of this research addressed these gaps, using mild ozone doses of

30 and 50 mg/L. The objectives of the second phase (Chapter 3) of this research were:

i) Assess the relative efficacy of ozone and peroxone in terms of NA degradation by examining four main conditions: (1:2) peroxone treatment by the addition of 20 mg/L of H_2O_2 to 50 mg/L utilized ozone. Same H_2O_2 concentration was used for the (1:1) peroxone treatment that was conducted using ozone at 30 mg/L. Both utilized ozone doses were also conducted alone (i.e., without H_2O_2) to compare their performances;

ii) Examine the significance of H_2O_2 addition to ozone by elucidating the degradation pathways with and without •OH scavenger using tert-butyl alcohol;

iii) Evaluate the individual influence of carbon and Z numbers as well as to examine, for the first time, their joined effect on the structure reactivity toward O_2-NA and O_x-NA degradation;

iv) Determine the best doses for both ozone and H_2O_2 as well as the optimum peroxone molar ratio (mol H_2O_2/mol O_3) using several markers, including the degradation of NAs (O_2-NA and O_x-NA concentrations) per oxidant utilization, ion-mobility spectroscopy (IMS), fluorophore organic compounds removal; and toxicity assessment of the treated OSPWs.

The third phase of the research applied the knowledge gained from the preceding phase about the effectiveness of peroxone in degrading NA species and

addresses the research gaps related to the residual toxicity. In addition, several indices and markers were introduced to enhance the monitoring of the treated waters.

The main objectives of the third phase (Chapter 4) were as follows:

v) to examine the differences in compositions of treated OSPW compared to raw OSPW by monitoring the distributions of different classes of NAs including O_2, O_3, O_4, O_2S, O_3S, N_2O_x and others after oxidation and assess the susceptibility of treated water for further biodegradation after different AOPs.

vi) to characterize the treated OSPW and to observe theses variations using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) and Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS);

vii) to explore the relative differences between different processes (ozone versus peroxone) in transforming NA species;

viii) to elucidate the variations in water characteristics after AOP treatment using several indices and markers such as mineralization, cyclicity, biodegradability enhancement as well as a toxicity toward *Vibrio fischeri*.

The aims of the fourth phase were to fill the gaps about the kinetics of ozonation processes in OSPW given that limited studies have previously examined with main focus on model NA compounds and even none of studies was in peroxone in addition to address the gap related to the *In vitro* toxicity.

Based on the knowledge gained from the preceding phases about the peroxone effectiveness in degrading NA species; other peroxone treatment conditions with different molar ratio and different mild and small oxidants doses were used to assess the optimal monitoring criteria of the treatments and their effectiveness. The fourth phase objectives (Chapter 5) in the research were as follows:

ix) to examine the differences and similarities in performance between different peroxone conditions with different molar ratios and oxidant doses in order to select the best minimal /or economical oxidant doses that can accomplish efficient removal for the different classes of NAs.

x) to elucidate the reaction kinetics of OSPW NA species especially the classical NAs in both O_3 and O_3/H_2O_2 processes and to determine the reaction rate constants for the different NA species

xi) to explore and compare the efficiency of toxicity reduction between different processes (ozone versus peroxone) using goldfish primary kidney macrophages (PKMs);

xii) to characterize the treated OSPW as well as to observe the variations in the relative abundance of the species in the negative and positive electrospray ionization mode using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS).

1.3 Thesis Organization

This thesis consists of six chapters. A general introduction about the research background as well as the research objectives and its significance are presented in Chapter 1. Specifically, it encompasses a brief review of the oil sands and the motivation for the current research with brief insights about the AOPs and the OSPW characterization and treatments, research objectives, and thesis organization. The methodologies and the detailed experimental procedures, results, and discussions are presented separately in each chapter (Chapters 2-5).

Chapter 2 presents the characterization of the NAs using two different methods and two different pretreatments. The two different analytical methods are ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) and Fourier transformation infrared (FTIR) spectroscopy while the two different extraction methods are liquid–liquid extraction (LLE) using dichloromethane (DCM) and solid-phase extraction (SPE). The total NAs (sum of classical NAs and oxidized NAs) represented as O_x-NAs as well as acid extractable fraction (AEF) from OSPW were analyzed. The influence of selectivity between the samples' extraction/pretreatment method (SPE and LLE) was investigated while showing the differences in characterization between FTIR and UPLC-TOFMS with different water sources and time. Additionally, the chapter presents the similarities and differences between different water samples (i.e., groundwater wells in the oil sands area in addition to samples collected from oil sands tailings ponds) and different standards: OSPW extract standard versus commercial NA standard.

Chapter 3 explores the outlook of adding hydrogen peroxide (H_2O_2) in the ozonation process as an AOP, during the remediation OSPW that can lead to the increase of the hydroxyl radical (•OH) production, as an unselective oxidizer. The similarities/differences between the different treatments in terms of cost, toxicity reduction, best utilization of the oxidants, as well as differences with previous studies, relative effectiveness of treatments in removing specific structures were covered for the sake of further practical implementations. Two molar ratios using two mild utilized ozone doses of 30 and 50 mg/L with the addition of 20 mg/L of H_2O_2 were used to accomplish (1:2) and (1:1) peroxone treatments. The same ozone doses were conducted alone to maintain consistency and to compare the performance of the different processes. To propose hypothetical pathways and the contribution in degradation from both the selective ozonation reactions and the •OH, tert-butyl alcohol was added to suppress the •OH route.

Chapter 4 introduces several indicators and markers to monitor the NA removal and differences between the treatment performance. As a useful approach in OSPW remediation, the variations and abundance of different classes and compounds after treatments were monitored. The variations in the compositions of classical and heteroatomic NAs after treatment using AOPs (i.e., ozone and peroxone with and without ·OH scavenger TBA) and two different mass spectrometry characterization methods; UPLC-TOFMS and FTICR-MS, were examined. Ratios such as (O_2S:O_3S:O_4S and O_2:O_4) as well as BOD_5/COD and A/C were used to reveal the changes in composition. The changes in biodegradability indices and dissolved organic carbon were used to examine the

extent of the OSPW recalcitrance to propose different approaches either AOPs coupled with biological processes as a pre- or post-treatment. The similarities of the treated water characteristics with natural water sources were also highlighted. Toxicity toward *Vibrio fischeri* was investigated to reflect and deduce the residual toxic effects after AOPs. The reductions of the toxic-responsible compounds as well as the corresponding reduction in toxicity were also examined. The generated by-products/or compounds were introduced as useful indicators to evaluate the treatment performance that would allow selecting the best multi-barrier approaches and eventual guidelines in terms of species reductions.

Chapter 5 examines the optimum conditions covered in Chapters 2 and 3 in addition to other peroxone conditions with different molar ratios and small oxidant doses to compare the relative performance and removal levels in order to select the best minimal /or economical oxidant doses that can accomplish efficient removal for the different classes of NAs. More insights about the treatments kinetics and the different analysis using different ionization modes were provided. The OSPW toxicity toward goldfish PKMs was analyzed before and after treatment.

Chapter 6 illustrates the major conclusion of the research presented in Chapters 2-5. Additionally, future recommendations for further research are encompassed in this last chapter. Finally, the Appendix section presents some of the experimental methodologies, with supplementary tables and figures as referred in the main chapters.

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

Armstrong, S.A., Headley, J.V., Peru, K.M. and Germida, J.J. (2009) Differences in phytotoxicty and dissipation between ionized and nonionized oil sands naphthenic acids in wetland plants. Environmental Toxicology and Chemistry 28(10), 2167-2174.

Audenaert, W.T.M. (2012) Ozonation and UV/hydrogen peroxide treatment of natural water and secondary wastewater effluent: experimental study and mathematical modelling, Ghent University, Belgium.

Beltrán, F.J. (2004) Ozone Reaction Kinetics for Water and Wastewater Systems, Lewis Publishers, Boca Raton, Fla.

Beltrán, F.J., Encinar, J.M. and Alonso, M.A. (1998) Nitroaromatic Hydrocarbon Ozonation in Water. 1. Single Ozonation. Industrial & Engineering Chemistry Research 37(1), 25-31.

Brown, L.D. and Ulrich, A.C. (2015) Oil sands naphthenic acids: A review of properties, measurement, and treatment. Chemosphere 127, 276-290.

(CEATAG), C.E.A.T.A.G. (1998) Naphthenic acids Background Information Discussion Report, p. pp. 65., Alberta Department of Energy: Edmonton, AB, Canada,.

Dorn, P.B., Van Compernolle, R., Mueller, G.R., Sun, P.J., Glaze, D.E., Hwang, J.C. and Hansen, S.R. (1992) Toxicity identification and derivation of a water quality based effluent limit for a west coast refinery.In Toxicity Reduction: Evaluation and Control, pp. 183–204, Ford, D.L. Ed.; Technomic Pub Inc.: Lancaster, PA, USA.

ERCB, E.R.C.B. (2012) Alberta's Energy Reserves 2011 and Supply/Demand Outlook 2012-2021. Government of Alberta, C., AB, Canada, 2012 (ed).

Frank, R.A., Fischer, K., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van der Kraak, G. and Solomon, K.R. (2009) Effect of Carboxylic Acid Content on the Acute Toxicity of Oil Sands Naphthenic Acids. Environmental Science & Technology 43(2), 266-271.

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

Garcia-Garcia, E., Ge, J.Q., Oladiran, A., Montgomery, B., Gamal El-Din, M., Perez-Estrada, L.C., Stafford, J.L., Martin, J.W. and Belosevic, M.

(2011a) Ozone treatment ameliorates oil sands process water toxicity to the mammalian immune system. Water Res. 45(18), 5849-5857.

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

Gottschalk, C., Libra, J. and Sau, A. (2010) Ozonation of Water and Waste Water : A Practical Guide to Understanding Ozone and its Applications, Wiley-VCH.

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

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

Hagen, M.O., Katzenback, B.A., Islam, M.D.S., Gamal El-Din, M. and Belosevic, M. (2014) The Analysis of Goldfish (*Carassius auratus L.*) Innate Immune Responses After Acute and Subchronic Exposures to Oil Sands Process-Affected Water. Toxicological Sciences 138(1), 59-68.

Han, X.M., 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.

He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., El-Din, M.G., Giesy, J.P. and Wiseman, S.B. (2012a) Toxicity of untreated and ozone-treated oil sands process-affected water (OSPW) to early life stages of the fathead minnow (Pimephales promelas). Water Res. 46(19), 6359-6368.

He, Y., Wiseman, S.B., Wang, N., Perez-Estrada, L.A., El-Din, M.G., Martin, J.W. and Giesy, J.P. (2012b) Transcriptional responses of the brain-gonad-liver axis of fathead minnows exposed to untreated and ozone-treated oil sands process-affected water. Environ Sci Technol 46(17), 9701-9708.

He, Y., Wiseman, S.B., Zhang, X., Hecker, M., Jones, P.D., El-Din, M.G., Martin, J.W. and Giesy, J.P. (2010) Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line. Chemosphere 80(5), 578-584.

Headley, J.V., Peru, K.M., Fahlman, B., Colodey, A. and McMartin, D.W. (2013a) Selective solvent extraction and characterization of the acid extractable fraction of Athabasca oils sands process waters by Orbitrap mass spectrometry. International Journal of Mass Spectrometry 345-347, 104-108.

Headley, J.V., Peru, K.M., Mohamed, M.H., Frank, R.A., Martin, J.W., Hazewinkel, R.R.O., Humphries, D., Gurprasad, N.P., Hewitt, L.M., Muir,

D.C.G., Lindeman, D., Strub, R., Young, R.F., Grewer, D.M., Whittal, R.M., Fedorak, P.M., Birkholz, D.A., Hindle, R., Reisdorph, R., Wang, X., Kasperski, K.L., Hamilton, C., Woudneh, M., Wang, G., Loescher, B., Farwell, A., Dixon, D.G., Ross, M., Pereira, A.D., King, E., Barrow, M.P., Fahlman, B., Bailey, J., McMartin, D.W., Borchers, C.H., Ryan, C.H., Toor, N.S., Gillis, H.M., Zuin, L., Bickerton, G., McMaster, M., Sverko, E., Shang, D., Wilson, L.D. and Wrona, F.J. (2013b) Chemical fingerprinting of naphthenic acids and oil sands process watersA review of analytical methods for environmental samples. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 48(10), 1145-1163.

Hoigne, J. and Bader, H. (1983) Rate constants of reactions of ozone with organic and inorganic compounds in water .1. Non-dissociating organic compounds. Water Research 17(2), 173-183.

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

Huang, R., Sun, N., Chelme-Ayala, P., McPhedran, K.N., Changalov, M. and Gamal El-Din, M. (2015b) Fractionation of oil sands-process affected water using pH-dependent extractions: a study of dissociation constants for naphthenic acids species. Chemosphere 127, 291-296.

Hwang, G., Dong, T., Islam, M.S., Sheng, Z.Y., Perez-Estrada, L.A., Liu, Y. and El-Din, M.G. (2013) The impacts of ozonation on oil sands process-

affected water biodegradability and biofilm formation characteristics in bioreactors. Bioresource Technology 130, 269-277.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and Gamal El-Din, M. (2014) Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Sci. Total Environ. 493, 282-290.

Jivraj, M.N., MacKinnon, M. and Fung, B. (1995) Naphthenic acids extraction and quantitative analyses with FT-IR spectroscopy. Syncrude Canada Ltd. Research Department, Edmonton, Canada

Jones, D., Scarlett, A.G., West, C.E., Frank, R.A., Gieleciak, R., Hager, D., Pureveen, J., Tegelaar, E. and Rowland, S.J. (2013) Elemental and spectroscopic characterization of fractions of an acidic extract of oil sands process water. Chemosphere 93(9), 1655-1664.

Leshuk, T., Wong, T., Linley, S., Peru, K.M., Headley, J.V. and Gu, F. (2016) Solar photocatalytic degradation of naphthenic acids in oil sands process-affected water. Chemosphere 144, 1854-1861.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. (2006) Mass spectrometric and toxicological assays of Athabasca oil sands naphthenic acids. Water Research 40(4), 655-664.

Martin, J.W. (2015) The Challenge: Safe release and reintegration of oil sands process-affected water. Environmental Toxicology and Chemistry 34(12), 2682-2682.

Martin, J.W., Barri, T., Han, X.M., Fedorak, P.M., El-Din, M.G., Perez, L., Scott, A.C. and Jiang, J.T. (2010a) Ozonation of Oil Sands Process-Affected Water Accelerates Microbial Bioremediation. Environ. Sci. & Technol. 44(21), 8350-8356.

Martin, J.W., Barri, T., Han, X.M., Fedorak, P.M., El-Din, M.G., Perez, L., Scott, A.C. and Jiang, J.T. (2010b) Ozonation of Oil Sands Process-Affected Water Accelerates Microbial Bioremediation. Environmental Science & Technology 44(21), 8350-8356.

Martin, J.W., Han, X.M., Peru, K.M. and Headley, J.V. (2008) Comparison of high- and low-resolution electrospray ionization mass spectrometry for the analysis of naphthenic acid mixtures in oil sands process water. Rapid Communications in Mass Spectrometry 22(12), 1919-1924.

McQueen, A.D., Kinley, C.M., Hendrikse, M., Gaspari, D.P., Calomeni, A.J., Iwinski, K.J., Castle, J.W., Haakensen, M.C., Peru, K.M., Headley, J.V. and Rodgers Jr, J.H. (2017) A risk-based approach for identifying constituents of concern in oil sands process-affected water from the Athabasca Oil Sands region. Chemosphere 173, 340-350.

Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W. and Giesy, J.P. (2015) Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process-Affected Water. Environ. Sci. Technol. 49(20), 12395-12404.

Nyakas, A., Han, J., Peru, K.M., Headley, J.V. and Borchers, C.H. (2013) Comprehensive analysis of oil sands processed water by direct-infusion fourier-transform ion cyclotron resonance mass spectrometry with and without offline UHPLC sample prefractionation. Environ. Sci. & Technol. 47(9), 4471-4479.

Peng, H., Sun, J., Alharbi, H.A., Jones, P.D., Giesy, J.P. and Wiseman, S.B. (2016) Peroxisome Proliferator-Activated Receptor γ is a Sensitive Target for Oil Sands Process-affected Water: Effects on Adipogenesis and Identification of Ligands. Environ. Sci. & Technol.

Peng, J., Headley, J.V. and Barbour, S.L. (2002) Adsorption of single-ring model naphthenic acids on soils. Canadian Geotechnical Journal 39(6), 1419-1426.

Perez-Estrada, L.A., Han, X.M., Drzewicz, P., El-Din, M.G., Fedorak, P.M. and Martin, J.W. (2011) Structure-Reactivity of Naphthenic Acids in the Ozonation Process. Environmental Science & Technology 45(17), 7431-7437.

Quesnel, D.M., Oldenburg, T.B.P., Larter, S.R., Gieg, L.M. and Chua, G. (2015) Biostimulation of Oil Sands Process-Affected Water with Phosphate Yields Removal of Sulfur-Containing Organics and Detoxification. Environ. Sci. & Technol. 49(21), 13012-13020.

Rogers, V.V., Liber, K. and MacKinnon, M.D. (2002a) Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48(5), 519-527.

Rogers, V.V., Wickstrom, M., Liber, K. and MacKinnon, M.D. (2002b) Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Toxicological Sciences 66(2), 347-355.

Rowland, S.J., Pereira, A.S., Martin, J.W., Scarlett, A.G., West, C.E., Lengger, S.K., Wilde, M.J., Pureveen, J., Tegelaar, E.W., Frank, R.A. and Hewitt, L.M. (2014) Mass spectral characterisation of a polar, esterified fraction of an organic extract of an oil sands process water. Rapid Communications in Mass Spectrometry 28(21), 2352-2362.

Rowland, S.J., Scarlett, A.G., Jones, D., West, C.E. and Frank, R.A. (2011a) Diamonds in the Rough: Identification of Individual Naphthenic Acids in Oil Sands Process Water. Environmental Science & Technology 45(7), 3154-3159.

Rowland, S.J., West, C.E., Jones, D., Scarlett, A.G., Frank, R.A. and Hewitt, L.M. (2011b) Steroidal Aromatic 'Naphthenic Acids' in Oil Sands Process-Affected Water: Structural Comparisons with Environmental Estrogens. Environmental Science & Technology 45(22), 9806-9815.

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., Young, R.F. and Fedorak, P.M. (2008a) Comparison of GC-MS and FTIR methods for quantifying naphthenic acids in water samples. Chemosphere 73(8), 1258-1264.

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

Siminiceanu, I., Bartalis, I. and Arany, E. (2012) Enhancement of phenol oxidation by ozone in wastewater. II: Kinetic modeling. Environmental Engineering and Management Journal 11(2), 449-455.

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

Suty, H., De Traversay, C. and Cost, M. (2004) Applications of advanced oxidation processes: present and future. Water Science and Technology 49(4), 227-233.

Thomas, K.V., Langford, K., Petersen, K., Smith, A.J. and Tollefsen, K.E. (2009) Effect-Directed Identification of Naphthenic Acids As Important in Vitro

Xeno-Estrogens and Anti-Androgens in North Sea Offshore Produced Water Discharges. Environmental Science & Technology 43(21), 8066-8071.

Wang, C., Huang, R., Klamerth, N., Chelme-Ayala, P. and Gamal El-Din, M. (2016a) Positive and negative electrospray ionization analyses of the organic fractions in raw and oxidized oil sands process-affected water. Chemosphere 165, 239-247.

Wang, C., Klamerth, N., Messele, S.A., Singh, A., Belosevic, M. and Gamal El-Din, M. (2016b) Comparison of UV/hydrogen peroxide, potassium ferrate(VI), and ozone in oxidizing the organic fraction of oil sands process-affected water (OSPW). Water Res. 100, 476-485.

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. Environ. Sci. & Technol. 47(12), 6518-6526.

Wiseman, S.B., He, Y., Gamal-El Din, M., Martin, J.W., Jones, P.D., Hecker, M. and Giesy, J.P. (2013) Transcriptional responses of male fathead minnows exposed to oil sands process-affected water. Comp Biochem Physiol C Toxicol Pharmacol 157(2), 227-235.

Xue, J., Zhang, Y., Liu, Y. and Gamal El-Din, M. (2016) Treatment of raw and ozonated oil sands process-affected water under decoupled denitrifying

anoxic and nitrifying aerobic conditions: a comparative study. Biodegradation 27(4), 247-264.

Young, R.F., Orr, E.A., Goss, G.G. and Fedorak, P.M. (2007) Detection of naphthenic acids in fish exposed to commercial naphthenic acids and oil sands process-affected water. Chemosphere 68(3), 518-527.

Young, R.F., Wismer, W.V. and Fedorak, P.M. (2008) Estimating naphthenic acids concentrations in laboratory-exposed fish and in fish from the wild. Chemosphere 73(4), 498-505.

Zhang, K., Wiseman, S., Giesy, J.P. and Martin, J.W. (2016) Bioconcentration of Dissolved Organic Compounds from Oil Sands Process-Affected Water by Medaka (Oryzias latipes): Importance of Partitioning to Phospholipids. Environ. Sci. & Technol.

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

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. Sci. Total Environ. 427, 364-372.

2 QUANTIFICATION OF OIL SANDS ORGANIC ACIDS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY: IMPACTS OF THE EXTRACTION AND CALIBRATION METHODS[1]

2.1 Introduction

The oil sands industry in Northern Alberta, Canada produces large amounts of oil sands process-affected water (OSPW) (Energy Resources Conservation Board 2012, Shell Canada Limited 2016). OSPW is currently stored in tailings ponds to permit the recycling of water, and due to their toxicity to aquatic organisms (Hagen et al. 2014, Sun et al. 2014, Martin 2015, Van den Heuvel 2015, Mahaffey and Dubé 2016). OSPW is a highly complex mixture of suspended solids, salts, metals, and organic compounds (i.e., naphthenic acids (NAs), oil, grease and other hydrocarbons) (McQueen et al. 2017). The characterization of the organic fraction of OSPW alone is a great challenge because of the thousands of organic compounds present in OSPW (Barrow et al. 2010) which can hardly be characterized by simple methods while mass

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spectrometric techniques coupled with chromatographic can provide some options (Rowland et al. 2011a, Rowland et al. 2011b, Ross et al. 2012, Shang et al. 2013, Woudneh et al. 2013, Pereira et al. 2013b, Barrow et al. 2015, Huang et al. 2015a).

NAs are natural constituents of bitumen and have been reported to be the main contributor to the acute and chronic toxicity of OSPW (Verbeek et al. 1994, Morandi et al. 2015, Yue et al. 2015). NAs are a complicated mixture of carboxylic acids with the general formula of $C_nH_{2n+Z}O_x$, where n denotes the carbon number, Z the hydrogen deficiency number (zero or a negative even integer), and x the number of oxygen atoms. Recent advances in analytical techniques and methods have revealed that the NAs comprise of not only classical NAs (x=2) but also oxidized NAs with $x \geq 3$ (Huang et al. 2015a), as well as some other species such as aromatic NAs (Jones et al. 2012, Reinardy et al. 2013, Scarlett et al. 2013). Furthermore, it has been reported that NAs may contain heteroatoms such as nitrogen or sulphur atoms in the molecule (Headley et al. 2013b, Headley et al. 2015, Zhang et al. 2015).

To date, there are a couple of methods available for the extraction and pre-treatment of NAs and naphthenic acid fraction compounds (NAFCs) from water samples like OSPW for analysis. The first is a, a protocol for liquid–liquid extraction (LLE) using dichloromethane (DCM) was developed by Syncrude Canada Ltd (Jivraj MN 1995, Rogers et al. 2002) and well implemented in a number of studies (Rogers et al. 2002, Scott et al. 2008, Headley et al. 2013a, Huang et al. 2015b). The second common sample clean-up method used in OSPW

sample preparation is the solid-phase extraction (SPE) method (Headley et al. 2013a, Yue et al. 2015, Yue et al. 2016).

In addition to sample pre-treatment, there are several different instrument methods used in OSPW NA analysis, such as ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS), Fourier transfer ion cyclotron resonance mass spectrometry (FTICR-MS), and Fourier transformation infrared (FTIR) spectroscopy methods. Both UPLC-TOFMS and FTICR-MS are used to identify and categorize the composition and explore the profile of NA species or their relative abundance in the water samples based on Z, n and x numbers. Alternatively, FTIR has been implemented to measure the acid extractable fraction or NAFCs (i.e., organic compounds isolated from OSPW during the LLE and SPE sample clean-up processes). Although the estimates of FTIR method are not specific to individual NAs and lack the ability to resolve carbon numbers and Z families, FTIR results are still implemented as surrogate parameters to monitor the efficiency of water treatments, OSPW water quality, and NA degradation (Gamal El-Din et al. 2011, Zubot et al. 2012). The correlation between NAs and NAFCs was previously studied (Martin et al. 2008, Han et al. 2009) and observed using treated process water samples, where the determination of the NAFCs should encompass all NA species among other compounds (Islam et al. 2014). However, there is a lack of knowledge whether the correlation holds true with groundwater (GW) with different concentration levels and sample matrices (Grewer et al. 2010, Ross et al. 2012). The classical and oxidized NAs have been found to represent ~64% of the composition of

NAFCs (Nyakas et al. 2013). The commercial NAs (e.g., Fluka or Merichem mixtures) have been widely used as NA calibration standard to quantify NAs (Headley et al. 2010a, Mishra et al. 2010, Hindle et al. 2013, Sun et al. 2014). However, the variations in their composition between production lots might have an impact on the mixture composition and hence the quantification method (West et al. 2011, Hindle et al. 2013).

Therefore, the main objective of this study was to compare two different analytical methods (a non-mass spectrometry (MS) method [FTIR] and high resolution MS method [UPLC-TOFMS]) with different extraction methods (LLE and SPE) to analyze O_x-NAs (sum of classical NAs and oxidized NAs) and NAFCs using statistical multivariate analysis. The specific objectives aimed to: i) examine the influence of selectivity between the samples' extraction/pre-treatment method (SPE and LLE); ii) show the differences in characterization between FTIR and UPLC-TOFMS with different water sources; and iii) explore the similarities and differences between OSPW extract standard and commercial NA extract used as calibration standards. The correlation of these different methods and techniques would help the research community to adopt the best tools available for sample preparation and analysis.

2.2 Materials and methods

2.2.1 Water samples

Water samples were collected from ten different locations within the Shell Canada Limited's Albian Sands oil sands mining operations located in northeastern Alberta, Canada. Three samples (labeled OSPW-1 to OSPW-3) were collected from oil sands tailings ponds, in addition to seven groundwater samples collected from either different aquifers or locations and/or depths in the same oil sands area and labeled as GW-4 to GW-10. Two of the OSPW samples are the supernatant (i.e., collected from the zone of clear water) of the external tailings facility (ETF) for both the Jackpine Mine (OSPW-1) and Muskeg River Mine (OSPW-2) ETFs while the third OSPW sample (OSPW-3) is recycled water that is directed back into the extraction process. The recycled water is collected from a recycle pond is the combination of the two ETFs water as well as other water sources from site. The groundwater and OSPW samples were stored at 4 °C until use. The same 10 samples were collected from the same locations at different times: June, August, and October of 2015 for a total of 30 samples.

2.2.2 Chemical and reagents

Sulfuric acid (H_2SO_4) and dichloromethane (DCM) Optima grade used in the extraction process were from Fisher Scientific (ON, Canada). Formic acid and Fluka commercial NA standards were purchased from Sigma-Aldrich (ON, Canada). In our study, we used Fluka commercial NAs because they have been implemented in several studies (McMartin et al. 2004, Headley et al. 2009,

Mishra et al. 2010) due to its comprehensive characteristics and compositions (Rudzinski et al. 2002, Barrow et al. 2004, Scott et al. 2005, Headley et al. 2010a). Optima-grade water, methanol, and acetonitrile (Fisher Scientific, ON, Canada) were used for the instrument analysis. Isolute® layered SPE columns (6 mL ENV+) were purchased from Biotage, (NC, USA).

2.2.3 Sample preparation

2.2.3.1 Liquid-liquid extraction (LLE)

Due to the surfactant properties of NAs and to avoid dissolution of organic contaminants on the surfaces after contacting with DCM, glass laboratory wares and Teflon^{TM} were used in all experiments (Rogers et al. 2002). OSPW and groundwater samples were centrifuged to remove suspended particles (Rogers et al. 2002). Each sample was divided into four working aliquots of 100 mL (sample weight ≈100 g). The pH of each sample was adjusted to pH 2 using H_2SO_4 for further extraction. Each adjusted centrifuged water sample of 100 mL in every beaker was extracted using two times of 50 mL DCM, where the entire dried residues were recorded for calculating the fraction weight. Air flushing/drying unit was used to dry the extract. After shaking and venting the mixture for 3 minutes, the mixture was left for another 3 minutes to assure complete separation. The solvent: sample ratio was 1:2, as stated in the original protocol by Jivraj et al. (1995).

2.2.3.2 Solid-phase extraction (SPE)

Similar to LLE, water samples were divided into four aliquots of 100 mL after centrifugation. The samples were acidified to pH 1 using formic acid before extraction. An ENV+ (Biotage) cartridge was used as received and conditioned with 5 mL water, followed by 5 mL of methanol and finally with 10 mL of water. The 100 mL sample was loaded into the column and the eluent went to the waste. Then, the sample in the column was rinsed (i.e., eluent to waste) with 5 mL of water. After that, 6 mL of methanol was added to elute the fraction out from the column. The 6 mL methanol was evaporated and dried using air. The factions were then used for further analysis of UPLC-TOFMS and FTIR.

2.2.4 Analytical methods

2.2.4.1 Ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) analysis

Chromatographic separations were performed using a Waters UPLC Phenyl BEH column (1.7 μm, 150 mm × 1 mm), with mobile phases of 10 mM ammonium acetate in water (A), 10 mM ammonium acetate in 50/50 methanol/acetonitrile (B), and the injection volume of 10 μL. The elution gradient was 0−2 min, 1% B; 2−3 min, increased from 1% to 60% B; 3−7 min, from 60% to 70% B; 7−13 min, from 70% to 95% B; 13−14 min, from 95% to 1% B, and hold 1% B until 20 min to equilibrate column with a flow rate of 100 μL/min. The column temperature was set at 50 °C and the sample temperature at 10 °C. Samples were analyzed using the UPLC-TOFMS (Synapt G2, Waters) with the

TOF analyzer in high-resolution mode (mass resolution is 40000) and the investigated mass range of 100-600 (m/z). The electrospray ionization (ESI) source was operated in negative ion mode to measure NA concentrations in the samples (Pereira et al. 2013a). Data acquisition was controlled using MassLynx (Waters) and data extraction from spectra was performed using TargetLynx (Waters). This method was developed previously for NA semi-quantification based on the signal of a compound versus the signal of spiked internal standard (Wang et al. 2013, Huang et al. 2015b).

A pre-calibrated OSPW extract (Environment and Climate Change Canada, Saskatoon, SK) was used as standard for preparation of the external standard calibration curve with 5, 10, 25, 50, 75, 100 mg/L in 50/50 acetonitrile/water (Martin et al. 2008, Headley et al. 2013a). Duplicate pretreated samples were prepared for injection; however, a single injection was used due to the superior accuracy, constant reliability and precision of the UPLC-TOFMS technique as reported in previous studies (Martin et al. 2010, Hwang et al. 2013, Sun et al. 2014). Because the SPE or LLE fractions were concentrated for 100 times from 100 mL sample to 1 mL fraction, discrete dilution times were applied to different samples with the solvent 50/50 acetonitrile/water, based on the weight of each fraction, to fit the measured concentrations into the dynamic range of the external curve. The SPE or LLE extractions were necessary to remove the sample matrix and to concentrate the samples in order to estimate the NA concentration using the external calibration curve.

2.2.4.2 Fourier transform infrared (FTIR) spectroscopy analysis

FTIR quantification of NAFCs was conducted using a Nicolet 8700 FTIR spectrometer. The fixed path length of KBr liquid cell was 3 mm. Purge gas generator from Parker Balston Model 75-52 was used while running the samples. Omnic Software was used to acquire and process the spectrum. The sample spectrum was recorded for 128 scans after a 7-minute purge. The peak height or absorbance was recorded at both wavelengths of 1743 and 1706 cm^{-1}. The concentration of NAFCs in the water samples was calculated based on a prepared calibration curve and the total of recorded peak heights (Scott et al. 2008). All samples were analyzed in duplicate. The Fluka standards prepared for the calibration curve are provided in Table B1 in Appendix B.

Two calibration curves using two sets of standards were prepared as provided in the Appendix A (Figures A1 and A2). The first calibration curve was established from a commercial mixture of NAs (Fluka) (Figure A1) while the second curve was produced using the OSPW extract similar to UPLC-TOFMS (Figure A2). The quantification of NAFCs was estimated using both OSPW extract and Fluka standard. The appropriateness of estimating the NAFCs and NAs concentration from the curve of commercial NA mixtures has been previously reported (Martin et al. 2008, Scott et al. 2008).

2.2.5 Statistical analysis

Statistical analyses were performed using R 3.3 software. The normality of the data was checked by Shapiro–Wilk test. Kruskal-Wallis and Mann-Whitney-Wilcoxon tests were performed for non-normally distributed data with a

significance level of 0.05. Mann-Whitney-Wilcoxon and Kruskal-Wallis tests are the non-parametric alternatives to T-test and ANOVA, respectively. The null hypothesis is that all samples are similar or come from same population while the alternative hypothesis is that not all samples come from same population. Data were grouped with regard to the sampling locations, instrumental methods, standard used in calibration (FTIR-Fluka vs FTIR-OSPW Extract vs UPLC-TOFMS-OSPW Extract), and sample pre-treatments or extraction method (LLE vs SPE). For instance, the terminologies used in this study are as follows: FTIR-SPE-Fluka refers to analysis of sample by FTIR pretreated by SPE and using Fluka as standard; FTIR-LLE-OSPW refers to analysis of sample by FTIR pretreated by LLE and using OSPW extract as standard; TOF-SPE-OSPW and TOF-LLE-OSPW refer to analysis of samples by UPLC-TOFMS pretreated by either SPE or LLE, respectively and using OSPW extract as standard.

2.3 Results and discussion

Efforts have been made to investigate the differences and variations between the profiles of industrial processed water (e.g., OSPW) and natural waters (e.g., groundwater) (Ross et al. 2012, Frank et al. 2014). While the studies about the water quality in the oil sands region (Ross et al. 2012, Frank et al. 2014) aimed to deduce chemical indicators or surrogate parameters to monitor the variations of the water characteristics as well as to investigate suspected seepage or natural biodegradation, our study focused on comparing different analytical tools to measure the concentrations of NAs and NAFCs (Figure 2.1).

Different water samples (i.e., OSPW samples: 1, 2 and 3; groundwater samples (GW): 4, 5, 6, 7, 8, 9 and 10) were collected in three replicates. The variations of O_2-NA, O_x-NAs and NAFCs concentrations for each batch with time are illustrated in Table 2.1. Figure 2.2 also shows the concentrations of O_x-NAs and NAFCs for all samples (i.e., OSPW samples: 1, 2 and 3; GW: 4, 5, 6, 7, 8, 9 and 10) in each replicate. Overall, the Kruskal-Wallis test was implemented to assess the differences between all samples as one group and based on their source (OSPW or GW). The results showed that there was no significant difference between samples 1, 2, and 3 (OSPW samples; $p > 0.05$), but statistical differences existed between groundwater samples ($p < 0.05$; Table B2). Table B2 in Appendix B illustrates the summary of Kruskal-Wallis test results.

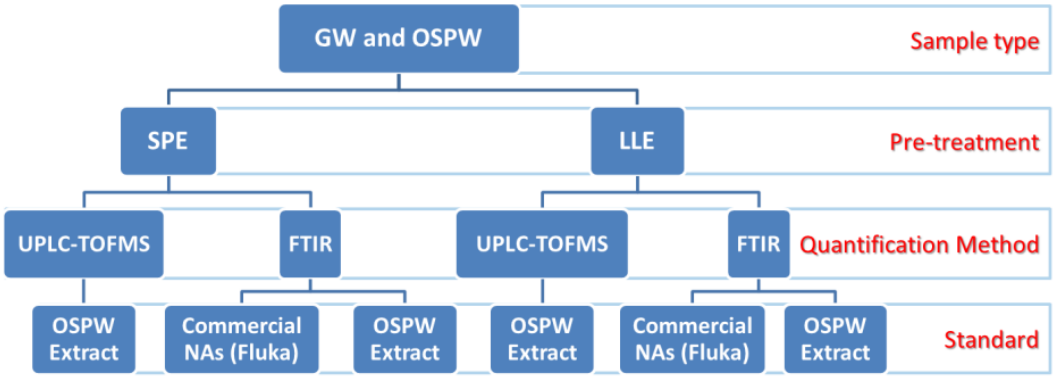

Figure 2.1. Schematic diagram showing the different pre-treatment methods and analyses performed in the present study, including the different water samples. Abbreviations are listed as follows: groundwater, GW; oil sand process-affected water, OSPW; solid-phase extraction, SPE; liquid-liquid extraction, LLE; ultra-performance liquid chromatography time-of-flight mass spectrometry, UPLC-TOFMS; Fourier transform infrared spectroscopy, FTIR; and naphthenic acids, NAs.

Table 2.1. Classical NAs (O_2-NAs) and sum of classical NAs and oxidized NAs (O_x-NAs). NA concentrations in (mg/L) were estimated by UPLC-TOFMS while naphthenic acid fractions compounds (NAFCs) concentrations in (mg/L) were estimated by FTIR for all groundwater and OSPW samples.

Sample #	UPLC-TOFMS				FTIR			
	OSPW Extract standard				Fluka standard		OSPW Extract standard	
	TOF-LLE	TOF-SPE	TOF-LLE	TOF-SPE	FTIR-LLE	FTIR-SPE	FTIR-LLE	FTIR-SPE
	O_2-NAs (mg/L)		O_x-NAs (mg/L)		NAFCs (mg/L)			
OSPW-1	20.067±1.263	20.077±2.512	32.933±3.126	36.117±2.808	33.6±2.4	40.8±18.0	87.1±5.8	102.5±28.1
OSPW-2	28.353±1.875	28.557±3.740	43.510±2.803	46.447±3.885	40.7±2.7	60.3±3.2	105.5±7.2	149.1±18.4
OSPW-3	22.290±1.240	21.727±2.492	34.993±1.789	36.240±2.779	34.1±1.7	50.0±6.2	88.3±5.2	119.4±11.6
GW-4	4.287±0.419	4.993±0.175	6.080±0.642	7.477±0.454	4.9±0.6	4.4±1.3	13.7±1.7	9.9±5.6
GW-5	6.330±1.387	7.250±0.852	8.950±1.817	10.877±1.233	7.3±0.6	6.5±2.9	19.9±1.6	15.5±7.5
GW-6	10.233±0.540	9.337±0.839	14.913±0.670	14.430±1.403	12.3±0.5	11.1±0.9	32.5±1.3	27.2±3.1
GW-7	7.717±1.262	9.573±0.296	12.467±2.128	15.940±1.897	12.6±1.0	15.5±3.6	33.2±2.7	38.7±11.1
GW-8	7.953±0.602	8.980±1.214	11.350±0.707	12.627±1.144	11.0±1.4	10.7±1.4	29.3±3.6	26.4±3.5
GW-9	0.640±0.072	0.627±0.665	1.080±0.135	1.547±1.442	1.0±0.2	0.7±1.7	3.5±0.8	1.1±1.2
GW-10	0.927±0.029	1.030±0.203	1.650±0.082	2.443±0.598	2.1±0.2	3.5±2.3	6.3±0.9	7.7±8.9

Notes:

- Solid-phase extraction is denoted as SPE; and liquid-liquid extraction is denoted as LLE. OSPW samples: OSPW-1, OSPW-2 and OSPW-3; Groundwater samples are denoted as GW-4, GW-5, GW-6, GW-7 GW-8, GW-9, and GW-10.
- FTIR-LLE and FTIR-SPE refer to the analysis of a sample by FTIR and pretreated by LLE and SPE, respectively; TOF-LLE and TOF-SPE refer to the analysis of a sample by UPLC-TOFMS and pretreated either by LLE or SPE, respectively.

- Error bars are standard errors based on the sample size (n) = 3 or triplicate samples collected over three months (June, August and October 2015).
- Sources of OSPW: OSPW-1 and 2 are collected from external tailings facility from different mine locations while OSPW-3 is recycled water.
- Sources of GW: Groundwater samples are collected from different Basal and channel aquifers from different mine locations.

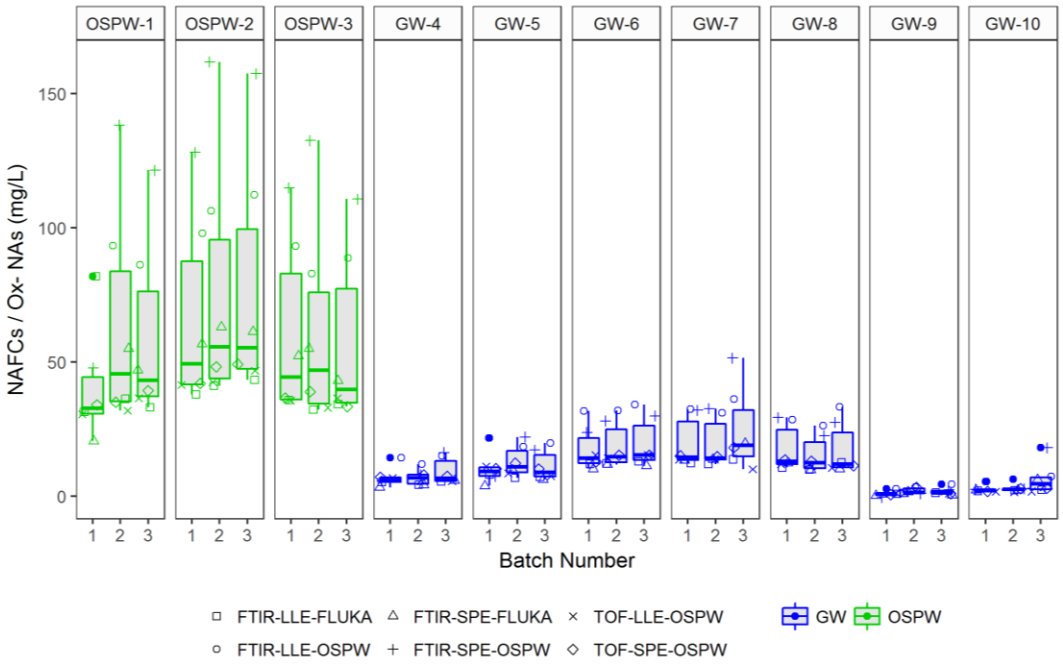

Figure 2.2. Change of O_x-NAs [sum of NAs at ($2 \leq x \leq 6$) or sum of classical (O_2) NAs and oxidized NAs; as measured by UPLC-TOFMS analyses] and naphthenic acid fractions compounds (NAFCs as measured by FTIR analyses) concentrations in the three sample batches or replicates (1 = June, 2 = August, and 3 = October 2015) at each location using the different pre-treatment methods and all analyses. Top of the figure: OSPW samples: 1, 2 and 3; Groundwater (GW) samples: 4, 5, 6, 7, 8, 9 and 10. Horizontal lines represent first quartile, medians, and third quartiles define the boxes, while the bottom and top tails represent the 10^{th} and 90^{th} percentiles. Data points for each boxplot are randomly placed to minimize points overlapping. Notes: Data points are presented as Instrument-Sample Preparation-Calibration standard. TOF = UPLC-TOFMS, SPE = Solid-phase extraction, LLE = liquid-liquid extraction, Fluka = Fluka commercial NA, and OSPW = oil sands process-affected water NA extract.

2.3.1 Differences between LLE and SPE

The O_x-NAs as well as NAFCs concentrations in mg/L detected with the SPE and LLE sample pre-treatment are displayed for the different water samples in Figure 2.3. With respect to O_x-NAs and NAFCs concentration, it can be observed that the recovery of the SPE was almost similar to LLE in lower values or GW samples. However, higher values could be observed using SPE compared to LLE for values higher than 30 mg/L (e.g., OSPW samples 1, 2 and 3). As shown in Table B3 in Appendix B, the SPE/LLE ratio exceeded 1 in most of samples, especially at high concentrations. For instance, the ratio for OSPW-1 in replicate 1 was 1.02 and 1.50 in UPLC-TOFMS (denoted as TOF in Figures) and FTIR, respectively, while the ratio for same sample in replicate 2 was 1.14 and 1.53 in UPLC-TOFMS, and FTIR, respectively. This high ratio in most of samples suggested the higher recovery in SPE compared to LLE. These findings agree with Headley et al. (2013a), who reported the low selectivity of (ENV+) SPE, allowing more species and components to be extracted. Similar findings were reported by Juhascik and Jenkins (2009), who reported high recoveries by SPE compared to LLE. The authors attributed the discrepancy between the extraction methods (i.e., SPE vs LLE) due to the possibility of partial/or minor loss for some components in the pretreated analyte based on the differences in selectivity of each component for instance the efficient extraction of weakly acids and other compounds by SPE compared to LLE.

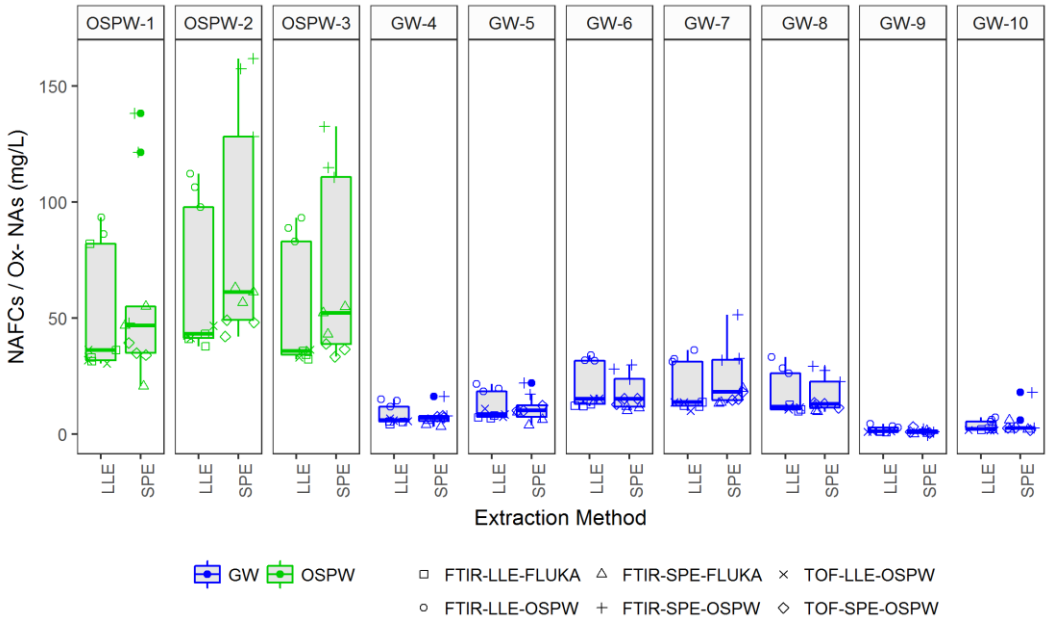

Figure 2.3. Differences between solid-phase extraction (SPE) and liquid-liquid extraction (LLE) pre-treatment using box plot of O_x-NAs [sum of NAs at ($2 \leq x \leq 6$) or sum of classical (O_2) NAs and oxidized NAs as measured by UPLC-TOFMS analyses] and naphthenic acid fractions compounds (NAFCs; as measured by FTIR analyses) for the different water samples; OSPW samples: 1, 2 and 3, groundwater (GW) samples: 4, 5, 6, 7, 8, 9 and 10. Horizontal lines represent first quartile, medians, and third quartiles define the boxes, while the bottom and top tails represent the 10^{th} and 90^{th} percentiles. Data points for each boxplot are randomly placed to minimize points overlapping. For each location, the sample size (n) = 3 or triplicate samples were collected over three months (June, August and October 2015). Notes: Data points are presented as Instrument-Sample Preparation-Calibration standard. TOF = UPLC-TOFMS, Fluka = Fluka commercial NA, and OSPW = oil sands process-affected water NA extract.

Additional to the extraction performance of SPE vs LLE there are operational and logistical differences to consider between the two methods. It has been found that SPE is relatively fast and convenient compared to LLE (Juhascik and Jenkins 2009, Mohamed et al. 2015, Mitra 2004) and it is useful as an analytical tool in monitoring compounds of interest /or full characterization of emerging contaminants in the environmental samples (Headley et al. 2013a). Additionally, the upsides of SPE over LLE are the lack of operator errors as well as the significant efforts applied during the sample preparation using LLE while the fractionation using SPE is based on the resins in the separation column, sorbents type and flow rate of the water sample. Other disadvantages of LLE include errors and losses that can arise during the separation of the organic phase and the consumption of large volumes of solvents (Juhascik and Jenkins 2009). Our findings indicated that there was no statistical difference between SPE and LLE results for individual samples and for all samples as one group for a given quantification method, i.e. there was no difference between the LLE and SPE samples which were quantified by FTIR technique using Fluka standard (Mann-Whitney-Wilcoxon P-value >0.05) (Figures 2.4 and 2.5). Although there was no a statistical significant difference between the SPE and LLE on quantifying either the NAFCs or O_x-NA, it can be noticed that SPE method usually produced higher recoveries than the LLE method (Table 2.1).

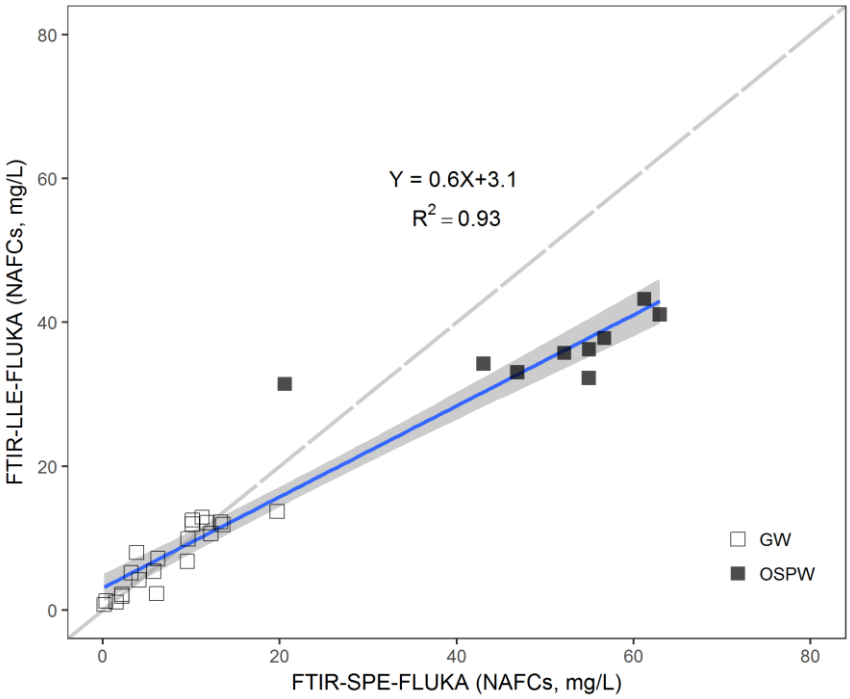

Figure 2.4. Comparison between the determination of naphthenic acid fractions compounds (NAFCs) after SPE and LLE pre-treatment using FTIR and Fluka as standard. Note: Groundwater (GW) and oil sand process-affected water (OSPW); solid-phase extraction (SPE); and liquid-liquid extraction (LLE). The grey zone represents the 95% confidence level for the regression. For each location, the sample size (n) = 3 or 3 replicates per sample location collected over three months (June, August and October 2015). FTIR-LLE-Fluka and FTIR-SPE-Fluka refer to the analysis of sample by FTIR pretreated by LLE and SPE, respectively, and using Fluka as standard.

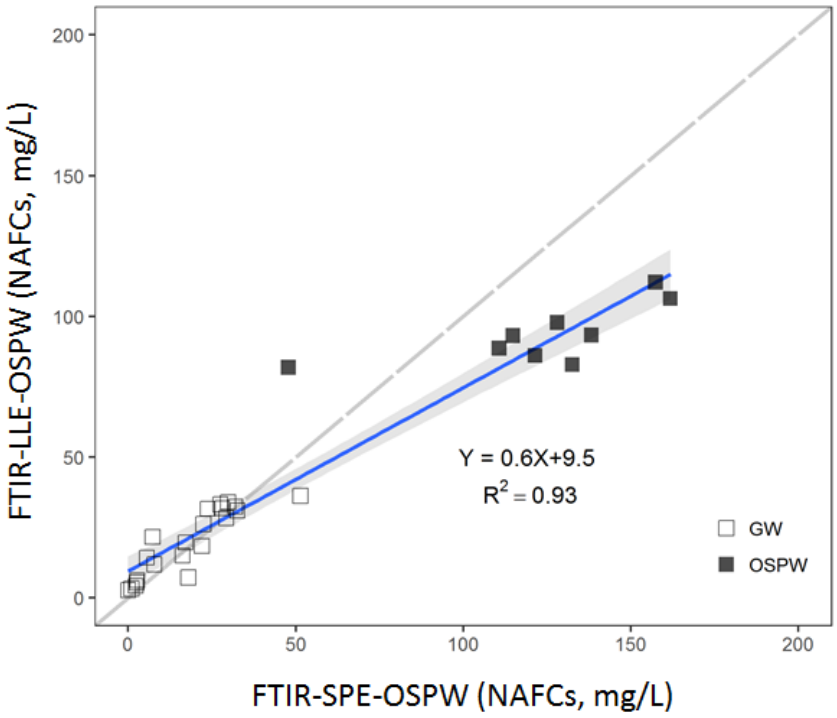

Figure 2.5. Comparison between the determination of naphthenic acid fractions compounds (NAFCs) using FTIR after SPE and LLE pretreatment and using OSPW extract as standard. Note: solid-phase extraction is denoted as SPE; and liquid-liquid extraction is denoted as LLE. The grey zone represents the 95% confidence level. For each sample location, the sample size (n) = 3 or triplicate samples were collected over three months (June, August and October 2015). Notes: X-axis and Y-axis categories are presented as Instrument-Sample Preparation-Calibration standard. OSPW = oil sands process-affected water NA extract.

With regards to the of O_2 species, significant similarity in concentrations of both LLE and SPE can be observed as depicted in Table 2.1. For instance, OSPW-1 in TOF-LLE-OSPW and TOF-SPE-OSPW yielded 20.067±1.263 mg/L and 20.077±2.512 mg/L, respectively, while GW-10 yielded 0.927±0.029 mg/L and 1.030±0.203 mg/L in TOF-LLE-OSPW and TOF-SPE-OSPW, respectively. As well, using Tukey simultaneous statistical test, no significant differences were observed ($p=0.937> 0.05$). On the other hand, the differences in most oxidized species (i.e., O_3-NAs and O_4-NAs) between the SPE and LLE using UPLC-TOFMS could be considered minimal due to their low portions (for instance: the sum of the abundance of the O_3-NAs and O_4-NAs in LLE versus SPE respectively; OSPW-1: 26% vs 27% and GW-6: 36% vs 34%)); furthermore acute toxicity towards the bacteria *Vibrio fischeri* was previously associated with O_2 species (Yue et al. 2015, Morandi et al. 2016) rather than oxidized species (Yue et al. 2015). Therefore, we focused our discussion on O_2 species as the primary component of interest in O_x-NAs. Figure 2.6 illustrates the percentage of the relative abundance of O_2−NAs for all samples using UPLC-TOFMS after the different pre-treatment conditions. With respect to the GW samples, the O_2 species contributed to 63.4±7.3% and 55.8±15.3% of the O_x-NAs in the LLE and SPE, respectively, while for the OSPW samples, the contribution was 63.1±2.1% and 58.5±3.0% in the LLE and SPE respectively (Figure 2.6). The two current observations relevant to the O_2 species either by the significant similarity of concentrations in both LLE and SPE as well as high recovery in the abundance of O_2 species in LLE may be due to the hydrophobicity influence of these species.

Few aspects can be highlighted to clarify these observations: (1) In the liquid chromatography, the separation mechanism depends on two principal factors the Van der Waals force and hydrophobicity (Bataineh et al. 2006), while Yue et al. (2015) reported that the most hydrophobic fraction of OSPW did encompass relatively higher amount of O_2 species. (2) The transport between water and hydrophobic extractants such as DCM counts on the hydrophobic impact or solvophobic impact (Mitra 2004). The hydrophobic impact can be considered as a selectivity parameter which can influence the appearance and disappearance of specific species. The absence of some species after polydimethylsiloxane extractions was reported because of their low hydrophobicity (Zhang et al. 2015). Overall using the three replicates of samples, we determined the non-significance in the differences between SPE and LLE at 95% confidence level (p-value of 0.67). However, deep and comparative insights are warranted in the coming sections to show the differences/similarities in the limits of detection/quantitation and the detection reliability using different standards and different quantification methods.

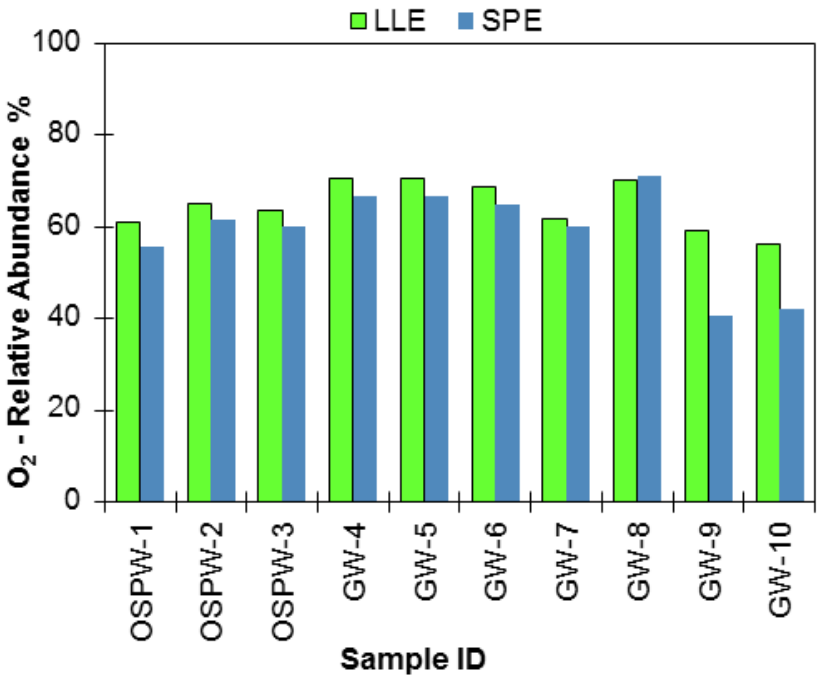

Figure 2.6. Relative abundance of classical NAs (O_2-NAs), for all samples using UPLC-TOFMS with different pretreating conditions liquid-liquid extraction (LLE) and solid-phase extraction (SPE). OSPW samples: 1, 2 and 3; groundwater (GW) samples: 4, 5, 6, 7, 8, 9 and 10.

2.3.2 Quantification analysis

2.3.2.1 Fourier transform infrared (FTIR) spectroscopy

Figure 2.7 displays the concentrations of NAFCs measured by FTIR using both standards Fluka and OSPW extract. The SPE revealed higher concentrations compared to LLE using both standards. All OSPW samples and most of GW samples had higher concentration in SPE versus LLE while the SPE concentration was 0.97 - 1.48 folds higher than LLE. The NAFCs for OSPW samples ranged from 33.6 to 60.3 mg/L while the GW was between 0.7 and 15.5 mg/L. Using different standards to measure NAFCs concentrations, the OSPW extract always

produced higher NAFCs compared to Fluka. This could be attributed to the difference in composition of the OSPW extract and the Fluka mixture. The OSPW extract might contain more fractions or structural isomers. For instance, the OSPW extract has more cyclic isomers (Martin et al. 2008) and less branched (Han et al. 2008) than the commercial NAs. Therefore, we can hypothesize and anticipate the particularity of OSPW extract as a standard in fully characterizing and reflecting wide distributions and compositions of the samples compared to commercial standards (i.e., higher concentration values due to thorough detection and characterization of more isomers). The statistical analysis of NAFCs for all samples showed similarity between SPE and LLE based on Fluka and OSPW extract calibration curve (p-value> 0.05) as depicted in Figures 2.7 and 2.5, respectively. However, in regards to GW only, the LLE as well as SPE methods had very close values unlike the OSPW samples; SPE tended to produce higher value than LLE.

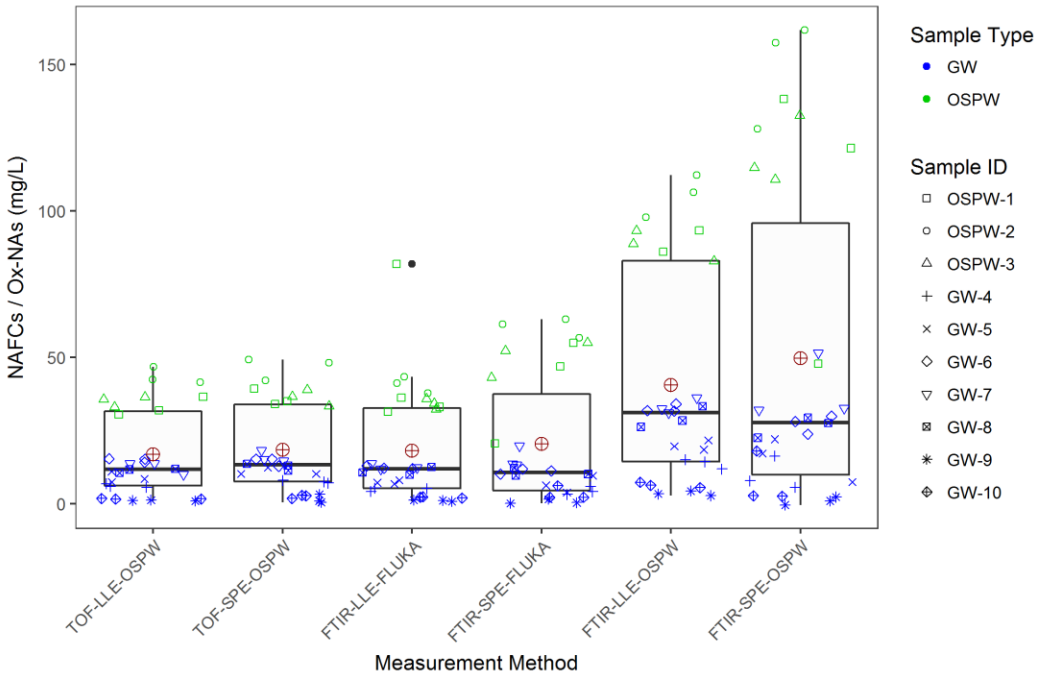

Figure 2.7. Box plot comparing the quantification of O_x-NAs [sum of NAs at ($2 \leq x \leq 6$) or sum of classical NAs and oxidized NAs as measure by UPLC-TOFMS] using solid-phase extraction (SPE) pretreated samples; and liquid-liquid extraction (LLE) pretreated samples as well as quantification of naphthenic acid fractions compounds (NAFCs; as measured by FTIR) using Fluka standard and OSPW extract with the two pre-treatment methods (SPE and LLE). OSPW samples: 1, 2 and 3; Groundwater (GW) samples: 4, 5, 6, 7, 8, 9 and 10. For each sample location, the sample size (n) = 3 or triplicate samples were collected over three months (June, August and October 2015). The mean is denoted as circled plus ⊕. Horizontal lines represent first quartile, medians, and third quartiles define the boxes, while the bottom and top tails represent the 10th and 90th percentiles. Data points for each boxplot are randomly placed to minimize points overlapping. Notes: X-axis categories are presented as Instrument-Sample Preparation-Calibration standard. TOF = UPLC-TOFMS, Fluka = Fluka commercial NA, and OSPW = oil sands process-affected water NA extract.

2.3.2.2 UPLC-TOFMS

As shown in Figure 2.7, consistent O_x-NAs concentrations detected by UPLC-TOFMS can be observed for both LLE and SPE pre-treatment using different water sources. Statistically, the results showed that the O_x-NAs obtained from the UPLC-TOFMS method using the SPE and LLE, were not significantly different as indicated by the Mann-Whitney-Wilcoxon p-value > 0.05 (Table 2.2 and Figure 2.8). OSPW is a very complex mixture (Rowland et al. 2011a) with thousands of individual structures (Anderson et al. 2012). Thus, no optimum technique has been reported so far to completely characterize (West et al. 2013, Noestheden et al. 2014) or to separate all compounds in OSPW (Scott et al. 2008, Headley et al. 2013b, Huang et al. 2015a). Misclassification of some minor acidic components was previously reported due to differences in selectivity between the direct injection electrospray ionization mass spectrometry and high-pressure liquid chromatography/high-resolution mass spectrometry (HPLC/HRMS) (Martin et al. 2008). The authors of the study suggested that the selectivity differences had a significant role on the characterization compared to other parameters and sensitivity of the mass spectrometry. With respect to standard, our decision to use OSPW extract only in calibration curve of UPLC-TOFMS was due to several reasons: 1) the acyclic O_2 species are more dominant in commercial mixture NAs compared to dominance of tricyclic and bicyclic species in OSPW fractions (Marentette et al. 2015). In addition, there is a lack of oxidized species in Fluka (i.e. further details in Section 2.3.2.3.); therefore, OSPW extract will provide more convenient composition with a considerable similarity to the real

world samples in terms of all species than commercial NA standard. 2) It is widely reported to use internal standards in UPLC-TOFMS (Bowman et al. 2014, Sun et al. 2014, Woudneh et al. 2013) rather than commercial NAs as external standards.

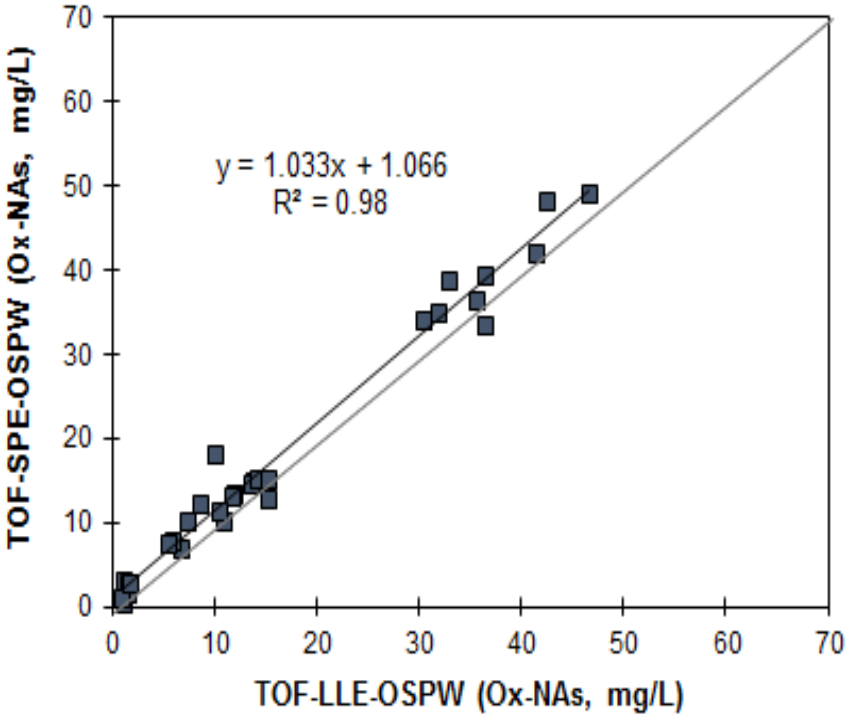

Figure 2.8. Comparison between UPLC-TOFMS determination of O_x-NAs after SPE and LLE pretreatment using OSPW extract as standard. Note: Solid-phase extraction (SPE); and liquid-liquid extraction (LLE); the confidence level is 95% for the regression. TOF-LLE-OSPW and TOF-SPE-OSPW refer to the analysis of sample by UPLC-TOFMS pretreated by LLE and SPE, respectively and using OSPW extract as standard.

Table 2.2. Comparison between the determination of naphthenic acid fractions compounds (NAFCs) by FTIR and determination of O_x-NAs by UPLC-TOFMS using OSPW extract and Fluka standards after samples pre-treatment with LLE and SPE.

Correlation	Conditions	Determination method (X)	Determination method (Y)	Equation	Determination coefficient (R^2)
SPE vs LLE	**UPLC-TOFMS (O_x-NAs) (OSPW extract)**	TOF-**LLE**-OSPW	TOF-**SPE**-OSPW	Y=1.033X+1.066	0.98
SPE vs LLE	**FTIR (NAFCs), (OSPW extract)**	FTIR-**SPE**-OSPW	FTIR-**LLE**-OSPW	Y=0.6X+9.5	0.93
SPE vs LLE	**FTIR (NAFCs), (Fluka)**	FTIR-**SPE**-Fluka	FTIR-**LLE**-Fluka	Y=0.6X+3.1	0.93
Fluka vs OSPW extract)	**FTIR (NAFCs), LLE**	FTIR- LLE-**Fluka**	FTIR-LLE-**OSPW**	Y=2.6X+0.9	0.99
Fluka vs	**FTIR (NAFCs), SPE**	FTIR- SPE-**Fluka**	FTIR-SPE-**OSPW**	Y=2.5X-0.6	0.99
UPLC-TOFMS vs FTIR	**(O_x-NAs) vs (NAFCs), & (OSPW extract), LLE**	**TOF-LLE**-OSPW	**FTIR-LLE**-OSPW	Y=2.476X+0.346	0.98
UPLC-TOFMS vs FTIR	**(O_x-NAs) vs (NAFCs), & (OSPW extract), SPE**	**TOF-SPE**-OSPW	**FTIR-SPE**-OSPW	Y=3.397X-12.829	0.93
UPLC-TOFMS vs FTIR	**(O_x-NAs) vs (NAFCs), &(OSPW extract) vs (Fluka), LLE**	**TOF-LLE**-OSPW	**FTIR-LLE**-Fluka	Y=1.017X+0.535	0.98
UPLC-TOFMS vs FTIR	**(O_x-NAs) vs (NAFCs), & (OSPW extract) vs (Fluka), SPE**	**TOF-SPE**-OSPW	**FTIR-SPE**-Fluka	Y=1.371X-4.911	0.93

Notes: Solid-phase extraction is denoted as SPE; and liquid-liquid extraction is denoted as LLE. OSPW samples: OSPW-1, OSPW-2 and OSPW-3; Groundwater samples are denoted as GW-4, GW-5, GW-6, GW-7 GW-8, GW-9, and GW-10. FTIR-LLE and FTIR-SPE refer to the analysis of a sample by FTIR and pretreated by LLE and SPE, respectively; TOF-LLE and TOF-SPE refer to the analysis of a sample by UPLC-TOFMS and pretreated either by LLE or SPE, respectively.

2.3.2.3 Calibration curves and appropriate standard

The differences between OSPW NA and the commercial Merichem NA extract has been investigated by Martin et al. (2008) and Sun et al. (2014) while analyzing water samples. Their findings suggested that the calibration plots generated from the Merichem preparation are likely suitable for estimating the concentrations of NAs from oil sands sources. However others still claim that further research is warranted to develop authentic/universal standards for calibration rather than commercial NAs (Scott et al. 2008, Zhao et al. 2012). The authentic standard can better represent the entire composition of OSPW and reflect all species. Thus, in this section, we highlighted the differences between the two calibration curves (prepared with two different NA standards) with regards to two aspects; 1) the composition of standard itself using UPLC-TOFMS; and 2) the correlation between the results of FTIR using the two standards separately in either LLE or SPE.

To understand the differences in the composition of two standards, samples of Fluka and OSPW NA extract were analyzed using the UPLC-TOFMS. Figure 2.9 presents the percent distributions of carbon number (n) and hydrogen deficiency number (Z), in both standards. In Fluka mixture, the range of n was from 7 to 20 while Z numbers from 0 to – 12. However, in OSPW extract, the n was observed from 7 to 22 and Z numbers from 0 to -18. In addition, 93% of the Fluka standard compounds had Z values between 0 and 4 while 37% of the OSPW extract compounds were in the same region (Figure 2.9). The statistical Kruskal-Wallis test showed the dissimilarity between OSPW extract and Fluka (p-

value < 0.05). To determine the extent of difference between the composition of the OSPW extract and commercial Fluka NAs, the speciation of both standards is illustrated in Figures 2.10 and 2.11, respectively. For the O_2 species, the Fluka showed higher abundance in the lower Z and lower carbon (Figure 2.10-d). Conversely, the O_2 species in the OSPW extract had higher abundance with smaller Z and higher carbon (Figure 2.10-c). Carbon (13-22) at Z (-12, -14, -16 and -18) was significant in OSPW extract compared to Fluka. These findings are consistent with what was formerly observed by Headley et al. (2010a) about the higher molecular weights of OSPW NAs compared to Fluka (Armstrong et al. 2008, Headley et al. 2010b). With respect to oxidized NAs (i.e., species ($3 \leq x \leq 6$)) low abundance of these species could be observed in Fluka (Figure 2.11 b,d). Similarly, a negligible abundance of oxidized species has been reported for Merichem commercial NA extract (Sun et al. 2014).

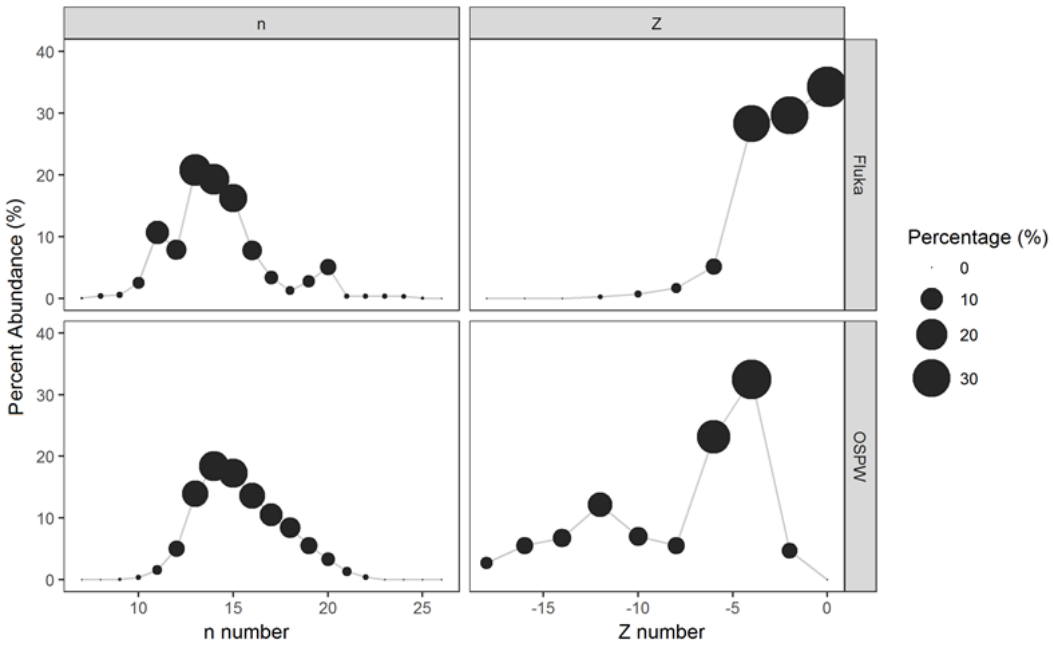

Figure 2.9. Percent abundance (%) of carbon number (n) and Z distribution for OSPW NA extract and Fluka NA standard. NA general formula: $C_nH_{2n+Z}O_x$ where the number of carbons, the number of hydrogen lost, the number of oxygen are represented by n, Z and x respectively. ($7 \leq n \leq 26$), ($0 \leq -Z \leq 18$), and ($2 \leq x \leq 6$). OSPW Ext. refers to OSPW extract.

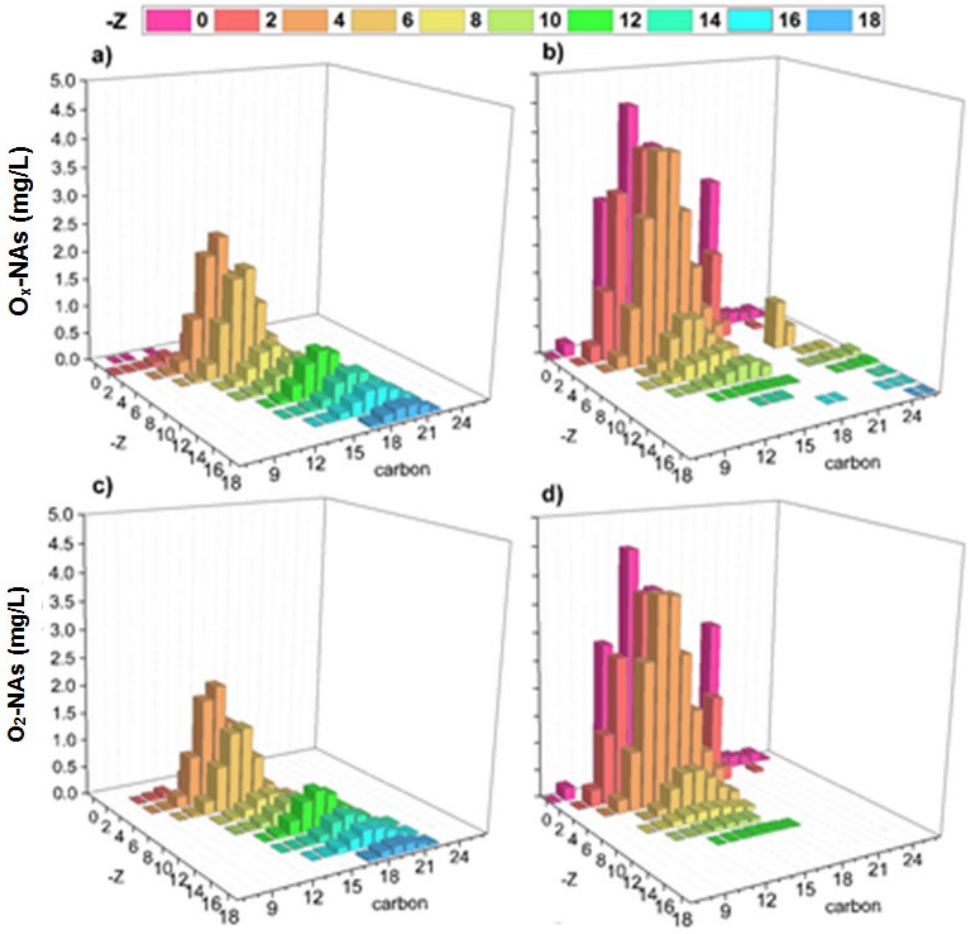

Figure 2.10. Profiles of O_x-NAs and classical (O_2-NAs) species of OSPW extract (left panels a,c) and Fluka standard (right panels b,d).

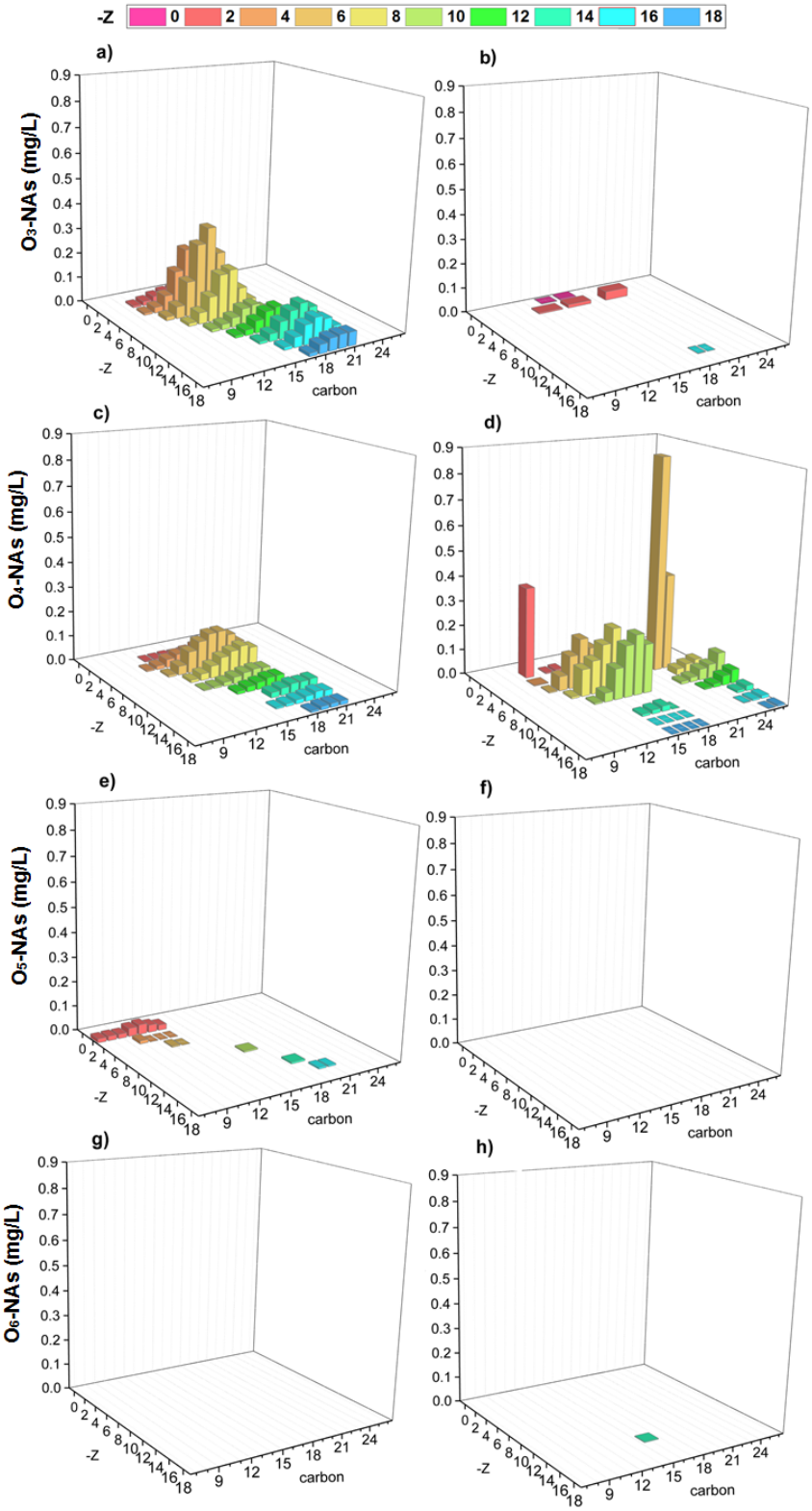

Figure 2.11. Profiles of oxidized NA or NA species (3≤x≤6) of OSPW extract (left panels a,c,e,g) and Fluka standard (right panels b,d,f,h).

The Mann-Whitney-Wilcoxon test showed that there was a significant difference between the NAFCs values produced from FTIR-OSPW and FTIR-Fluka (Mann-Whitney-Wilcoxon p-value <0.05). However, by comparing the two sets of data, it suggested a linear relationship between the two data sets. A linear regression between the two standards suggest the FTIR-OSPW extract results were 2.56 and 2.47 old higher than FTIR-Fluka for the LLE and SPE, respectively, regardless of the type of the water. Although these results were significantly different, similar coefficient of determination (R^2=0.99) was observed in both in LLE and SPE as shown in Figures 2.12 and 2.13 and tabulated in Table 2.2.

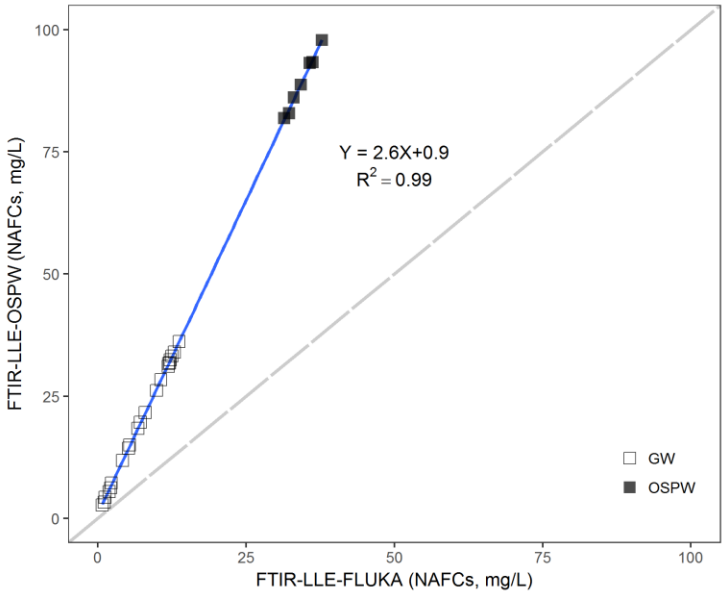

Figure 2.12. Comparison between naphthenic acid fractions compounds (NAFCs) concentrations using Fluka and OSPW standards after LLE. Note: Groundwater (GW) and oil sand process-affected water (OSPW); and liquid-liquid extraction (LLE). For each sample location, the sample size (n) = 3 or triplicate samples collected over three months (June, August and October 2015). The 95% confidence level lies under the line and is very close. Notes: X-axis and Y-axis categories are presented as Instrument-Sample Preparation-Calibration standard. Fluka = Fluka commercial NA, and OSPW = oil sands process-affected water NA extract.

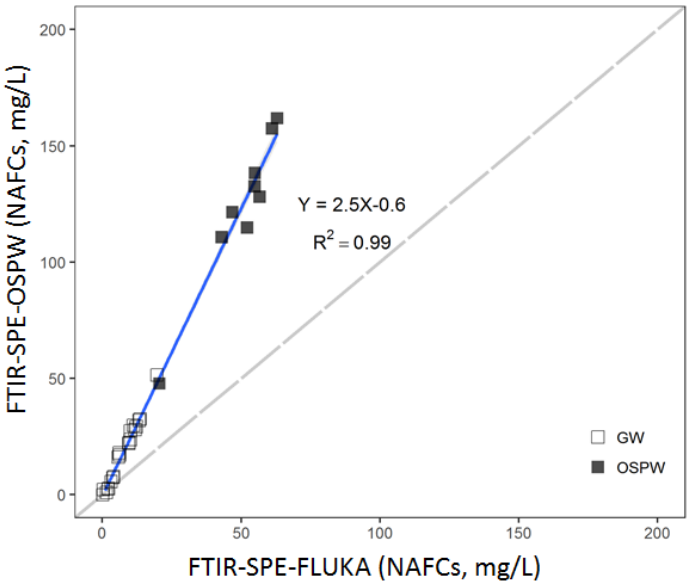

Figure 2.13. Comparison between standards: Fluka (Fluka commercial NA) and OSPW (oil sands process-affected water NA extract) using FTIR results (naphthenic acid fractions compounds (NAFCs)) in SPE. Notes: X-axis and Y-axis categories are presented as Instrument-Sample Preparation-Calibration standard. Notes: Groundwater (GW), oil sand process-affected water (OSPW); and solid-phase extraction (SPE). For each sample location, the sample size (n) = 3 or triplicate samples were collected over three months (June, August and October 2015)

2.3.2.4 UPLC-TOFMS versus FTIR

Figures 2.14 and 2.15 show the relationship between results of UPLC-TOFMS (O_x-NA) and FTIR (NAFCs) based on OSPW extract after LLE and SPE respectively while all correlations is shown in Table 2.2. The linear regression in most of the correlations (refer to Table 2.2) in addition to the former plots (Figures 2.14 and 2.15) showed a good range for the determination coefficient with R^2=0.92-0.98. Based on OSPW extract standard, the FTIR results were about

2.47 and 3.4 fold higher than UPLC-TOFMS results in both the LLE and SPE, respectively, regardless of the type of the water.

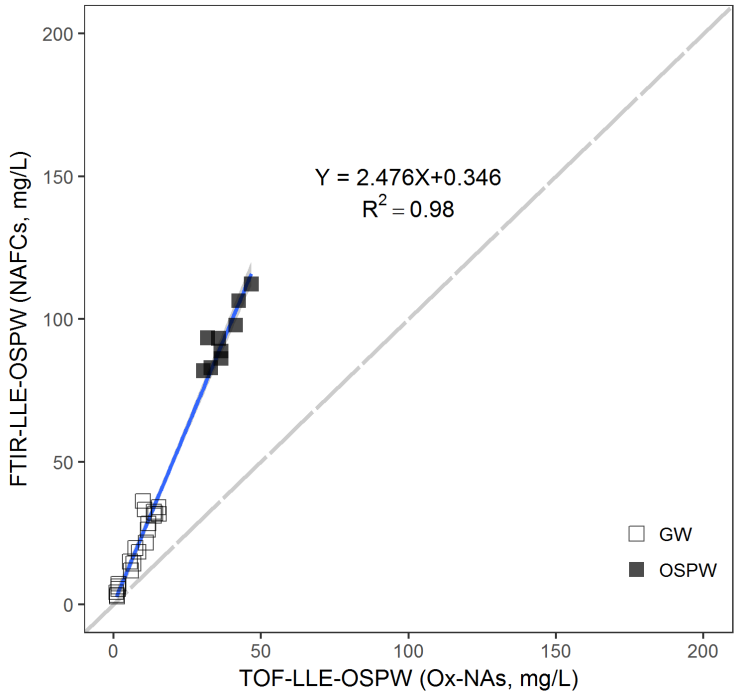

Figure 2.14. Comparison between the determination of naphthenic acid fractions compounds (NAFCs) by FTIR-OSPW extract and determination of UPLC-TOFMS (O_x-NAs) using OSPW extract after samples LLE pre-treatment. Notes: Groundwater (GW) and oil sand process-affected water (OSPW); and liquid-liquid extraction (LLE). The 95% confidence level lies under the line and is very close. O_x-NAs refer to the sum of classical NAs (i.e., O_2) and oxidized NAs (i.e., O_3, O_4, O_5, and O_6, etc.). Notes: X-axis and Y-axis categories are presented as Instrument-Sample Preparation-Calibration standard. TOF = UPLC-TOFMS and OSPW = oil sands process-affected water NA extract. For each sample location, the sample size (n) = 3 or triplicate samples were collected over three months (June, August and October 2015)

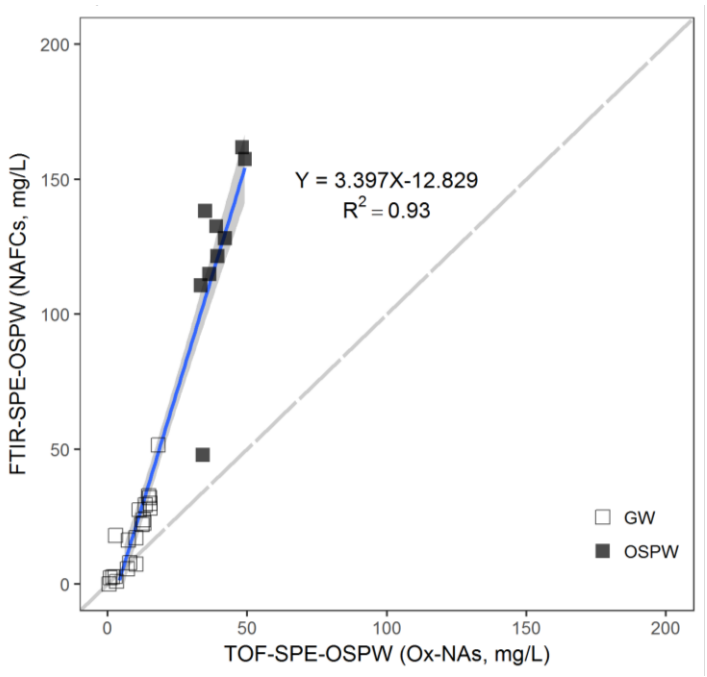

Figure 2.15. Comparison between the determination of UPLC-TOFMS (O_x-NAs) and (naphthenic acid fractions compounds (NAFCs)) by FTIR using OSPW extract after samples pre-treatment with solid-phase extraction (SPE). Notes: Groundwater (GW) and oil sand process-affected water (OSPW). The grey zone represents the 95% confidence level. For each sample location, the sample size (n) = 3 or triplicate samples were collected over three months (June, August and October 2015). O_x-NAs refer to the sum of classical NAs (i.e., O_2) and oxidized NAs (i.e., O_3, O_4, O_5, and O_6, etc.). Notes: X-axis and Y-axis categories are presented as Instrument-Sample Preparation-Calibration standard. TOF = UPLC-TOFMS and OSPW = oil sands process-affected water NA extract.

Distinct from the OSPW extract and estimating the NAFCs by FTIR using Fluka standard, the FTIR-Fluka results were about 1.01-1.37 times UPLC-TOFMS (O_x-NA) for LLE and SPE as exhibited in (Figures 2.16 (a, b)). The key point in these results is the evidence of the strong correlation and close similarity between the FTIR-Fluka and UPLC-TOFMS especially in LLE. The findings agreed with previous studies that used the FTIR measurement in terms of NAFCs as surrogate parameter for NAs as an indicator for treatment effectiveness (Gamal El-Din et al. 2011, Zubot et al. 2012, Islam et al. 2014). Additionally, the FTIR-Fluka after LLE could be a better alternative to high cost UPLC-TOFMS analysis and greatest representative to measure the NAs in water samples. Although slight differences in concentrations could be observed, there was a significant linear relationship between FTIR and UPLC-TOFMS in our study as reported by Zhao et al. (2012) and confirmed our decision to use only OSPW extract calibration curve in UPLC-TOFMS detection. In summary, the commercially-accessible FTIR method could be used as an affordable substitute for analysis of water samples for NAs.

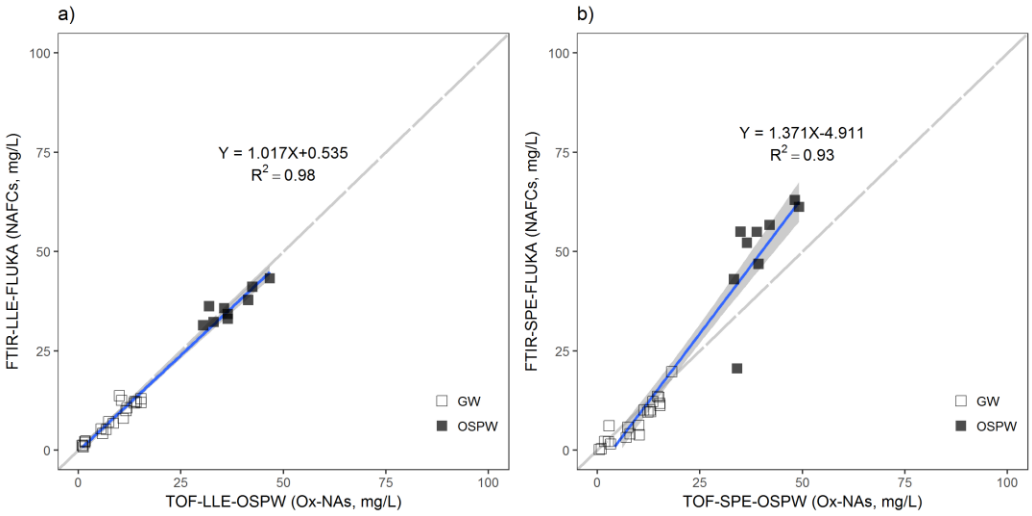

Figure 2.16. Comparison between the determination of naphthenic acid fractions compounds (NAFCs) by FTIR-Fluka and determination of O_x-NAs (sum of classical NAs (i.e., O_2) and oxidized NAs (i.e., O_3, O_4, O_5, and O_6, etc.)) by UPLC-TOFMS using OSPW extract after samples pre-treatment: a) LLE and b) SPE. Notes: Groundwater (GW) and oil sand process-affected water (OSPW); solid-phase extraction (SPE); and liquid-liquid extraction (LLE). For each sample location, the sample size (n) = 3 or triplicate samples collected over three months (June, August and October 2015).The grey zone represents the 95% confidence level. Notes: X-axis and Y-axis categories are presented as Instrument-Sample Preparation-Calibration standard. TOF = UPLC-TOFMS, OSPW = oil sands process-affected water NA extract, and Fluka = Fluka commercial NA.

2.4 Conclusions

This study presented insights about the analysis of OSPW and groundwater samples with FTIR and UPLC-TOFMS measurements using two standards after SPE or LLE pre-treatments. We elucidated the similarities as well as the differences between these techniques to have a better understanding about the impact of pre-treatment and quantification standards on the reliability of the results.

1. For most of the samples, regardless the water source (OSPW or GW) and quantification methods (UPLC-TOFMS, FTIR), higher recovery of both O_x-NA and NAFCs in SPE was achieved compared to LLE (i.e., 1.0-1.4 fold high in SPE based on its less selectivity).
2. Similar concentrations of O_2 species were observed in both LLE and SPE with higher abundance of O_2 species in LLE (e.g., in the three OSPW samples, (63.1±2.1%) in LLE compared to (58.5±3.0%) in SPE). The increase of O_2 species abundance using LLE was due to the high impact of the hydrophobicity in which the conveyance of acids from water to DCM increased.
3. Comparing two calibration standards, relative dissimilarity in the compositions of commercial Fluka NA mixture versus OSPW extract as well as abundance of some classes were perceived. However, a very strong correlation was observed in concentrations of the LLE pretreated samples measured by both the FTIR analysis with Fluka standard and UPLC-TOFMS using OSPW extract standard.

Based on this study, SPE with ENV+ cartridge is recommended based on efficiency, repeatable detection and maximum recovery of abundant species. In addition, the findings of this study highlight the possibility of using the results of FTIR-Fluka as surrogate parameters and preliminary tools: (i) to monitor the total NA concentrations in different water matrices at different concentration levels (i.e. low levels such as groundwater and high levels such as OSPW); (ii) to assess the environmental pollution loading by monitoring the water quality of point and

non-point sources; and (iii) to assess the efficiency of different water treatment and reclamation approaches for process waters.

The continuous development of low cost and standardization of analytical techniques (i.e., detection methods, samples preparation and authentic standards) is warranted to characterize complicated matrices such as OSPW and total NAs.

2.5 References

Anderson, J.C., Wiseman, S.B., Wang, N., Moustafa, A., Perez-Estrada, L., El-Din, M.C., 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. Environ. Sci. & Techn.46(1), 486-493.

Armstrong, S.A., Headley, J.V., Peru, K.M. and Germida, J.J. (2008) Phytotoxicity of oil sands naphthenic acids and dissipation from systems planted with emergent aquatic macrophytes. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 43(1), 36-42.

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., El-Din, M.C., 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. Environ.Sci. & Techn. 46(1), 486-493.

Barrow, M.P., Peru, K.M., Fahlman, B., Hewitt, L.M., Frank, R.A. and Headley, J.V. (2015) Beyond naphthenic acids: environmental screening of water from natural sources and the athabasca oil sands industry using atmospheric pressure photoionization fourier transform ion cyclotron

resonance mass spectrometry. Journal of The American Society for Mass Spectrometry 26(9), 1508-1521.

Barrow, M.P., Headley, J.V., Peru, K.M. and Derrick, P.J. (2004) Fourier transform ion cyclotron resonance mass spectrometry of principal components in oilsands naphthenic acids. Journal of Chromatography A 1058(1–2), 51-59.

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

Bowman, D.T., Slater, G.F., Warren, L.A. and McCarry, B.E. (2014) Identification of individual thiophene-, indane-, tetralin-, cyclohexane-, and adamantane-type carboxylic acids in composite tailings pore water from Alberta oil sands. Rapid Communications in Mass Spectrometry 28(19), 2075-2083.

Energy Resources Conservation Board (2012) Energy Resources Conservation Board Tailings Management Assessment Report: Oil Sands Mining Industry , (posted date: June 12, 2013), from Alberta Energy Regulator, Accessed online April 2017: http://osipfiles.alberta.ca/datasets/158/TailingsManagementAssessmentReport2011-2012.pdf.

Frank, R.A., Roy, J.W., Bickerton, G., Rowland, S.J., Headley, J.V., Scarlett, A.G., West, C.E., Peru, K.M., Parrott, J.L., Conly, F.M. and Hewitt, L.M. (2014) Profiling Oil Sands Mixtures from Industrial Developments and Natural Groundwaters for Source Identification. Environ. Sci. & Technol. 48(5), 2660-2670.

Gamal El-Din, M., Fu, H.J., Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Drzewicz, P., Martin, J.W., Zubot, W. and Smith, D.W. (2011) Naphthenic acids speciation and removal during petroleum-coke adsorption and ozonation of oil sands process-affected water. Sci. Total Environ. 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? Sci. Total Environ. 408(23), 5997-6010.

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

Hagen, M.O., Katzenback, B.A., Islam, M.D.S., Gamal El-Din, M. and Belosevic, M. (2014) The analysis of goldfish (Carassius auratus L.) innate immune responses after acute and subchronic exposures to oil sands process-affected water. Toxicological Sciences 138(1), 59-68.

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

Headley, J.V., Du, J.L., Peru, K.M. and McMartin, D.W. (2009) Electrospray ionization mass spectrometry of the photodegradation of naphthenic acids mixtures irradiated with titanium dioxide. J. of Environ.l Sci. and Health Part A 44(6), 591-597.

Headley, J.V., Kumar, P., Dalai, A., Peru, K.M., Bailey, J., McMartin, D.W., Rowland, S.M., Rodgers, R.P. and Marshall, A.G. (2015) Fourier transform ion cyclotron resonance mass spectrometry characterization of treated athabasca oil sands processed waters. Energy & Fuels 29(5), 2768-2773.

Headley, J.V., Peru, K.M., Adenugba, A.A., Du, J.-L. and McMartin, D.W. (2010a) Dissipation of naphthenic acids mixtures by lake biofilms. J. of Environ. Sci.and Health, Part A; 45(9), 1027-1036.

Headley, J.V., Peru, K.M., Fahlman, B., Colodey, A. and McMartin, D.W. (2013a) Selective solvent extraction and characterization of the acid extractable fraction of Athabasca oils sands process waters by Orbitrap mass spectrometry. International J0 of Mass Spec. 345–347, 104-108.

Headley, J.V., Peru, K.M., Mishra, S., Meda, V., Dalai, A.K., McMartin, D.W., Mapolelo, M.M., Rodgers, R.P. and Marshall, A.G. (2010b) Characterization of oil sands naphthenic acids treated with ultraviolet and microwave radiation by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun. Mass Spectrom. 24(21), 3121-3126.

Headley, J.V., Peru, K.M., Mohamed, M.H., Frank, R.A., Martin, J.W., Hazewinkel, R.R.O., Humphries, D., Gurprasad, N.P., Hewitt, L.M., Muir, D.C.G., Lindeman, D., Strub, R., Young, R.F., Grewer, D.M., Whittal, R.M., Fedorak, P.M., Birkholz, D.A., Hindle, R., Reisdorph, R., Wang, X., Kasperski, K.L., Hamilton, C., Woudneh, M., Wang, G., Loescher, B., Farwell, A., Dixon, D.G., Ross, M., Pereira, A.D., King, E., Barrow, M.P., Fahlman, B., Bailey, J., McMartin, D.W., Borchers, C.H., Ryan, C.H., Toor, N.S., Gillis, H.M., Zuin, L., Bickerton, G., McMaster, M., Sverko, E., Shang, D., Wilson, L.D. and Wrona, F.J. (2013b) Chemical fingerprinting of naphthenic acids and oil sands process watersA review of analytical methods for environmental samples. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 48(10), 1145-1163.

Hindle, R., Noestheden, M., Peru, K. and Headley, J. (2013) Quantitative analysis of naphthenic acids in water by liquid chromatography-accurate mass time-of-flight mass spectrometry. J. Chroma. A 1286, 166-174.

Huang, R., McPhedran, K.N. and Gamal El-Din, M. (2015a) Ultra Performance liquid chromatography ion mobility time-of-flight mass spectrometry characterization of naphthenic acids species from oil sands process-affected water. Environ. Sci. Technol.

Huang, R., Sun, N., Chelme-Ayala, P., McPhedran, K.N., Changalov, M. and Gamal El-Din, M. (2015b) Fractionation of oil sands-process affected water using pH-dependent extractions: A study of dissociation constants for naphthenic acids species. Chemosphere 127, 291-296.

Hwang, G., Dong, T., Islam, M.S., Sheng, Z.Y., Perez-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 Technol. 130, 269-277.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and Gamal El-Din, M. (2014) Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Sci. Total Environ. 493, 282-290.

Jivraj MN, M.M., Fung B. (1995) Naphthenic acids extraction and quantitative analyses with FT-IR spectroscopy. Syncrude analytical methods manual 4th ed. Edmonton, Canada: Syncrude Canada Ltd. Research Department.

Jones, D., West, C.E., Scarlett, A.G., Frank, R.A. and Rowland, S.J. (2012) Isolation and estimation of the 'aromatic' naphthenic acid content of an oil sands process-affected water extract. J.l of Chroma.y A 1247, 171-175.

Juhascik, M.P. and Jenkins, A.J. (2009) Comparison of liquid/liquid and solid-phase extraction for alkaline drugs. J. of Chroma. Science 47(7), 553-557.

Mahaffey, A. and Dubé, M. (2016) Review of the composition and toxicity of oil sands process-affected water. Environmental Reviews 25(1), 97-114.

Marentette, J.R., Frank, R.A., Bartlett, A.J., Gillis, P.L., Hewitt, L.M., Peru, K.M., Headley, J.V., Brunswick, P., Shang, D. and Parrott, J.L. (2015) Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (Pimephales promelas) embryos. Aquatic Toxic..y 164, 108-117.

Martin, J.W. (2015) The Challenge: Safe release and reintegration of oil sands process-affected water. Environmental Toxicology and Chemistry 34(12), 2682-2682.

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

Martin, J.W., Han, X.M., Peru, K.M. and Headley, J.V. (2008) Comparison of high- and low-resolution electrospray ionization mass spectrometry for the

analysis of naphthenic acid mixtures in oil sands process water. Rapid Communications in Mass Spectrometry 22(12), 1919-1924.

McMartin, D.W., Headley, J.V., Friesen, D.A., Peru, K.M. and Gillies, J.A. (2004) Photolysis of naphthenic acids in natural surface water. J. of Environ. Sci. and Health Part A 39(6), 1361-1383.

McQueen, A.D., Kinley, C.M., Hendrikse, M., Gaspari, D.P., Calomeni, A.J., Iwinski, K.J., Castle, J.W., Haakensen, M.C., Peru, K.M., Headley, J.V. and Rodgers Jr, J.H. (2017) A risk-based approach for identifying constituents of concern in oil sands process-affected water from the Athabasca Oil Sands region. Chemosphere 173, 340-350.

Mitra, S. (2004) Sample Preparation Techniques in Analytical Chemistry, (Vol. 237) pp. 37-138, John Wiley & Sons, Inc (Ed. 2004).

Mohamed, M.H., Wilson, L.D., Shah, J.R., Bailey, J., Peru, K.M. and Headley, J.V. (2015) A novel solid-state fractionation of naphthenic acid fraction components from oil sands process-affected water. Chemosphere 136, 252-258.

Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W. and Giesy, J.P. (2015) Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process-Affected Water. Environ. Sci. & Technol. 49(20), 12395-12404.

Noestheden, M.R., Headley, J.V., Peru, K.M., Barrow, M.P., Burton, L.L., Sakuma, T., Winkler, P. and Campbell, J.L. (2014) Rapid characterization

of naphthenic acids using differential mobility spectrometry and mass spectrometry. Environ. Sci. & Technol. 48(17), 10264-10272.

Nyakas, A., Han, J., Peru, K.M., Headley, J.V. and Borchers, C.H. (2013) Comprehensive analysis of oil sands processed water by direct-infusion fourier-transform ion cyclotron resonance mass spectrometry with and without offline UHPLC sample prefractionation. Environ. Sci. & Technol. 47(9), 4471-4479.

Pereira, A.S., Bhattacharjee, S. and Martin, J.W. (2013a) Characterization of oil sands process-affected waters by liquid chromatography orbitrap mass spectrometry. Environ. Sci. & Technol. 47(10), 5504-5513.

Pereira, A.S., Islam, M.D.S., Gamal El-Din, M. and Martin, J.W. (2013b) Ozonation degrades all detectable organic compound classes in oil sands process-affected water; an application of high-performance liquid chromatography/obitrap mass spectrometry. Rapid Commun. Mass Spectrom. 27(21), 2317-2326.

Reinardy, H.C., Scarlett, A.G., Henry, T.B., West, C.E., Hewitt, L.M., Frank, R.A. and Rowland, S.J. (2013) Aromatic Naphthenic Acids in Oil Sands Process-Affected Water, Resolved by GCxGC-MS, Only Weakly Induce the Gene for Vitellogenin Production in Zebrafish (Danio rerio) Larvae. Environ. Sci.& Technol. 47(12), 6614-6620.

Rogers, V.V., Liber, K. and MacKinnon, M.D. (2002) Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48(5), 519-527.

Ross, M.S., Pereira, A.D., Fennell, J., Davies, M., Johnson, J., Sliva, L. and Martin, J.W. (2012) Quantitative and Qualitative Analysis of Naphthenic Acids in Natural Waters Surrounding the Canadian Oil Sands Industry. Environ. Sci. & Technol. 46(23), 12796-12805.

Rowland, S.J., Pereira, A.S., Martin, J.W., Scarlett, A.G., West, C.E., Lengger, S.K., Wilde, M.J., Pureveen, J., Tegelaar, E.W., Frank, R.A. and Hewitt, L.M. (2014) Mass spectral characterisation of a polar, esterified fraction of an organic extract of an oil sands process water. Rapid Commun.in Mass Spec. 28(21), 2352-2362.

Rowland, S.J., West, C.E., Scarlett, A.G. and Jones, D. (2011a) Identification of individual acids in a commercial sample of naphthenic acids from petroleum by two-dimensional comprehensive gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 25(12), 1741-1751.

Rowland, S.J., West, C.E., Scarlett, A.G., Jones, D. and Frank, R.A. (2011b) Identification of individual tetra- and pentacyclic naphthenic acids in oil sands process water by comprehensive two-dimensional gas chromatography/mass spectrometry. Rapid Commun.in Mass Spec.25(9), 1198-1204.

Rudzinski, W.E., Oehlers, L. and Zhang, Y. (2002) Tandem mass spectrometric characterization of commercial naphthenic acids and a Maya crude oil. Energy & Fuels 16(5), 1178-1185.

Mishra, S., V.M., Dalai, A., McMartin, D., Headley, J. and Peru, K. (2010) Photocatalysis of Naphthenic Acids in Water. Water Resource and Protection 2(7), 644-650 doi: 610.4236/jwarp.2010.27074.

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 93(2), 415-420.

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. Environ. Sci. & Technol. 39(21), 8388-8394.

Scott, A.C., Young, R.F. and Fedorak, P.M. (2008) Comparison of GC-MS and FTIR methods for quantifying naphthenic acids in water samples. Chemosphere 73(8), 1258-1264.

Shang, D., Kim, M., Haberl, M. and Legzdins, A. (2013) Development of a rapid liquid chromatography tandem mass spectrometry method for screening of trace naphthenic acids in aqueous environments. J Chromatogr A 1278, 98-107.

Shell Canada Limited, (2016) Oil Sands Performance Report 2015 (Dated April 22, 2016). Accessed online April 2017: http://www.shell.ca/en_ca/energy-

and-innovation/oil-sands/oil-sands-performance-report/_jcr_content/par/textimage_78af.stream/1463441702776/00cfc57a8625b41538ec24a2fc9d2e7160147b85367300b8dd8b9cb735aa1736/she-2055-oil-sands-performance-report-2015-final1.pdf.

Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M., Hagen, M., Tierney, K.B., Belosevic, M. and Gamal El-Din, M. (2014) Advanced analytical mass spectrometric techniques and bioassays to characterize untreated and ozonated oil sands process-affected water. Environ. Sci. & Technol. 48(19), 11090-11099.

Van den Heuvel, M.R. (2015) In Response: An academic perspective on the release of oil sands process–affected water. Environmental Toxicology and Chemistry 34(12), 2682-2684.

Verbeek, A.G., Mackay, W.C. and Mackinnon, M.D. (1994) Proceedings of the Twentieth Annual Aquatic Toxicity Workshop.

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

West, C.E., Jones, D., Scarlett, A.G. and Rowland, S.J. (2011) Compositional heterogeneity may limit the usefulness of some commercial naphthenic acids for toxicity assays. Sci. of the Total Environ.09(19), 4125-4131.

Woudneh, M.B., Coreen Hamilton, M., Benskin, J.P., Wang, G., McEachern, P. and Cosgrove, J.R. (2013) A novel derivatization-based liquid chromatography tandem mass spectrometry method for quantitative characterization of naphthenic acid isomer profiles in environmental waters. J Chromatogr A 1293, 36-43.

Yue, S., Ramsay, B.A., Brown, R.S., Wang, J. and Ramsay, J.A. (2015a) Identification of estrogenic compounds in oil sands process waters by effect directed analysis. Environ. Sci. & Technol. 49(1), 570-577.

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J. (2015b) Toxicity and composition profiles of solid phase extracts of oil sands process-affected water. Sci. Total Environ. 538, 573-582.

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J.A. (2016) Biodegradation and detoxification of naphthenic acids in oil sands process affected waters. Sci. Total Environ. 572, 273-279.

Zhang, K., Pereira, A.S. and Martin, J.W. (2015) Estimates of Octanol–Water partitioning for thousands of dissolved organic species in oil sands process-affected water. Environ. Sci. & Technol. 49(14), 8907-8913.

Zhao, B., R. Currie and H. Mian (2012) Catalogue of analytical methods for naphthenic acids related to oil sands operations. Oil Sands Research and

Information Network, University of Alberta, School of Energy and the Environment, Edmonton, Alberta. OSRIN Report No. TR-21.65 pp.

Zubot, W., MacKinnon, M.D., Chelme-Ayala, P., Smith, D.W. and El-Din, M.G. (2012) Petroleum coke adsorption as a water management option for oil sands process-affected water. Sci. Total Environ. 427, 364-372.

3 UNDERSTANDING THE SIMILARITIES AND DIFFERENCES BETWEEN OZONE AND PEROXONE IN THE DEGRADATION OF NAPHTHENIC ACIDS: COMPARATIVE PERFORMANCE FOR POTENTIAL TREATMENT [2]

3.1 Introduction

The enormous economical processes of bitumen extraction in the Canadian oil sands have led to the generation of large volumes of oil sands process-affected water (OSPW), which may cause environmental impacts on the surrounding region (Kelly et al. 2010). OSPW is stored on site in tailings and end-pit ponds that expand in footprint as the industry grows. OSPW is known to have acute and chronic toxicity to a variety of organisms (Gosselin et al. 2010) which has been attributed mostly to naphthenic acids (NAs) (Han et al. 2009, Headley and McMartin 2004). NAs are a group of alicyclic and aliphatic compounds with a general formula of $C_nH_{2n+Z}O_x$, where n represents the carbon number, Z (0 or negative even integer) the hydrogen deficiency number, and x the number of oxygen atoms present (x=2 for classical NAs (currently O_2-NAs) and x≥3 for oxidized NAs (oxy-NAs)) while their sum (classical and oxidized) is denoted as O_x-NAs. Nyakas et al. (2013) reported that O_x-NAs represent 64% of the organic

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acid-extractable fraction (AEF) in OSPW, while the sulfur-containing NA (S-NA) and nitrogen-containing NA (N-NA) species represent 31% among other organic compounds found in OSPW (Grewer et al. 2010).

Several treatment processes are currently being investigated at the bench-scale level to reduce/eliminate the OSPW toxicity to allow its eventual release into the environment. The use of ozonation (O_3) has been shown to be effective in degrading OSPW NAs, resulting in a partial (He et al. 2010, Martin et al. 2010) or complete toxicity reduction to *Vibrio fischeri* (Garcia-Garcia et al. 2011). Wang et al. (Wang et al. 2013) found reduced toxicity of OSPW after ozonation for *in vitro* and *in vivo* mice assays and complete removal of toxicity to *Vibrio fischeri*. However, previous studies used high and wide ranges of ozone doses from 20 mg/L (Sun et al. 2014) to 100 (Afzal et al. 2014, Perez-Estrada et al. 2011, Wang et al. 2016) and 360 mg/L (Wang et al. 2013), with the doses exceeding 100 mg/L not being cost-effective for the treatment of large volumes of OSPW. Anderson et al. (Anderson et al. 2011) found a negative influence of ozonated OSPW with high dose (80 mg/L) based on increasing toxicity to *Chironomus dilutes* despite the NA reduction, indicating the formation of toxic by-products post ozonation. Additionally, high ozone doses result in reduced NA degradation efficiency (e.g., lower degradation with higher doses). Gamal El-Din et al. (Gamal El-Din et al. 2011) reported that 0.6 mg/L AEF were oxidized per mg/L utilized ozone for doses $\leq$ 80 mg/L; however, at O_3 doses higher than 100 mg/L, the degradation declined to only 0.3 mg/L. Similarly, Wang et al. (Wang et al. 2013), found that for O_3 doses below 50 mg/L the degradation efficiency was 0.5 mg/L O_x-NAs

degraded per mg/L utilized ozone, while for O_3 doses exceeding 50 mg/L the degradation efficiency was only 0.05 mg/L NAs per mg/L ozone. Similar findings were reported by Islam et al. (Islam et al. 2014a); increasing the ozone dose to 100 mg/L led to a sharp AEF decrease; however, for ozone doses ≥ 100 mg/L, the removal reached a plateau. Likewise, the authors found that the removal efficiency of various NA species decreased considerably from 50 up to 170 mg/L (2014b). Beside the negative impact of high ozone doses on the degradation, it extends to the influence of the structure reactivity compared to the low doses (i.e., the structures with higher carbons and Z showed higher reactivity at ozone lower than 50 mg/L) (Wang et al. 2013). Clearly, the optimization of the ozonation process is needed given the high costs of ozonation, coupled with inefficient degradation at high doses (Ternes et al. 2003), which limits the feasibility of its use in large-scale industrial treatments. Therefore, the determination of optimum dosage to achieve required levels of removal, especially in complex water matrices such as OSPW, is required.

The limiting factor of the ozonation process is the production of •OH which readily reacts with almost any organic compound unselectively to increase degradation compared to molecular O_3 (Lee et al. 2013). The •OH increase can be accomplished by introducing hydrogen peroxide (H_2O_2) in the ozonation process (an advanced oxidation process or AOP) (2014, Oh et al. 2014). Afzal et al. (Afzal et al. 2014) investigated the feasibility of the peroxone (H_2O_2:O_3) process, using O_3 dose of 85 mg/L, to degrade model NA compounds including limited preliminary experiments for OSPW. Although focusing mainly on the model

NAs, the authors found that the peroxone process may be useful for OSPW treatment, but indicated the need of further research.

Given the limitations of the previous research and based on our knowledge due to the lack of comprehensive studies for peroxone applications in OSPW, a significant step forward is taken with this research in determining the peroxone feasibility for OSPW treatment and considerations about using minimum oxidant doses. The typical ozone dose for wastewater in terms of chemical oxygen demand (COD) is 0.23 – 0.8 mg ozone per mg COD (Di Iaconi 2012, Jagadevan et al. 2013). While the raw COD in our OSPW sample is 216 mg/L, therefore our typical range of ozone dose is 49.7 – 172.8 mg/L. Interestingly, Pocostales et al. (Pocostales et al. 2010) showed that the H_2O_2 addition to ozone doses increased the •OH yield, while the typical applied molar peroxone ratio for wastewaters and reuse applications is 1:2 (H_2O_2:O_3) (Paillard et al. 1988, Pisarenko et al. 2012, Pocostales et al. 2010). Similarly, Rosenfeldt et al. (Rosenfeldt et al. 2006) examined the 1:2 ratio, in addition to 1:1, in their investigation of •OH formation using different AOPs. Thus, it can be hypothesized that combining 50 mg/L utilized ozone with specific amount of H_2O_2 at specific ratio might be beneficial for the degradation and detoxification of OSPW.

In the present study, peroxone (1:2) treatment was assessed by the addition of 20 mg/L of H_2O_2 to 50 mg/L utilized ozone. The same H_2O_2 concentration was used for the peroxone (1:1) treatment that was conducted using ozone of 30 mg/L. Both utilized ozone doses were also conducted alone (i.e., without H_2O_2) to compare the results with those obtained during peroxone treatments. The main

objective of this study was to investigate the impact of the peroxone treatment on the degradation of NA species and OSPW toxicity reduction toward *Vibrio fischeri*. The specific objectives were as follows: (i) to assess the relative efficacy of ozone and peroxone in terms of NA degradation; (ii) to grasp the significance of H_2O_2 addition to ozone by elucidating the degradation pathways with/without •OH scavenger; (iii) to study the individual impact of carbon and Z as well as to examine, for the first time, their combined effect on the structure reactivity toward O_2-NA, and O_x-NA degradation; and (iv) to determine the best ozone and H_2O_2 doses as well as resulting peroxone molar ratio (mol H_2O_2/mol O_3) using several metrics, including degradation of NAs (O_2-NA and O_x-NA concentrations) per oxidant utilization, ion-mobility spectroscopy (IMS), fluorophore organic compounds removal; and toxicity assessment of the treated OSPWs.

3.2 Materials and methods

3.2.1 Chemical and reagents

Hydrogen peroxide (H_2O_2; 30% w/w), sulfuric acid (H_2SO_4; 95-98% w/w), bovine liver catalase (2950 units/mg), Optima grade dichloromethane (DCM), tert-butyl alcohol (TBA) and phenol (BioXtra >99.5%), were obtained from Fisher Scientific Co. (Edmonton, AB, Canada) and used as received. In addition, titanium(IV) oxysulfate was obtained from Sigma-Aldrich. Ultra-dry oxygen for ozone generation and purging were obtained from Praxair (Edmonton, AB, Canada). OSPW from an active tailings pond was received in 2014 from Fort

McMurray, Alberta, Canada, and stored in 200 L high-density polyethylene barrels in the dark at 4 °C.

3.2.2 Ozone and peroxone experiments

Ozone and peroxone semi-batch experiments were performed in 4-L reactors at the natural pH of OSPW (8.4±0.1; Table 2.1) at room temperature (20±1 °C). Specific H_2O_2 stock solution was prepared for the peroxone experiments and the required H_2O_2 dose was added prior to ozone exposure. Treatments included: (a) control using only 20 mg/L H_2O_2; (b) 30 mg/L ozone dose; (c) 50 mg/L ozone dose; (d) peroxone 1:1 (20 mg/L H_2O_2: 30 mg/L O_3); and (e) peroxone 1:2 (20 mg/L H_2O_2: 50 mg/L O_3). In addition, those former conditions were repeated after adding a specific amount of TBA (25 mM) to scavenge the •OH. Ozone was produced by an ozone generator (AGSO 30 Effizon, WEDECO AG Water Technology, Herford, Germany), and monitored throughout the experiments in both the feed and off-gas lines using two identical ozone monitors (HC-500, PCI-WEDECO AG Water Technology, Herford, Germany). Note that all ozone doses are determined as utilized doses (i.e., utilized refers to the net quantified ozone at the end of the treatments, while residual ozone measured using indigo method was very minimal) and the 20 mg/L H_2O_2 is the initial dose. After each experiment (around 10 minutes of ozonation with stable feed gas rate) the OSPW was purged with oxygen for 20 minutes to strip residual ozone and to stop further reactions (samples after quenching, during and after purging were analyzed and confirmed the reaction termination for quality control).

The schematic of the ozonation experiments and the entire setup is provided in Figure A3 in Appendix A. The utilized ozone dose/or transferred ozone dose (i.e., the transferred ozone in the treated water either decayed and consumed through direct reaction of molecular ozone with the NAs or decayed through auto decomposition to •OH) was calculated using the following equation:

$$\Delta O_3 = \int_0^t \frac{\left(Q_{G,in} C_{G,in} - Q_{G,out} Q_{G,out}\right)}{V_L} dt - C_L$$

Where:

- ΔO_3 (mg/L) = ozone concentration in the ozonated product;
- $C_{G,in}$ (mg/L) = ozone concentration in the feed gas;
- $C_{G,out}$ (mg/L) = ozone concentration in the off gas;
- C_L (mg/L) = residual ozone concentration;
- V_L (L)= effective reactor volume;
- $Q_{G,in}$ (L/min) = feed-gas flow rate;
- $Q_{G,out}$ (L/min)= off-gas flow rate; and
- t (min)= ozone contact time.

The detailed procedure for the ozonation process can be found elsewhere (Wang et al. 2013; Chelme-Ayala et al. 2011; Gamal El-Din et al. 2011).

Table 3.1. Water quality characteristics of raw OSPW (mg/L)

Parameter	Average (±SD)
pH (unitless)	8.4 ± 0.1
Total organic carbon (TOC)	56 ± 6.0
Chemical oxygen demand (COD)	216 ± 2.1
Dissolved organic carbon (DOC)	45.6 ± 6.0
DOC/TOC	0.8
Acid extractable organic fraction (AEF)	71.3 ± 0.6
UV_{254} (cm^{-1})	0.49 ± 0.02
Chloride	641± 27.4
Total solids (TS)	2681 ± 60
Alkalinity (Ca CO_3)	518 ± 60
Biochemical oxygen demand (BOD_5)	3.3 ± 1.6
Classical NAs (O_2-NAs)	35.5 ±0.8
NAs at x=3 only (O_3-NAs)	9.1 ±0.2
NAs at x=4 only (O_4-NAs)	10.2 ± 0.2
Oxidized NA* (oxy-NAs)*	19.3 ±0.2
Ox-NAs **	54.8±0.2

*Oxidized NA (oxy-NAs)= (sum of NAs at x≥3)

**Ox-NAs = (sum of classical and oxidized NAs)

3.2.3 Experimental analysis

H_2O_2 was determined by a UV-Vis spectrophotometer (Ultrospec 2100 pro, Biochrom, MA, USA) using the titanium(IV) oxysulfate method according to DIN 38402H15. Residual H_2O_2 was quenched at the end of peroxone experiment treatments using bovine liver catalase with 1 μM of H_2O_2 transformed by one unit of catalase per minute (Klamerth et al. 2013, Oh et al. 2014). A control experiment (blank) was implemented using H_2O_2 only.

All samples were filtered prior to analysis using 0.45 μm nylon filters (Supelco Analytical, Bellefonte, PA, USA) unless otherwise stated.

The concentrations of NA species were determined as a function of carbon and Z numbers using an ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) system (Waters, Milford, MA). Noteworthy that decreasing Z number (e.g., Z= -4 versus Z= -10) refers to increasing the hydrogen deficiency and increasing the number of rings.

The detected O_2 -NAs and oxy-NAs are based on the general or empirical formula $C_nH_{2n+Z}O_x$ (x=2, 3, 4) with carbon number ranging from 7 to 26 and Z from 0 to -18. Exact masses of NAs (m/z = ± 0.001) that fit the empirical formula $C_nH_{2n+Z}O_x$ were calculated for entirely combinations of carbon = 7 to 26, Z = 0 to -18 and x = 2 to 4 during the data analyses. 500 µL of centrifuged sample, 100 µL of 4 mg/L internal standard (ISD) (myristic acid-1-^{13}C) in methanol plus methanol of 400 µL (Fisher Scientific, ON) filled to a final volume of 1 mL was used as an injection solution. Mass spectrometry experiment was conducted in negative ion mode. The peak with a signal to noise ratio (S/N) more than 10 and mass difference less than 1 mDa was then integrated and its area was recorded. The ISD concentration was kept at 0.4 mg/L where its area was also subtracted along the NA species. Each individual NA species concentration (C_{NAs}) was estimated using equation (1). It was assumed that the ionization efficiency of NA species, are similar to ISD during the process of ion evaporation.

$$C_{(NAs)}=\frac{Area_{(NAs)}}{Area_{(ISD)}}\times C_{ISD} \qquad (1)$$

All experiments treatments were conducted in duplicate; however, only one of the duplicate samples was analyzed in UPLC-TOFMS analyses. Injecting only one sample proceeds the checking and confirmation of the consistency of the

two sets of the treated samples using other analyses such as ion mobility spectra, chemical oxygen demand and synchronous fluorescence spectra. Additionally, raw OSPW as well as standards were injected to adjust and confirm the calibration, the consistency, and the accuracy of the UPLC system. The analyses were conducted using a Waters Synapt G2 HDMS system (30,000 FWHM), equipped with an electrospray ionization source. MassLynx version 4.1 and TargetLynx version 4.1 were used to control the system and to analyze the data of target compounds, respectively. Tuning and calibration steps were performed using leucine enkephalin standard solutions with other chemicals. Tri-Wave® ion-mobility cell of 15 cm long, using nitrogen as the drift gas of purity > 99%, was used for the ion-mobility spectrometry (IMS). In brief, a transfer cell in IMS was responsible for collecting definite amount of ions with a helium gate to release the ions into the ion-mobility cell. The detailed procedure can be found elsewhere as reported previously (Shu et al. 2014, Sun et al. 2014).

Acute toxicity was measured using *Vibrio fischeri* 81.9% screening test protocol with a Microtox analyser (Model 500, Azur Environmental, Carlsbad, U.S.A.) (Chelme-Ayala et al. 2011, Islam et al. 2014b). The reduction of toxicity in terms of luminescence inhibition during incubation is a direct indication for toxicity decrease (Jones et al. 2011, Shu et al. 2014, Wang et al. 2013). P-values were calculated using Tukey Pairwise Comparisons in Minitab 17.

The acid extractable fraction (AEF) was determined using Fourier Transform Infrared Spectroscopy (FT-IR) method(Clemente and Fedorak 2005, Han et al. 2009), For each treatment, triplicate 50 mL aliquots were filtered and

acidified to pH 2-2.5 using H_2SO_4. In a 250 mL separatory funnel, 25 mL of DCM was added to the 50 mL processed sample and mixed well by shaking for 2 min to extract the acid extractable fraction (AEF) from the sample. This process was repeated and the resulting 50 mL DCM was dried in fume hood overnight. The dried samples were reconstituted in DCM before being assessed for AEF concentration via Fourier Transform Infrared Spectroscopy (FT-IR) method using a Nicolet 8700 FT-IR spectrometer (ThermoElectron Corporation, Waltham, USA). The absorbance intensities were measured at 1706 and 1743 cm^{-1} that are known as the carboxylic group intensities that would indicate organic compounds such as the NAs. More detailed information about the FT-IR analyses and the instrument can be found elsewhere (Gamal El-Din et al. 2011).

3.3. Results and discussion

3.3.1 Impact of treatments on NA degradation and pathways

The degradation of classical NAs (O_2-NAs) and O_x-NAs (sum of classical and oxidized NAs) for all treatment conditions are shown in Table 3.2. The highest degradation for O_2-NAs was observed in the peroxone (1:2) with 91%, followed by 50 mg/L ozone with 84%. The peroxone (1:1) and 30 mg/L ozone had similar removal of 77%. The highest removal (76%) of O_x-NAs was observed using the peroxone (1:2) process, followed by 50 mg/L ozone dose, peroxone (1:1), and 30 mg/L ozone dose with 63%, 59% and 58%, respectively (Table 3.2). Overall, the peroxone (1:2) treatment exhibited the highest degradation for O_2-NAs and O_x-NAs. Therefore, it is likely that the reduction of NA concentration is

due to the addition of H_2O_2 yielding a higher amount of •OH and the acceleration of ozone decay through the peroxone process as compared to conventional ozone (Lee et al. 2014).

Table 3.2. Percentage of degradation of O_2-NA species and degradation of O_x-NA species in mg/L per oxidant dose in mg/L under different treatment conditions.

Treatment	Degradation (%)		Degradation (mg/L of NAs per mg/L utilized O_3 dose)		Degradation (mg/L of NAs per mg/L initial H_2O_2 dose)[a]		Degradation (mg/L of NAs per mg/L utilized H_2O_2 dose)	
	O_2 -NAs	O_x-NAs	O_2 -NAs	O_x -NAs	O_2 -NAs	O_x-NAs	O_2 -NAs	O_x -NAs
Peroxone (1:2) [b]	91	76	0.65	0.83	1.64	2.08	3.28	4.17
Peroxone (1:2) +TBA	49	35	0.35	0.36	0.88	0.89	2.75	2.79
50 mg/L ozone	84	63	0.60	0.70	-	-	-	-
50 mg/L ozone +TBA	47	32	0.34	0.36	-	-	-	-
Peroxone (1:1) [c]	77	59	0.91	1.07	1.37	1.60	3.91	4.58
Peroxone (1:1) +TBA	39	26	0.46	0.46	0.69	0.69	5.60	5.60
30 mg/L ozone	77	58	0.92	1.06	-	-	-	-
30 mg/L ozone +TBA	51	34	0.61	0.62	-	-	-	-

[a] The initial concentration of H_2O_2 is 20 mg/L, residual concentration of H_2O_2 = 10.20 mg/L and 13.64 mg/L in peroxone (1:2) and peroxone (1:1), respectively. [b] Peroxone (1:2) (20 mg/L H_2O_2:50 mg/L O_3). [c] Peroxone (1:1) (20 mg/L H_2O_2:30

To grasp the significance of adding H_2O_2 to the ozone and to elucidate the possible degradation pathways, we spiked TBA as a scavenger for the •OH. Figures 3.1 and 3.2 show the concentration profiles of O_2-NAs and O_x-NAs, respectively, for the different treatment conditions (with and without TBA). As shown in Figures 3.1-g and 3.1-h, the O_2-NA concentration increased from 5.8 mg/L to 18.7 mg/L when TBA was used with 50 mg/L ozone and likewise the O_2-NA concentration increased from 3.1 mg/L to 18 mg/L in peroxone (1:2) + TBA. In all treatments, the degradation decreased by approximately half after adding TBA. For instance, the degradation of O_2-NAs and O_x-NAs in peroxone (1:2) decreased from 91% to 49% and 76% to 35%, respectively (Table 3.2). The reason can be attributed to the suppression of the •OH pathway (von Gunten and von Sonntag 2012). It can be observed that the direct reaction or molecular ozone pathway was responsible for degrading around 40-50% of the O_2-NAs and 26-34% of O_x-NAs (Table 3.2). Even though there is an enhancement in NA degradation in peroxone treatments due to the elevated yield of •OH, It is worth noting that the free radical intermediates could be an additional degradation pathway to the molecular ozone and •OH (Beltrán 2004).Schematics reactions of H_2O_2/O_3 chemistry for the $^{\bullet}OH$ and $^{\bullet}O_2^-$ species produced are illustrated in equations 1-3 [35, 37] and hypothetical the principal reactions for the oxidation by the generated ·OH and molecular ozone are as follows in equations 4-7:

$$H_2O_2 \rightarrow H^+ + HO_2^- \quad (1)$$

$$O_3 + HO_2^- \rightarrow \bullet OH + 2 \bullet O_2^- + O_2 \quad (2)$$

$$2O_3 + H_2O_2 \rightarrow 2 \bullet OH + 3O_2 \quad (3)$$

$O_3 + O_xNAs \rightarrow$ products (4)

$\bullet OH + O_xNAs \rightarrow$ products (5)

$\bullet OH + O_2NAs \rightarrow$ oxidized NAs (6)

$\bullet OH +$ oxidized NAs $\rightarrow$

oxidized NAs with higher oxygen + intermediates (7)

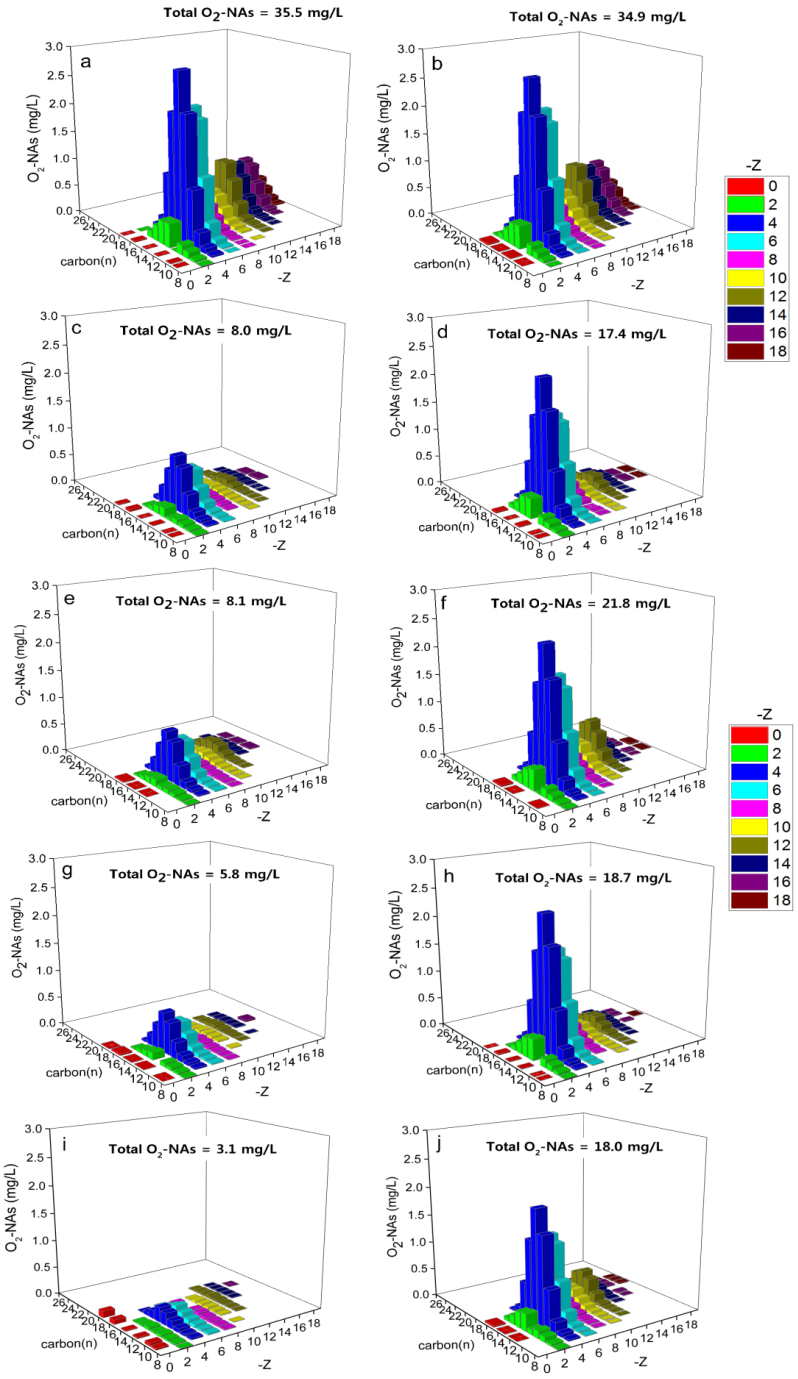

Figure 3.1. Concentration of O_2-NAs before and after different treatments: (a) raw OSPW; (b) control-hydrogen peroxide only; (c) 30 mg/L ozone; (d) 30 mg/L ozone + TBA; (e) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3); (f) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3) + TBA; (g) 50 mg/L ozone; (h) 50 mg/L ozone + TBA; (i) peroxone (1:2) (20 mg/L H_2O_2:50 mg/L O_3); and (j) peroxone (1:2) (20 mg/L H_2O_2:50 mg/L O_3)+ TBA. All ozone doses are utilized doses and the 20 mg/L H_2O_2 is the initial concentration.

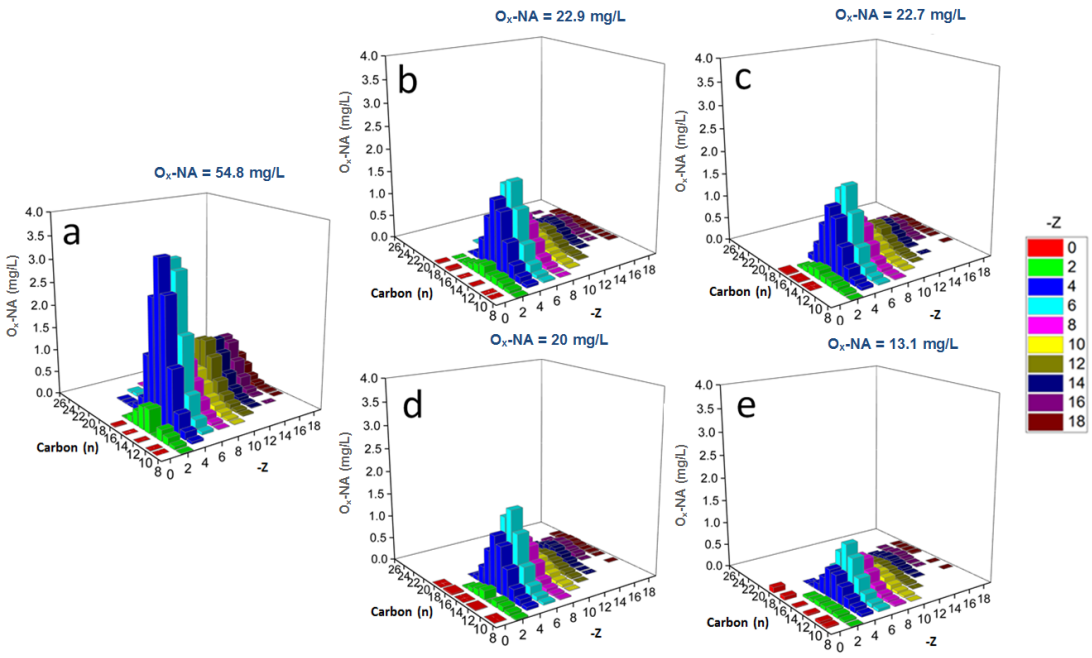

Figure 3.2. Concentration of O_x-NAs before and after different treatments: (a) raw OSPW; (b) 30 mg/L ozone; (c) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3); (d) 50 mg/L ozone; and (e) peroxone (1:2) (20 mg/L H_2O_2:50 mg/L O_3). All ozone doses are utilized doses and the 20 mg/L H_2O_2 is the initial concentration.

3.3.2 Impact of treatments on NA carbon (n) and Z numbers and their combined effect

The elimination of a contaminant by ozone treatment is determined by its reactivity (von Gunten and von Sonntag 2012), therefore, the understanding of the reactivity can be developed by structure reactivity rather than exact structure determination (Moloney 2009) or by monitoring how the compounds react, or by following the conversion of the reactants to intermediates and products (Moloney 2009).

The n and Z numbers of the NAs are indicative for their structure and as a consequence an indication of the structure-reactivity or structure-affinity toward oxidation. This provides an interesting set of metrics to assess the efficiency of NA degradation and treatment specificity (Figure 3.3, Tables 3.4 and 3.5). Overall, the peroxone (1:2) treatment has the highest degradation for the O_2-NA, O_3-NA, O_4-NA species and O_x-NAs based on both the n (Figure 3.3a,c,e,g) and Z numbers (Figure 3.3b,d,f,h) which can be attributed to the elevated production of •OH. Generally, the higher degradation levels are associated with higher n and |-Z| number in all treatments for the O_2-NA, O_3-NA, O_4-NA species and O_x -NAs (Tables 3.4 and 3.5). However, to understand more about the relationship between structure of the compounds and their affinity or their reactivity toward oxidation, we divided our discussion to three parts: the first part is focusing on the structure relationship with n (Figure 3.3a,c,e,g), then the second part with Z (Figure 3.3 (=b,d,f,h) and third part is related to the combined effect between n and Z (Figure 3.4 a,b,c,d,e,f,g,h).

For all treatments, there is a positive correlation between increasing n and increasing degradation for O_2-NAs and O_x-NAs with 50% or greater degradation for $n>10$ (O_2-NAs) and $n>12$ (O_x-NAs) (Figure 3.3a,c). Degradation higher than 50% for $n>16$ can be observed for the O_3-NA and O_4-NA species. Low degradation for O_3-NAs and O_4-NAs at $n<16$ can be due to the generation of oxidized NAs from parent compounds during the oxidation processes (Klamerth et al. 2015, Sun et al. 2014). The addition of TBA suppresses the •OH route in all treatments, apparently the •OH is highly reactive with the higher n more than the

lower n. However, the reduction in the degradation with TBA is higher at 12<n< 17 (i.e., ≈50%) compared to n<12 and n>18 (i.e., ≈30%). For instance, at n=16 and n=19 in O_x-NAs using peroxone (1:2) +TBA and 50 mg/L ozone+TBA, the degradation decreased around 44-49%, and around 33%, respectively (Table 3.5).

Similarly with Z number, the highest level of degradation (i.e., >80%) occurs with increasing the hydrogen deficiency at |-Z| ≥14 for O_x-NAs and at all |-Z| for O_2-NAs (Figure 3.3b,d). Unlike for O_3-NA and O_4-NA species, high degradation (i.e., >60%) happens at |-Z|≥14 only (Figure 3.3f,h). The differences in degradation of some species was previously reported due to the creation of some species (Pereira et al. 2013) from the oxidation and cleavage of higher n and |-Z| NAs. Our observations on real OSPW agree with the findings on model compounds from Pérez-Estrada et al. (2011) and Afzal, et al. (2012). The authors of these studies showed that model NA compounds with a higher number of rings (high |-Z| number) and higher n have higher reactivity toward oxidation. Spiking TBA highly influenced most of the degradation with Z. For instance, the degradation decreased by 40-50% for |-Z| ≤12 in O_x-NAs and O_2-NAs (Figure 3.3b,d). Likewise, a significant reduction in degradation of O_3-NA and O_4-NA species was observed after adding TBA. Overall, the high removals for larger |-Z| numbers are due to their larger amount of tertiary carbons, rings and possible double bonds which are more reactive toward oxidation (Afzal et al. 2012, Perez-Estrada et al. 2011). The moderate degradation in O_x-NAs influenced by very low degradation in O_3-NA and O_4-NA species compared to O_2-NA can be attributed to

the generation/production of oxidized NAs from parent O_2-NAs during oxidation (Klamerth et al. 2015, Sun et al. 2014).

The structure reactivity cannot be related only to molecular weight in terms of n or cyclicity in terms of Z but also should be related to their combined effect. Based on our knowledge, previous studies focused only on separate influences for n and Z with regards to classical NAs. In our study, we examined, for the first time, the synergic/combined impact of n and Z number for all NA species. It is useful to identify the degradation efficiency for NA species at each individual n at different Z values as shown in Figure 3.4 (a,b,c,d,e,f,g,h) for the O_2-NAs and Figure 3.5 for O_x-NAs. Overall, the degradation showed increasing trend with increasing n for each of the O_2-NAs and O_x-NAs, while higher |-Z| numbers showed increased degradation of the O_2-NAs at $n \geq 14$ and lower |-Z| number at $n < 14$.

The highest degradation trend with decreasing the hydrogen deficiency or |-Z| number for O_x-NAs was observed for peroxone (1:2) treatment (Figure 3.5d). The oxidation of NAs by the peroxone (1:2) process resulted in the complete degradation of higher molecular weight O_2-NAs (n = 15-20) (Figure 3.4f and Table 3.7). The degradation levels of O_2-NAs for every treatment are presented in Tables 3.6 to 3.9 where some negative degradation levels were observed. This indicates that these species were generated during the oxidation treatments as reported previously (Klamerth et al. 2015).

Comparing the O_2-NA degradation for 30 mg/L ozone and the peroxone (1:1) treatments at $n > 13$ for Z=-10,-12,-14, $n > 16$ for Z=-8 and n= 17 and 18 at

Z=-6, the degradation decreases after adding H_2O_2 (Figure 3.4a,b and Tables 3.7 and 3.8). Increasing the H_2O_2 concentration might not be advantageous under some conditions given that few possibilities to occur: 1- Similar levels of •OH are produced in either peroxone (1:1) compared to ozone alone due to the slow ozone decomposition at small ozone doses while the H_2O_2 addition is significant only at elevated ozone doses (Pocostales et al. 2010) 2- Scavenging effect is occurring from the peroxide at this condition (i.e., peroxone (1:1)). It has been reported that controlling the molar ratio of H_2O_2: O_3 to less than 0.5 can minimize both the scavenging effect of H_2O_2 and residual H_2O_2 (Wu et al. 2015). In other words, the increase of the ratio ≥0.45 had a negative impact on the overall removal due to the competing effects of peroxide and HO^-_2 (i.e., the dissociated product of peroxide at high pH) (Suh and Mohseni 2004). 3- High levels of generated •OH can be scavenged by the O_3 (Gottschalk et al. 2010) or the radical-radical coupling process (Glaze et al. 1987). Conversely, the impact of scavenging could not be confirmed/or ignored on this study due to the specificity of the current experiments in elucidating the overall differences as well as the requirement to have probe compound as an indication for the differences in the levels and yield of •OH. Our decision to use a fixed low H_2O_2 concentration compatible with two low levels of ozone doses (i.e., as per the water characteristics as mentioned earlier) at two well-known optimum molar ratios was to minimize the scavenging effect at higher H_2O_2 doses. The optimum H_2O_2 dose depends on the rate of ozone delivery to the system, the presence of the initiators and inhibitors in the water matrix and concentration of the target contaminants in the water (Suh and

Mohseni 2004) which confirms the relative complex role of H_2O_2 addition (Safarzadeh et al. 2001) due to its double character as an scavenger and an initiator in the different water matrix (Fernando 2003). The O_2-NA species for the 50 mg/L ozone (Figure 3.4e; Table 3.9) and peroxone (1:2) (Figure 3.4f; Table 3.6) had similar high degradation patterns as the other two treatments. To examine the O_2-NAs further, a 3-D representation of these species showing all n numbers for each Z number (0 to -18) is shown in Figure 3.6.

To attain deep insights about the relative differences between ozone and peroxone treatments with respect to the impact of structure (i.e., combined impact of n and Z numbers) and the mechanism of degradation (i.e., radical pathways versus molecular ozone), we highlighted some observations here.

The first observation is that the $|-Z|\geq 10$ in both O_2-NAs and O_x-NAs are preferentially removed in all treatments (see O_2-NAs in Figure 3.4 (a,b,e,f) and O_x-NAs in Figure 3.5 (a,b,e,f)). However, the degradation for these Z isomers in ozone treatments (i.e., 30 mg/L and 50 mg/L ozone) through molecular ozone pathway was significant compared to •OH pathway. This can be observed by the addition of TBA in both ozone treatments 30 mg/L and 50 mg/L ozone (see Fig. 3.4 c,g, for O_2-NAs and see Figure 3.5 c,g, for O_x-NAs). There were no statistical differences in the degradation of these Z isomers using ozone treatments either with or without TBA (p-value $0.096>0.05$) compared to other peroxone treatments (p-value $0.01<0.05$). This finding with an emphasis here in OSPW is consistent with Pérez-Estrada et al. (2011) and Afzal et al. (2012) studies about model compounds. The studies confirmed that higher tertiary C-H bonds and

higher number of rings have higher reactivity in ozonation (i.e., molecular ozone) (Pérez-Estrada et al. 2011) compared to less reactivity in UV/H_2O_2 (i.e., through •OH) (Afzal et al. 2012).

The second observation is that the addition of TBA to the four conditions (i.e., peroxone (1:2), 50 mg/L ozone, peroxone (1:1), and 30 mg/L ozone) did reduce partially the degradation while having the same trend of degradation increase with n of NAs. The reduction was significant in some treatments, especially with low molecular weights at n=9-14 (p-value $0.01<0.05$). For instance, in the peroxone (1:2) + TBA, the degradation of $-4\leq Z\leq -12$ at $11\leq n\leq 19$ were reduced considerably from 80% or more to 50% or less (Figure 3.3h). Likewise, there was resemblance between 50 mg/L ozone + TBA with peroxone (1:2) + TBA at $-4\leq Z\leq -8$; however, at Z=-10 and -12, slight reduction was observed. In addition, the Z= -14 at n>15 decreases marginally in all treatments with TBA.

It is interesting to note that the high reduction in high carbon is rationally attributed due to the increase of available hydrogen atoms for abstraction which typically increased the reactivity toward •OH. Nevertheless, the significant reduction in the smaller NAs degradation after adding TBA is not clear and might be attributed for two possibilities, first is due to reduction in •OH available for the alkyl substitutions of low n isomers (Perez-Estrada et al. 2011) and second; that the lower NAs are the residual compounds from degradation of the higher molecular weight NA (Vaiopoulou et al. 2015) and their rate of production is much more than their rate of degradation after adding TBA.

In summary, the current observations highlight the significance of studying n and Z individually as well as their combined impact on the degradation and the structure reactivity.

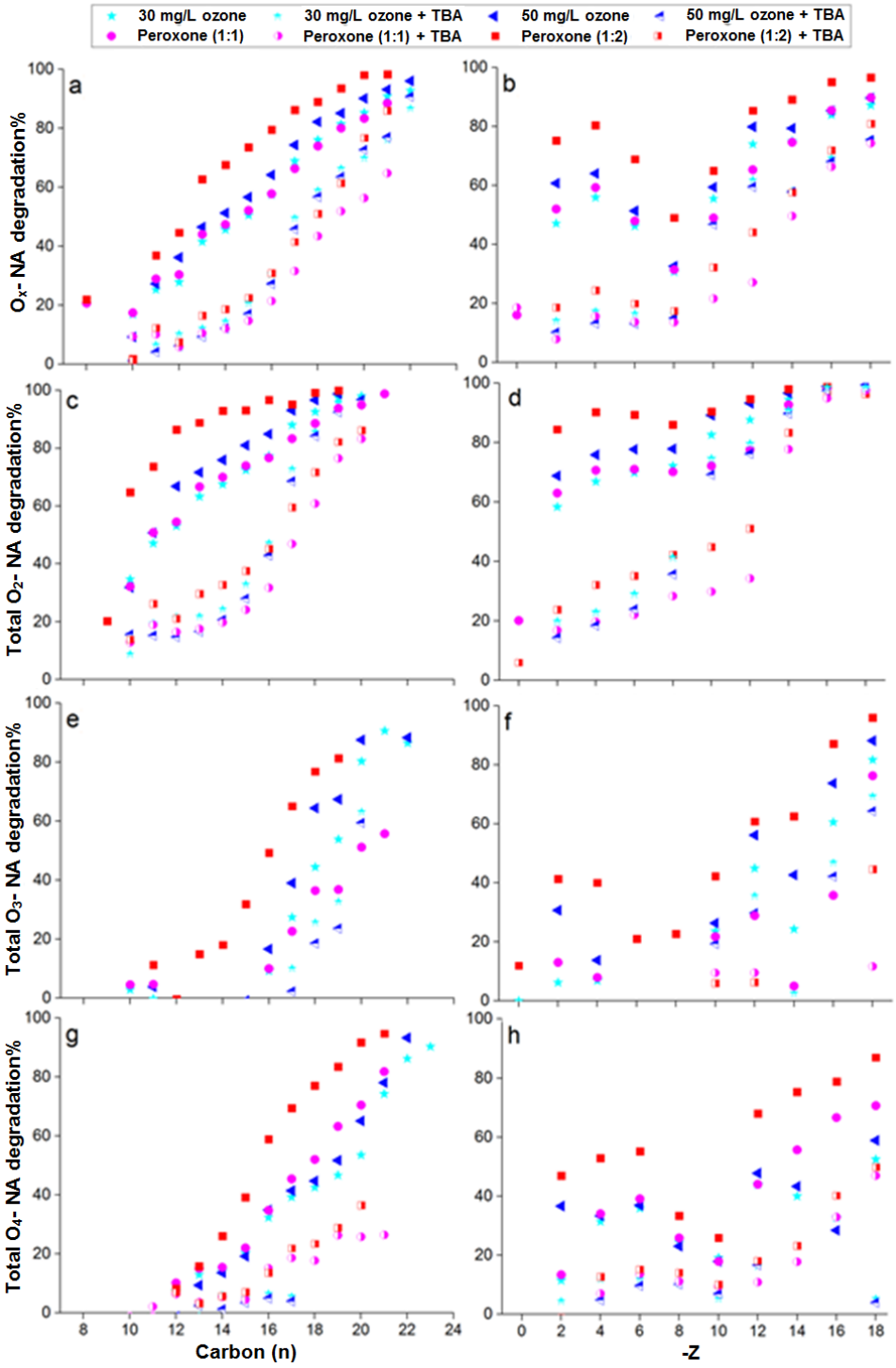

Figure 3.3. Carbon and Z number of NA species after various treatments in terms of: (a) and (b) O_x-NAs; (c) and (d) O_2-NAs; (e) and (f) O_3-NAs; and (g) and (h) O_4-NAs. O_x-NAs = (sum of classical and oxidized NAs).

Table 3.3. % of O_2, and O_x-NA degradation based on Z number.

NAs species	Treatment	Degradation % with -Z									
		0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**
O_2-NA	Peroxone (1:2)	-793.9	84.4	90.2	89.4	86	90.4	94.6	98	99.8	100
	50 mg/L ozone	-37.9	68.8	75.9	77.7	77.9	89.1	93.2	96.6	98.8	100
	Peroxone (1:1)	20.1	63	70.7	71	70.1	72.1	77.6	92.7	98.4	100
	30 mg/L ozone	-108.3	58.3	66.8	69.8	72.1	82.6	87.6	94.5	98.4	100
	Control - Peroxide only	-40.8	2	1	1.0	2.4	0	3	1.7	4	7.4

		Degradation % with -Z									
		0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**
O_x-NA	Peroxone (1:2)	-684	75	80	69	49	65	85	89	95	97
	50 mg/L ozone	-41	61	64	51	33	59	80	79	85	90
	Peroxone (1:1)	16	52	59	48	32	49	65	75	85	90
	30 mg/L ozone	-80	47	56	46	31	56	74	75	84	87
	Control - Peroxide only	-41	0	1	1	3	2	3	3	7	12

Table 3.4. % of O_2, and O_x-NA degradation based on carbon number.

NAs species	Treatment	Degradation % with Carbon number												
		7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
O_2-NA	Peroxone (1:2)	-21.3	-67.9	20.3	64.8	73.7	86.4	88.8	93.0	93.2	96.7	95.1	99.1	-
	50 mg/L ozone	-	-64.4	-37	31.9	50.7	66.9	71.6	75.9	81	84.9	93.1	96.7	98.8
	Peroxone (1:1)	-	-23.3	-16.8	32.2	50.9	54.5	66.7	70	73.9	76.7	83.3	88.6	93.8
	30 mg/L ozone	-	-84.2	-4	34.7	47.1	53.1	63.2	67.5	72.5	77.3	88	92.6	97.5
	Control - Peroxide only	-	18	-35.8	-10.6	-1.1	-1.4	0.9	2.6	0.6	2.6	2.2	2.8	2.7

		Degradation % with Carbon number												
		7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
O_x-NA	Peroxone (1:2)	-	22	-90	2	37	45	63	68	74	80	86	89	94
	50 mg/L ozone	-	-78	-70	9	27	36	47	51	57	64	74	82	85
	Peroxone (1:1)	-	21	-37	18	29	30	44	47	52	58	66	74	80
	30 mg/L ozone	-	-18	-35	17	25	28	42	46	51	58	69	76	81
	Control - Peroxide only	-	47	-30	-7	0	0	0	1.2	0	2	1	3	4

Table 3.5. % of O_2, and O_x-NA degradation based on carbon number after the addition of TBA.

NAs species	Treatment	Degradation % with Carbon number												
		7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
O_2-NA	Peroxone (1:2) +TBA	-	-62	-6	14	26	21	30	33	38	45	60	72	80
	50 mg/L ozone +TBA	-	-61	-28	16	15	15	17	21	28	43	69	84	-

		Degradation % with Carbon number												
		7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
O_x-NA	Peroxone (1:2) +TBA	-	22	-90	1	12	8	17	19	23	31	42	51	61
	50 mg/L ozone+TBA	-	-78	-28	9	10	6	11	12	15	20	32	44	52

** O_x-NAs = (sum of classical and oxidized NAs)

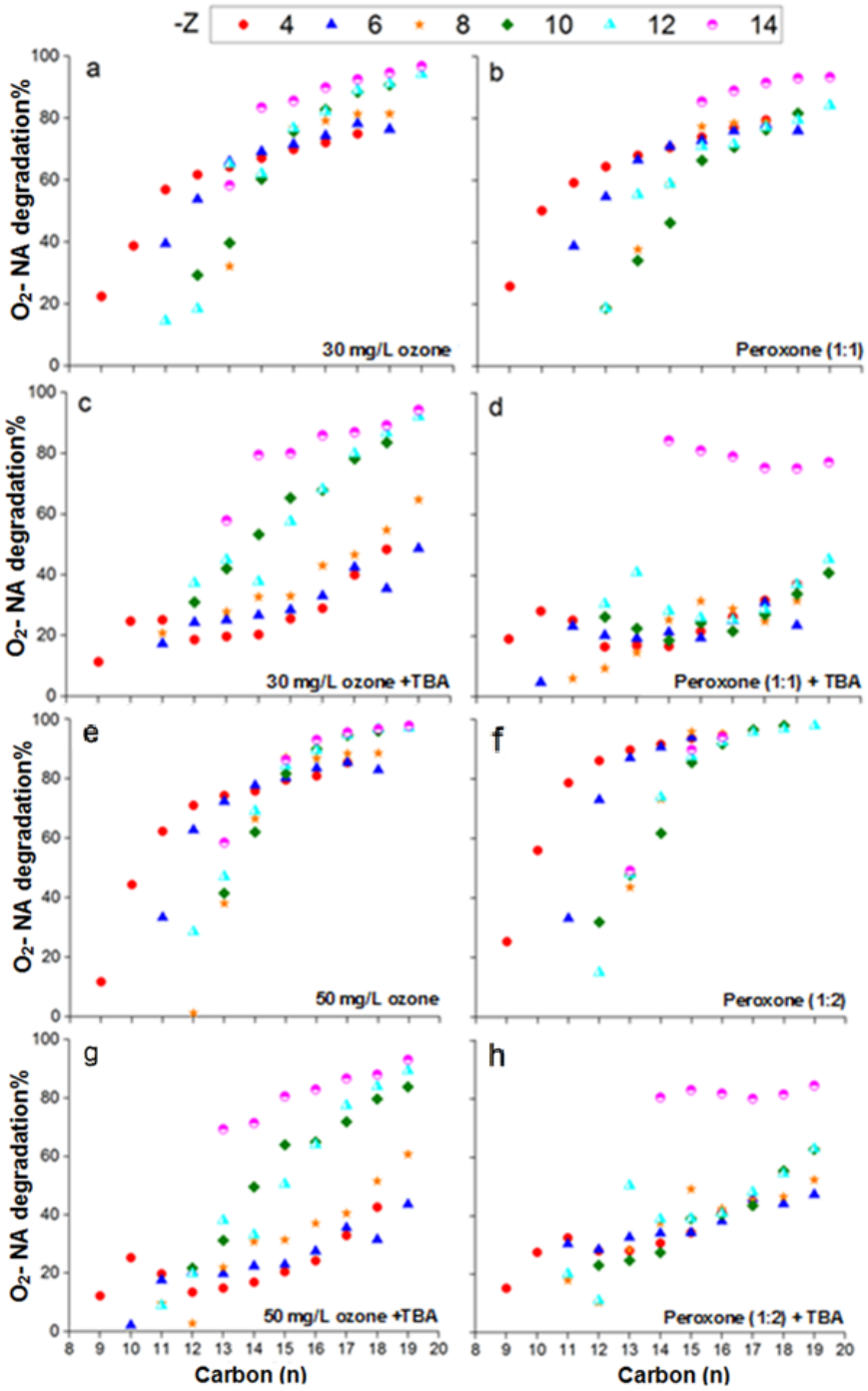

Figure 3.4. Combined effects of carbon and Z numbers on classical NAs (O_2-NAs) after various treatments: (a) 30 mg/L ozone; (b) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3); (c) 30 mg/L ozone + TBA; (d) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3) + TBA; (e) 50 mg/L ozone; (f) peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3); (g) 50 mg/L ozone + TBA; and (h) peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3) + TBA. All ozone doses are utilized doses and the 20 mg/L H_2O_2 is the initial concentration.

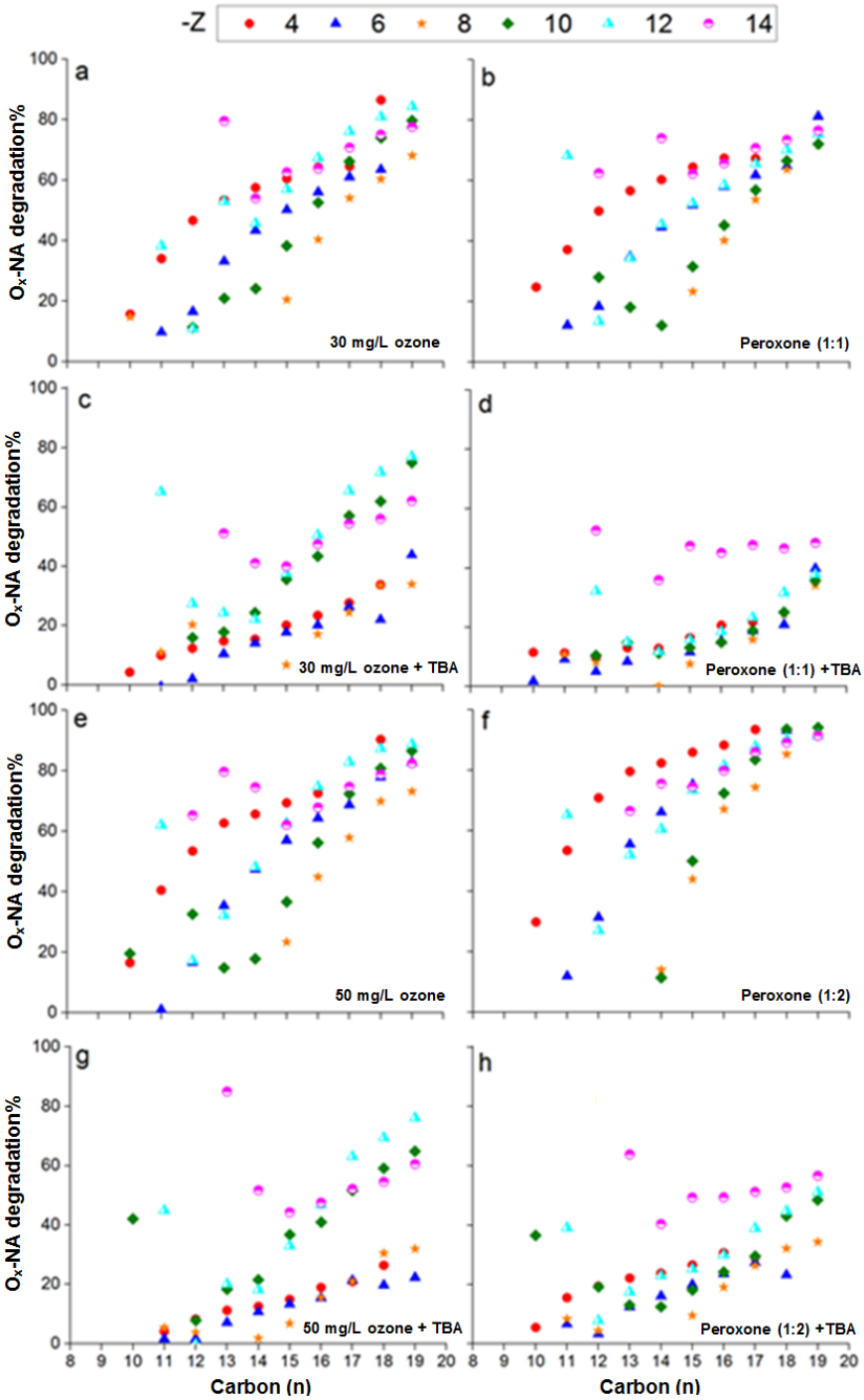

Figure 3.5. Combined effects of carbon and Z on O_x-NAs after various treatments (a) 30 mg/L ozone; (b) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3); (c) 30 mg/L ozone + TBA; (d) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3) + TBA; (e) 50 mg/L ozone; (f) peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3); (g) 50 mg/L ozone + TBA; and (h) peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3) + TBA. All ozone doses are determined as utilized doses and the 20 mg/L H_2O_2 is the initial concentration.** O_x-NAs = (sum of classical and oxidized NAs)

Table 3.6. % of O_2-NA degradation based on carbon and corresponding Z number at peroxone (1:2).

Carbon	-Z								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
7									
8		-21.3							
9		49.1	11.1						
10	-436.6	72.0	58.5	-49.1					
11		72.5	76.8	36.6	-61.8				
12	-842.3		85.8	71.9	-22.1	25.3	26.7		
13		88.0	90.1	86.2	36.3	39.6	58.3		
14	-1017.6	92.5	90.9	89.4	73.5	65.9	74.8		
15		93.6	93.2	93.1	100.0	85.2	88.4	89.3	
16	-262.0	100.0	94.3	94.2	100.0	90.9	92.6	94.9	
17			95.6	95.8	95.8	95.8	96.1	97.2	
18							96.5	98.3	99.2
19							97.2	99.0	

Table 3.7. % of O_2-NA degradation based on carbon and Z number at 30 mg/L ozone.

Carbon	-Z								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
7									
8		-84.2							
9		18.3	22.5						
10	84.9	45.0	38.8	-17.4					
11		49.9	57.0	39.5	-14.1		14.6		
12	-299.6		61.8	53.8	-13.4	29.3	18.4		
13		61.1	64.3	66.0	32.2	39.7*	65.3*	58.4	
14	-228.3	66.1	67.2	69.3	61.5*	60.3*	62.1*	83.5	
15		72.9	70.0	71.6	76.9	75.8*	76.8*	85.7*	
16	-73.2	75.6	72.2	74.4	79.2*	82.7*	82.2*	90.0*	85.1
17			75.0	78.3*	81.4*	88.6*	89.0*	92.6*	
18				76.5*	81.4*	90.8*	91.2*	94.7*	97.4
19							94.2*	96.9*	

* Refer to the difference in degradation at this particular Z and carbon without adding H_2O_2 and compared to peroxone process of ratio (1:1).

Table 3.8. % of O_2-NA degradation based on carbon and Z number at peroxone (1:1).

Carbon	-Z								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
7									
8		-23.3							
9		28.5	25.9						
10		46.8	50.3	-30.6					
11		57.6	59.3	38.9	-32.5				
12	23.0		64.5	54.8	-5.1	18.9	18.8		
13		69.8	68.2	66.7	37.8	34.2*	55.5*		
14	-191.1	71.8	70.7	71.1	58.9*	46.4*	59.0*		
15		74.9	74.1	73.0	77.4	66.5*	71.2*	85.6*	
16	0.6	76.4	77.1	76.0	78.5*	70.8*	71.7*	89.1*	89.1
17			79.6	78.2*	79.1*	76.4*	77.2*	91.6*	
18				76. 2*	80.9*	81.8*	79.6*	93.2*	97.6
19							84.3*	93.5*	

* Refer to the difference in degradation at this particular Z and carbon after adding H_2O_2 at peroxone process of ratio (1:1) compared to 30 mg/L O_3.

Table 3.9**.** % of O_2-NA degradation based on carbon and Z number at 50 mg/L ozone.

Carbon	-Z								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
7									
8		-64.4							
9		19.6	11.8						
10		47.8	44.4	-49.7					
11		57.9	62.3	33.4	-33.7				
12	-240.0		71.1	62.8	1.2		28.6		
13		70.0	74.3	72.4	38.1	41.5	47.1	58.6	
14	-352.8	76.4	75.9	77.8	66.5	62.1	69.2		
15		77.8	79.6	80.4	87.2	81.7	84.3	86.4	
16	-27.8	82.0	81.0	83.5	86.7	90.0	89.6	93.1	82.4
17			85.4	85.4	88.4	94.6	94.6	95.6	
18				82.9	88.6	96.0	96.6	96.7	
19							97.1	97.9	

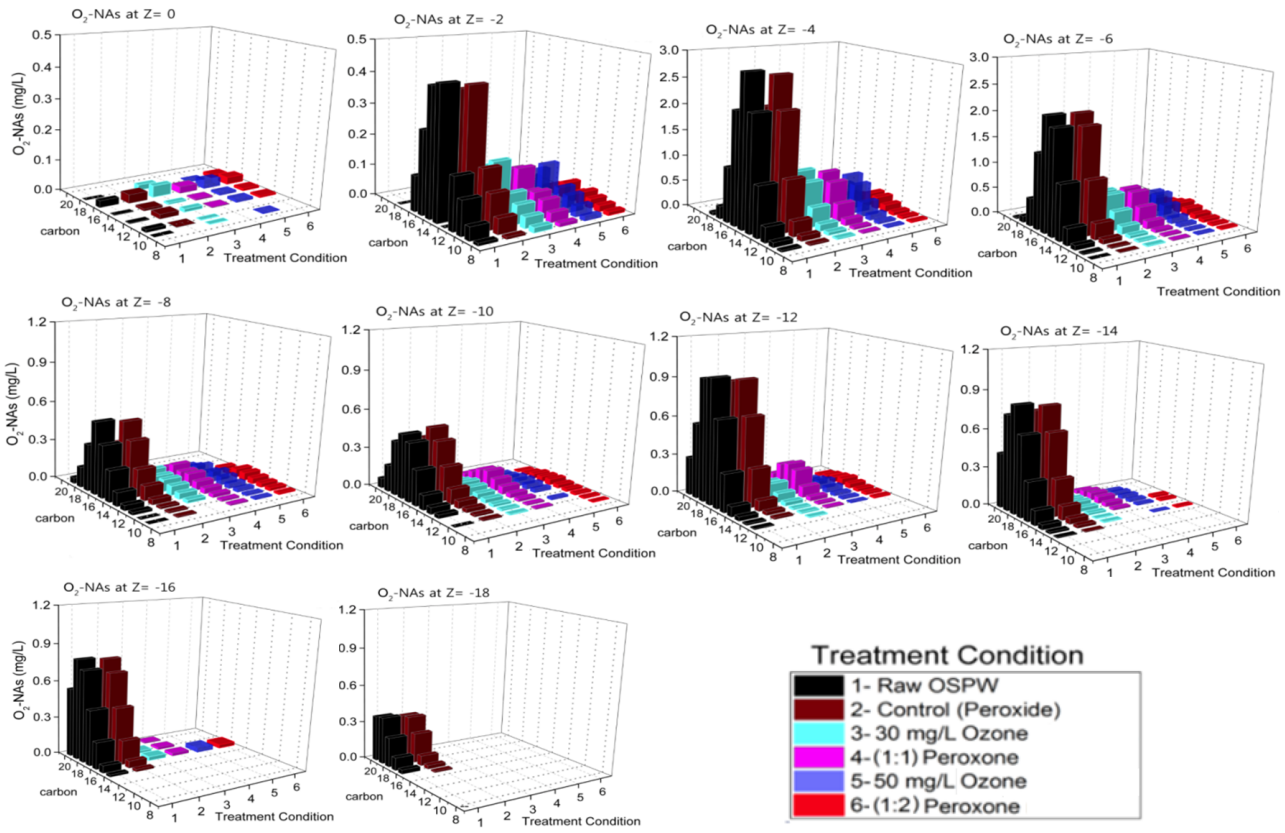

Figure 3.6. O_2-NA concertation profile based on Z from Z = 0 till Z = -18 after various treatments 30 mg/L ozone; peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3); 50 mg/L ozone; peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3). All ozone doses are determined as utilized doses and the 20 mg/L H_2O_2 is the initial concentration.

3.3.3 Ion-mobility spectroscopy images

IMS is a qualitative analytical method that can be used to characterize the NA groups in different matrices and different treatment conditions. The O_2-NAs, oxy-NAs ($x \geq 3$) and heteroatomic NAs (S–NA) are found in three distinctive clusters separated by their retention and drift time in the IMS as shown in Figure 3.7 and identified previously (Sun et al. 2014). The peroxone (1:2) process exhibited the highest removals for all clusters (Figure 3.7h) especially the O_2-NA cluster where no residual intensity is visible compared to the 'highest' intensity of clusters as indicated by yellow regions in raw OSPW. Overall, the removals decreased by: peroxone (1:2) > 50 mg/L ozone > 30 mg/L ozone ≈ peroxone (1:1). As for the TOFMS results, the reduction in NA species for the 30 mg/L ozone and the peroxone (1:1) treatments were similar (see Figure 3.7c,d). The current results are in agreement with the IMS reported for ozone-treated samples at 30 mg/L, (Sun et al. 2014) while the peroxone (1:2) treatment showed similar removal for each of the clusters reported by Afzal et al. utilizing a 100 mg/L ozone dose (Afzal et al. 2014). The authors suggested that high utilized ozone doses (≥100 mg/L) were required to reduce the O_2-NAs and S–NAs and that there was a lack of data for peroxone processes especially in actual wastewater matrices. Our study clearly shows the ability of 50 mg/L ozone dose coupled with 20 mg/L H_2O_2 to degrade both species (Figure 3.7). Interestingly, for all treatments the S–NA species were completed reduced indicating no intensities in any clusters of the IMS plots compared to raw OSPW and the control (Figures 3.7a and 3.7b,

respectively). Unfortunately, the S-NAs cannot yet be reliably quantified in the TOFMS due to lack of commercially available standards (Sun et al. 2014).

The addition of TBA leads to a enormous increase in the O_2-NAs clusters in all treatments (Figures 3.7e,f,i,j) compared to the treatment without TBA (Figures 3.7c,d,g,h). These observations are consistent with the TOFMS results about the significant role of the •OH in the degradation of these type of species. While using TBA, the S–NA species showed some differences in the degradation pathways between ozone and peroxone treatments. Slight intensities can be observed in the S–NA clusters in both 30 mg/L and 50 mg/L ozone (see Figures 3.7e,f) while considerable intensity in (1:1) and peroxone (1:2) (see Figures 3.7i,j). This difference can be attributed to the preferential reaction of the S species with molecular ozone other than •OH. Previously, it was reported the high reactivity of ozone to sulfoxides and sulfinic acids which ease their attack by ozone (Moloney 2009).

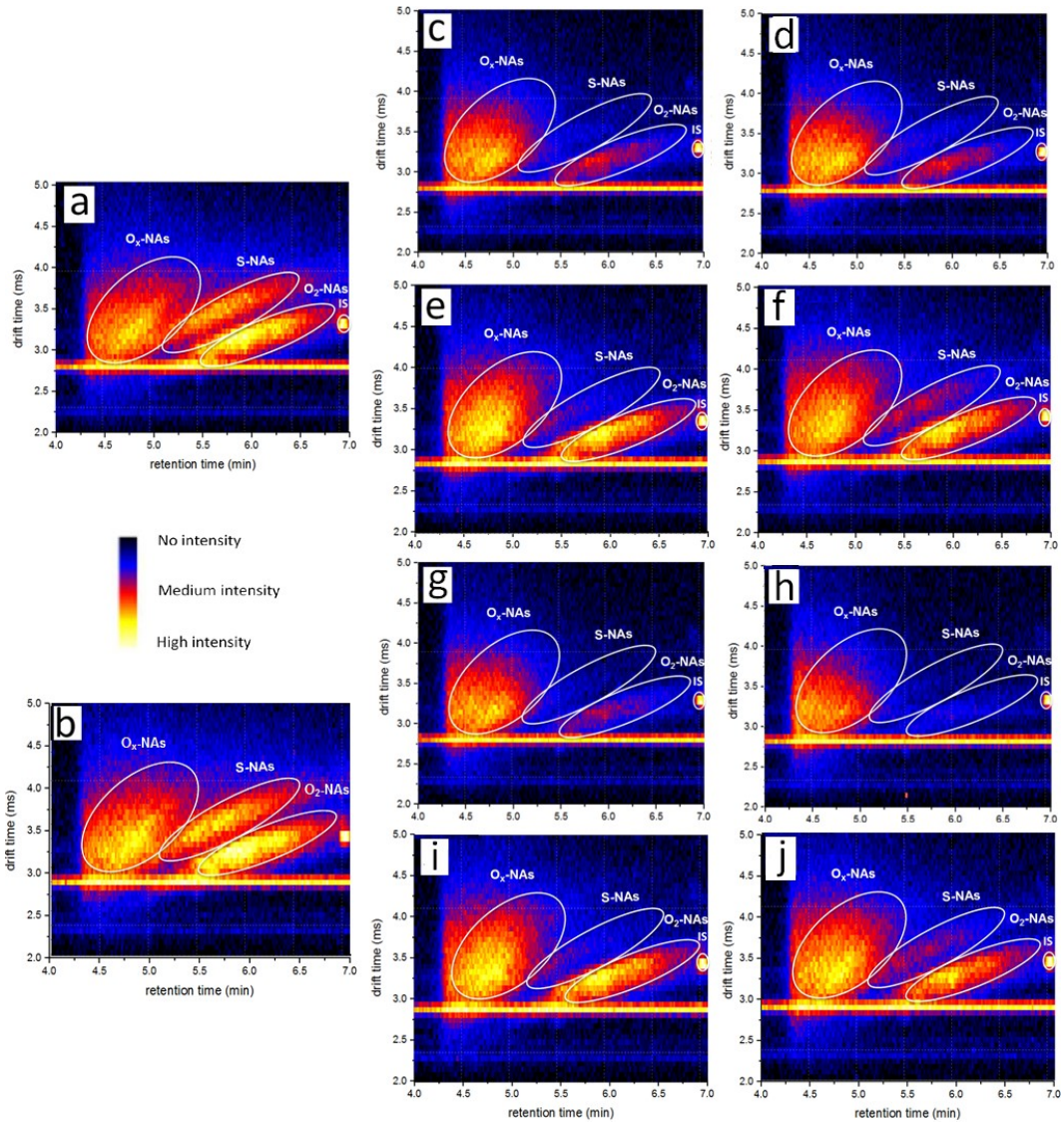

Figure 3.7. IMS plot at different treatment conditions. (a) raw OSPW, O_x-NAs (sum of classical and oxidized NAs = 54.8 mg/L; (b) Control, 20 mg/L hydrogen peroxide, O_x-NAs = 53.3 mg/L; (c) 30 mg/L ozone, O_x-NAs = 22.9 mg/L; (d) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3), O_x-NAs = 22.7 mg/L; (e) 30 mg/L ozone + TBA, O_x-NAs = 35.9 mg/L; (f) peroxone (1:1) (20 mg/L H_2O_2:30 mg/L O_3) +TBA, O_x-NAs = 40.3 mg/L; (g) 50 mg/L ozone, O_x-NAs = 20 mg/L; (h) peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3), O_x-NAs = 13.1 mg/L; (i) 50 mg/L ozone + TBA, O_x-NAs = 37.3 mg/L; and (j) peroxone (1:2) (20 mg/L H_2O_2:30 mg/L O_3) + TBA, O_x-NAs = 35.8 mg/L. The bright spot is the internal standard (IS) which is signified in each plot at $t_R \approx 7$ min and $t_D \approx 3$ ms. Colors specify the relative intensity, with the yellow colored clusters indicating the high abundant peak areas. All ozone doses are utilized doses and the 20 mg/L H_2O_2 is the initial concentration.

3.3.4 Reduction of fluorophore organic compounds

The synchronous fluorescence spectroscopy (SFS) profiles for raw and treated OSPW exhibits three distinctive peaks (I, II, III) that are representative of fluorophore organic compounds group in which one ring, two rings, and three aromatic rings are located at 260-280, 300-315 and 320-330 nm, respectively (Rowland et al. 2011, Tuan Vo 1978). Overall, the peroxone (1:2) and 50 mg/L ozone treatments were the most effective treatments while they showed the highest reductions in SFS with complete removals of peaks II and III and marked reduction in peak I (Figure 3.8a). On the other hand, the 30 mg/L ozone and peroxone (1:1) did not reduce effectively peak I as peaks II and II (Figure 3.8b). Additionally, the 30 mg/L ozone accomplished higher reductions compared to peroxone (1:1) in which peroxone (1:1) still exhibited peaks II and III (reduced relatively) and peak I (unreduced). Reasons for this lower reduction in the peroxone (1:1) are not known. However, it can be attributed to the scavenging of •OH at these oxidants doses as mentioned earlier in the NA degradation or the evidence of the significant role of the molecular ozone rather than the •OH in targeting those types of aromatic compounds. The presence of aromatic compounds associated with high molecular weights NAs can be correlated with SFS due to their fluorescence (Frank et al. 2009). Interestingly, our earlier findings confirmed the reduction of high molecular weight NAs as shown in Figures 3.1 and 3.2 alike here in the corresponding reduction of aromatic acids in Figure 3.8. The reason of the decrease of fluorescence after ozonation might be due to enhancing the electron withdrawing groups in aromatic compounds that

can weaken the structures and break down the chromophoric groups in the aromatic structure (Islam et al. 2014b). Although the amount of •OH is expected to be very high at pH 8 where the •OH is considered to be the main contributor in reducing these aromatic portion, it can be observed that the removal of some peaks increased again by spiking the TBA. For instance, peak III recovered by spiking TBA in peroxone (1:2) (see Figure 3.8a). This can be attributed to a competition between the different scavengers in the real water matrix that can occur with the spiked TBA which turns the reaction pathways through other organic radicals. As well, the typical reaction between ozone and aromatics is hydroxylation that can lead to the generation of phenol while continuously producing •OH that can elevate the degradation efficiency (Nothe et al. 2009).

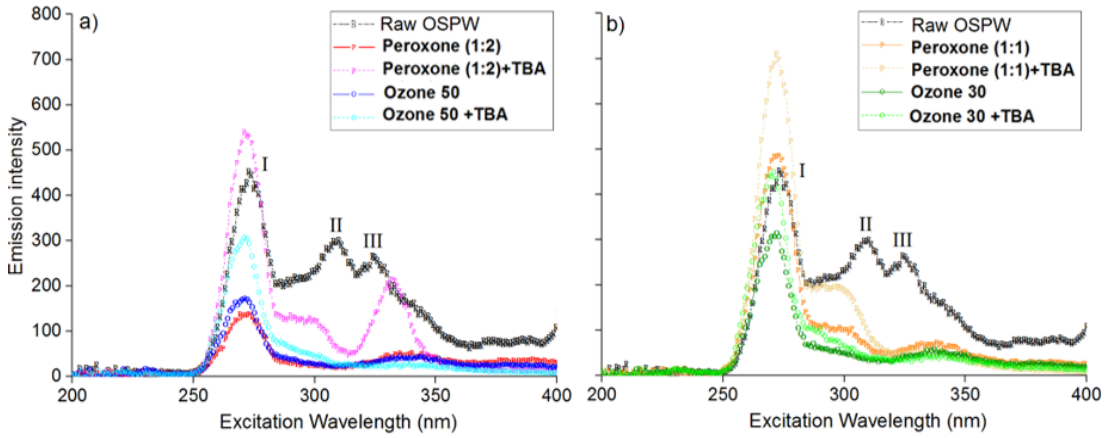

Figure 3.8. SFS plots after applying different utilized O_3 doses [30 mg/L and 50 mg/L] and peroxone, processes at different H_2O_2:O_3 ratios [(1:1) and (1:2)] with and without adding TBA as a ·OH scavenger; three distinctive peaks (I, II, III) are representing one ring, two rings, and three rings respectively. All ozone doses are utilized doses.

3.3.5 Toxicity assessment and practical implications

For practical implementations of the current study, we highlighted the differences between the treatments in terms of: toxicity reduction, best utilization of the oxidants, as well as similarities/differences with previous studies, relative effectiveness of treatments in removing specific structures, and the cost.

The reduction of toxicity toward *Vibrio fischeri* can be observed in all treatments (Table 3.10). However, the peroxone (1:2) leads to the highest reduction in toxicity of 50%. Despite the large decreases of the O_2-NAs (91%; Figure 3.1) and AEF (75%; Table 3.10) for peroxone (1:2) treatment, the reduction of toxicity was partial. This can be attributed to the increase (or unchanged) of the compounds or intermediates responsible for the toxicity. The peroxone (1:1) treatment was similar to the 30 mg/L treatment in decreasing toxicity by 31% versus 35% Table 3.10, while being statistically lower than the peroxone (1:2) treatment. Given the current data, the best process seems to be the peroxone (1:2) process in which the toxicity reduction was enhanced compared to 50 mg/L ozone. However, further detailed report is undergoing to examine the toxicity toward mammalian cells with respect to specific species.

Table 3.10. Parameters of OSPW before and after ozone and peroxone treatments.

Parameter (mg/L)	Before treatment	utilized O_3				Peroxone at Ratio			
		30 mg/L		50 mg/L		1:1		1:2	
		Treated	Degradation %	Treated	Degradation %	Treated	Degradation %	Treated	Degradation %
O_2-NAs	35.5	8.00	77	5.8	84	8.1	77	3.1	91
O_3-NAs	9.1	8.2	10	7.6	16	8.5	7	5.4	41
O_4-NAs	10.2	6.7	34	6.6	35	6.1	39	4.6	55
AEF	71.3± 0.6	43±0.7	39	39±0.5	46	42.4±0.4	41	17.5±0.5	75
pH	8.4± 0.1	8.3± 0.2		8.5± 0.1		8.6± 0.15		8.4± 0.2	
COD reduction %		16%		13%		8%		24%	
Toxicity reduction %		35		42		31		50	

Although the ozone dose is an important operating factor (Buffle et al. 2006), the feasibility of ozonation processes in municipal wastewater is usually evaluated in terms of kg ozone/kg contaminant removed or kg ozone supplied per COD reduced (Bes-Pía et al. 2004, Wu et al. 2012). Noteworthy that the COD reduction in OSPW is quite small (i.e., Table 3.10; COD<25%) compared to other parameters. Currently, the toxicological, physicochemical characteristics of the OSPW are required to assess and select the best options of OSPW treatments (McQueen et al. 2017a). In the meanwhile, clear identification for the constituents of concerns in OSPW (McQueen et al. 2017b) as well as their extents of removals is progressing and warranted while other non-typical parameters should be used. A helpful metric/or parameter to elucidate the differences between the four treatments is the degradation efficiency ratio considered as mg/L NAs oxidized per mg/L oxidant dose (Gamal El-Din et al. 2011, Wang et al. 2013). To compare ozone-only and peroxone treatments, we implemented this former metric to highlight the differences in the degradation efficiency using the same ratio as previous studies (Gamal El-Din et al. 2011, Islam et al. 2014b, Wang et al. 2013). The higher the value of the ratio is (i.e., high reduction of NAs in mg/L per mg/L dose of oxidant consumed), the best utilization of the oxidants in this treatment is. For the degradation of NA species (mg/L) per utilized O_3 dose (mg/L), the most abundant O_2-NAs and the O_x-NAs had the highest utilization efficiencies in the peroxone (1:1) (0.91 and 1.07) and 30 mg/L ozone treatments (0.92 and 1.06), respectively (Table 3.2). Of the remaining treatments, the peroxone (1:2) had higher removal efficiencies for O_x-NAs versus the 50 mg/L ozone only treatment

with removals ratio of 0.83 and 0.70, respectively (Table 3.2). The increase in ozone-only dose from 30 mg/L to 50 mg/L resulted in only a marginal increase (58% to 63%) in the degradation of O_x-NAs. This is an indicative of the decrease of the degradation efficiency (i.e., mg/L NAs per mg/L utilized ozone dose) at ozone >30 mg/L (0.92 to 0.60). As well, these results indicate that the ozonation efficiency increases at a lower ozone concentration than the previously reported cutoffs of 50 mg/L and 100 mg/L (Sun et al. 2014, Wang et al. 2013). It is worth noting that the utilization of H_2O_2 at peroxone (1:1) was more efficient than peroxone (1:2) (Table 3.2 for H_2O_2 utilization and Figure 3.9 for residual H_2O_2) with 4.58 mg/L versus 4.17 mg/L O_x-NAs degraded per mg/L of H_2O_2, respectively. The differences in degradation between current study compared to previous studies in terms of mg/L NAs per mg/L ozone are shown in Figure 3.10. Peroxone (1:2) process showed higher degradation efficiency with respect to oxidant utilization of 0.83 compared to 0.24 reported by Islam et al. (Islam et al. 2014b) at utilized ozone dose of 170 mg/L (Fig. 3.10 a,b,c). In addition, the peroxone (1:2) process was highly effective in degrading high initial NA concentrations O_x-NAs of 54.8 mg/L versus 45.7 mg/L (Islam et al. 2014b).

With regards to removing specific structures; overall peroxone (1:1) has similar or sometimes better effect in degradation compared to 50 mg/L ozone at $9< n <11$, and with less effect of 20% less at n= 12-15 (Figure 3.11). As well, the range of the rough cost estimates for the four treatments were 0.17-0.19 \$/m^3 at level of 30 mg/L ozone and 0.25-0.3 \$/m^3 at level of 50 mg/L ozone which indicated the similarities of the peroxone treatment with corresponding ozone

treatment at same ozone level (Table 3.11). To grasp more understanding about the significance of H_2O_2 addition at either of the two ozone levels, the energy requirements was averagely calculated as 22 kWh/kg and 10 kWh/kg for production of O_3 and H_2O_2, respectively. Based on a 90% degradation of both O_2-NAs and O_x-NAs, applying peroxone (1:2) was almost similar in energy requirement of O_2-NAs to 50 mg/L ozone (1.18 versus 1.17 kWh/m^3) and less expensive in O_x-NAs energy requirement compared to 50 mg/L ozone (1.43 versus 1.55 kWh/m^3). All energy calculations and assumptions from the previous studies (Rosenfeldt et al. 2006; Katsoyiannis et al. 2011; Wu et al. 2015) as well as current values are in Table 3.12. In summary, the energy requirement for the peroxone treatments was comparable to ozone alone at same level of ozone either at 30 mg/L or 50 mg/L (i.e., in kWh/m^3 of O_2-NAs degradation, 0.85 versus 0.76 with 12 % increase difference in peroxone (1:1) and 1% in peroxone (1:2)). In summary, the peroxone (1:1) and peroxone (1:2) explored different better strengths and effectiveness compared to ozone treatments in reducing toxicity, better oxidants utilization and removing specific structures beside significant decrease of the absolute bulk of the O_2-NAs and O_x-NAs with superiority in oxidized NAs (e.g. O_4-NAs). These findings not only strengthen the feasibility of adding H_2O_2 to enhance ozonation and remove specific structures at low oxidant doses but also elucidate the differences in the treatments performance based on removal extents, toxicity reduction, energy requirements and cost point of views.

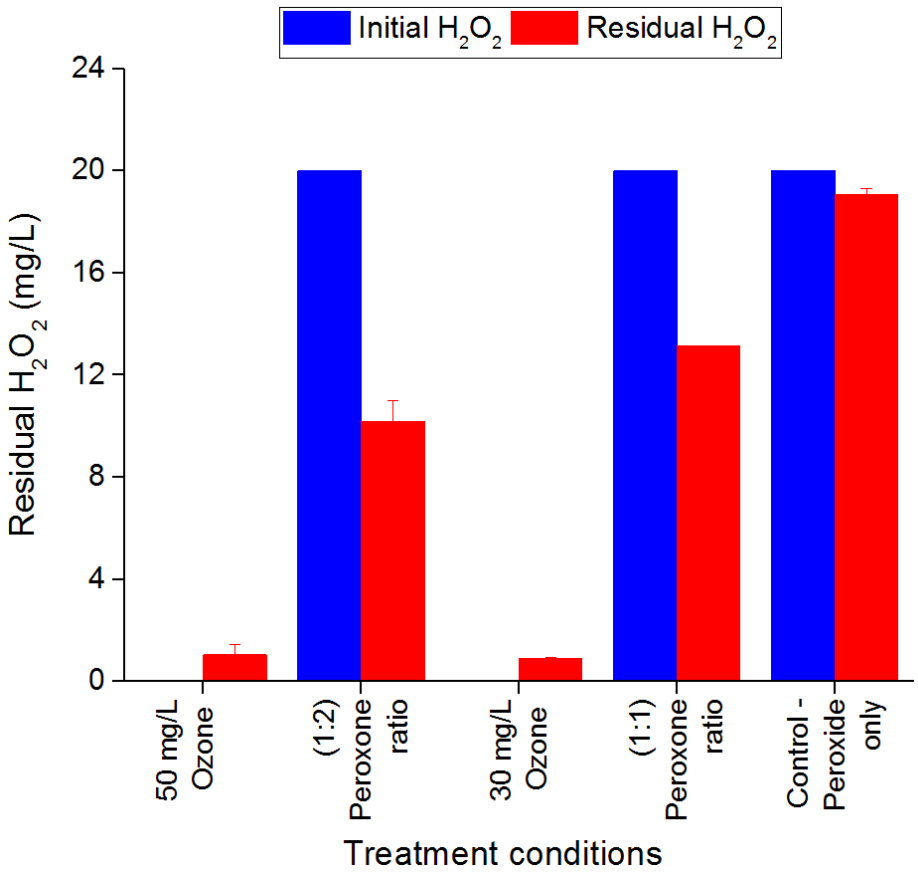

Figure 3.9. Initial and residual H_2O_2 after different peroxone (H_2O_2/O_3) ratios.

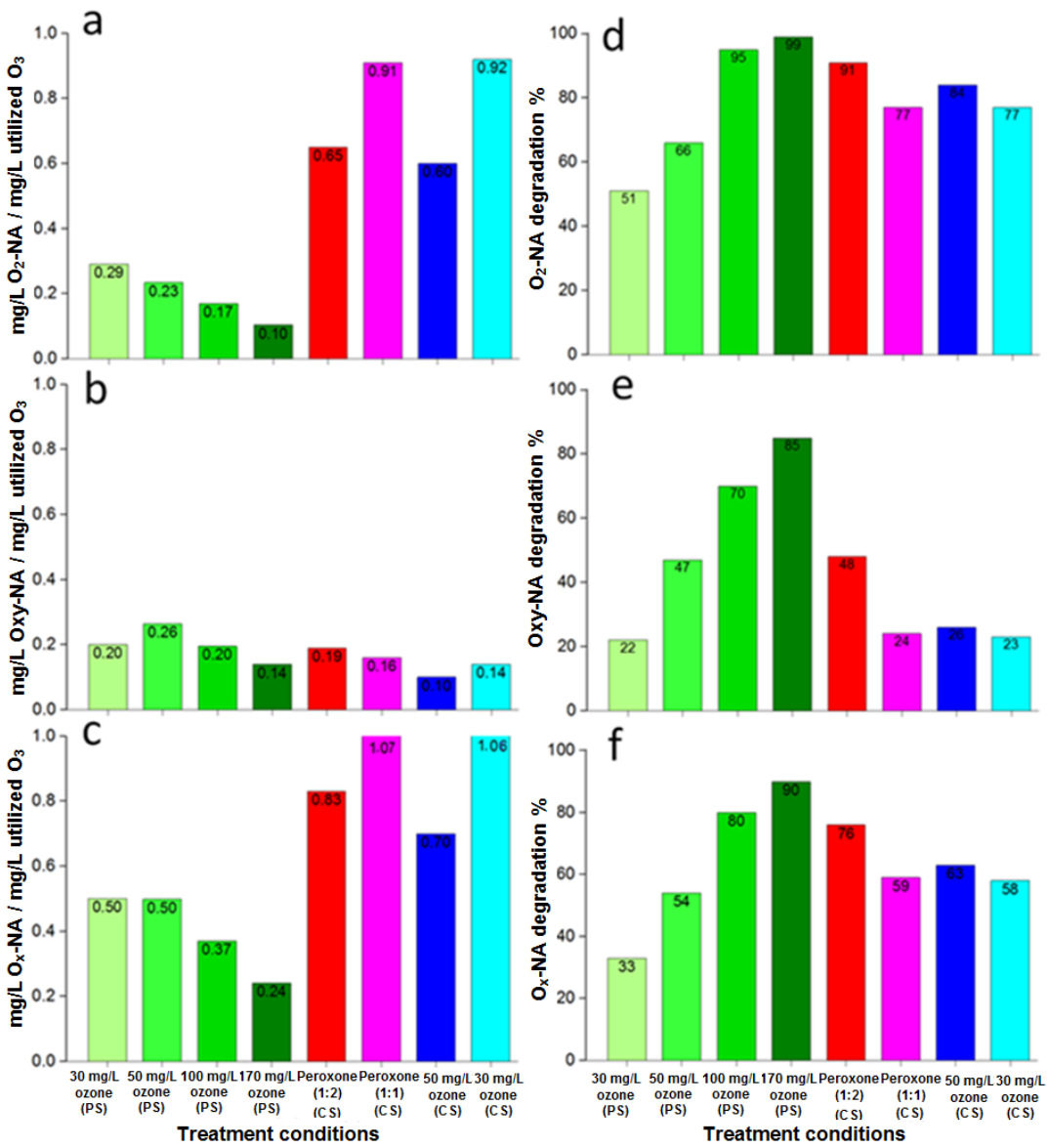

Figure 3.10. Comparisons of the ozone-only and peroxone treatments with previous studies using the metrics mg/L NAs per mg/L utilized ozone dose; a) O_2-NA; b) Oxy-NA; c) O_x -NA as well as degradation % d) O_2-NA; e) Oxy-NA; and f) O_x -NA. Note that: O_x-NAs = (sum of classical and oxidized NAs); initial O_x -NAs in CS = 54.8 mg/L while initial O_x -NAs in previous study (Islam et al. study) = 45.7 mg/L (Islam et al. 2014). Islam et al. "Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water" *Sci. of The Total Env.* **2014,** *493*, 282-290. Current study = (CS), Previous study = (PS).

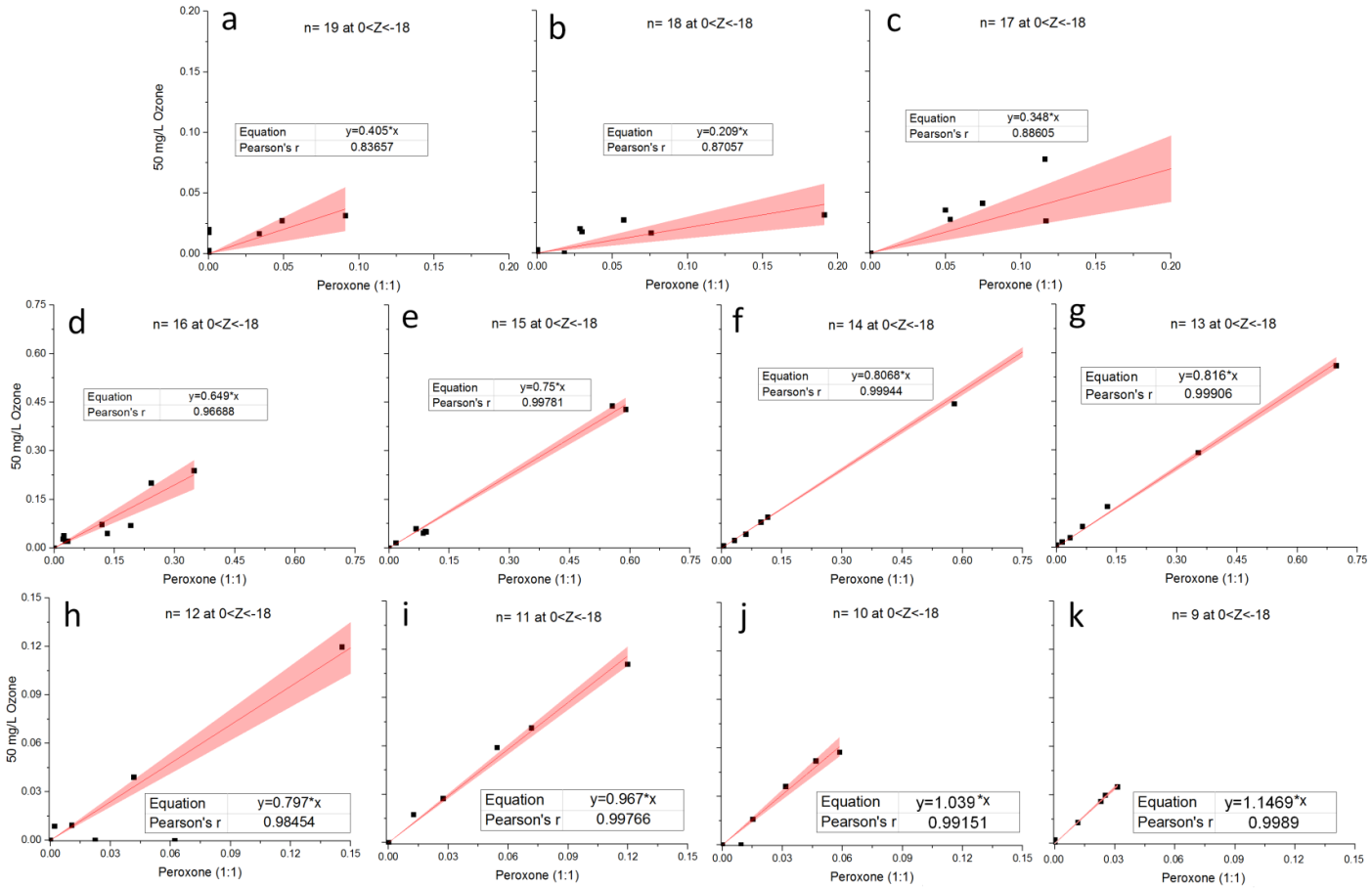

Figure 3.11. Correlations between the residual concentrations of O_2-NAs in 50 mg/L ozone versus peroxone (1:1) treatment at different carbon number (n). The rose zone represents the 95% confidence level.

Table 3.11. Estimated costs of the treated OSPW after ozone and peroxone treatments.

	30 mg/L Ozone	**Peroxone (1:1)**	**50 mg/L Ozone**	**Peroxone (1:2)**
Estimated capital expenditures, $/yr	302807.0	302807	413613.7	413613.7
Estimated operational and maintenance expenditures, $/yr	314800.5	380500	491755.8	557455.8
Total annual cost, $/yr	617607.5	683307.5	905369.5	971069.5
Estimated cost per treated water volume of, $/m^3	0.17	0.19	0.25	0.3

Assumptions for cost estimate:

1. Capacity =10,000,000 L/d
2. H_2O_2 (100%) chemical cost= 0.9-1.1 $/kg obtained from industrial sources
3. Interest rate= 0.06
4. Service life =15 years
5. The expenses values for the capital and operational and maintenance expenditures were estiamted as present worth in the current year 2016 after being adjusted with Engineering News Record index.
6. The cost of ozonation systems was estimated using two methods, first method by the equation developed for ozonation systems in water treatment facilities by McGivney and Kawamura (2008), second method devleoped by Sharma et al. (2013). The differences between the two methods were around 0-15% and the lowest values were execluded from the table. The chemical cost was only used to calculate the operational and maintenance expenditures including the ozone generation as well as considering the additional chemical cost of H_2O_2 in peroxone treatments.

Table 3.12. Estimated costs of the treated OSPW after ozone and peroxone treatments based on energy requirements at 90% degradation of each species

		30 mg/L ozone	Peroxone (1:1)	50 mg/L ozone	Peroxone (1:2)
Ozone dose kg/m^3	**O_2-NAs**	0.035	0.035	0.053	0.049
Hydrogen peroxide dose kg/m^3		N/A	0.008	N/A	0.010
Energy requirement kWh for kg ozone		0.764	0.772	1.172	1.081
Energy requirement kWh for kg hydrogen peroxide		N/A	0.082	N/A	0.097
Estimated energy per volume of treated water, kWh/m^3		0.76	0.85	1.17	1.18
Ozone dose kg/m^3	**O_x-NAs**	0.047	0.046	0.070	0.059
Hydrogen peroxide dose kg/m^3		N/A	0.011	N/A	0.012
Energy requirement kWh for kg ozone		1.024	1.014	1.550	1.307
Energy requirement kWh for kg hydrogen peroxide		N/A	0.108	N/A	0.118
Estimated energy per volume of treated water, kWh/m^3		1.02	1.12	1.55	1.43

Assumptions for cost estimate:

1. Energy requirement of hydrogen peroxide in kWh for 1 kg hydrogen peroxide is estimated based on the average energy requirement from different studies as 10 kWh for every kg hydrogen peroxide (Katsoyiannis et al. 2011,Rosenfeldt et al. 2006).
2. Ozone generation requirement in kWh for 1 kg ozone was reported as 18-26 kWh/kg ozone (Katsoyiannis et al. 2011, Wu and Englehardt 2015) and 15 kWh/kg ozone (Rosenfeldt et al. 2006). The values in the current calculation were estimated based on the average from previous studies as 22 kWh for 1 kg ozone.
3. 90% degradation for the species either O_2-NAs or O_x-NAs.

3.4 Conclusions

The peroxone process was assessed to determine its viability in degrading organic compounds and detoxifying OSPW. Overall, the peroxone (1:2) was found to be the most effective to degrade NAs (i.e., 91% of O_2-NAs and 76% of O_x-NAs), to reduce toxicity and to improve the ozonation efficiency confirming the value of the addition of H_2O_2 to increase the •OH. Furthermore, the superiority of •OH contribution enhanced the reduction of the oxidized species in peroxone (1:2) compared to 50 mg/L ozone (i.e., residual O_3-NAs; 5.4 mg/L in peroxone (1:2) versus 7.6 mg/L in 50 mg/L ozone compared to 9.1 mg/L in raw OSPW). However, the molecular ozone pathway after adding TBA still contributes to around 40-50% of the O_2-NAs and 26-35% of O_x-NAs degradation. Additionally, though the peroxone (1:2) had the highest degradation (i.e., lowest NAs concentration), compared to peroxone (1:1); the peroxone (1:1) (i.e., with 30 mg/L ozone) was more efficient in terms of oxidant utilization (peroxone (1:2) versus peroxone (1:1); 0.83 versus 1.07) and cost (0.30 versus 0.17) and targeting specific structure with similar competence to 50 mg/L ozone. Specific to the NAs, there was a demonstrated structure-reactivity relationship of degradation processes as represented by the combined impact of n and Z numbers. Using AOPs such as peroxone process will allow us to reduce the need for high ozone doses to treat OSPW for its future release to the environment. Further research is recommended to examine the peroxone process as a useful upstream treatment prior to biological treatment of OSPW.

3.5 References

Afzal, A., Chelme-Ayala, P., Drzewicz, P., Martin, J.W. and Gamal El-Din, M. (2014) Effects of Ozone and Ozone/Hydrogen Peroxide on the Degradation of Model and Real Oil-Sands-Process-Affected-Water Naphthenic Acids. Ozone Sci. Eng. 37(1), 45-54.

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

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. (2011) Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to chironomus dilutus. Environ. Sci. Technol. 46(1), 486-493.

Beltrán, F.J. (2004) Ozone reaction kinetics for water and wastewater systems, Lewis Publishers, Boca Raton, Fla.

Bes-Pía, A., Iborra-Clar, A., Mendoza-Roca, J.A., Iborra-Clar, M.I. and Alcaina-Miranda, M.I. (2004) Desalination strategies in South Mediterranean Countries Nanofiltration of biologically treated textile effluents using ozone as a pre-treatment. Desalination 167, 387-392.

Buffle, M.-O., Schumacher, J., Meylan, S., Jekel, M. and von Gunten, U. (2006) Ozonation and advanced oxidation of wastewater: Effect of O_3 dose, pH,

DOM and HO center dot-scavengers on ozone decomposition and HO center dot generation. Ozone Sci. Eng. 28(4), 247-259.

Chelme-Ayala, P., El-Din, M.G., Smith, D.W. and Adams, C.D. (2011) Oxidation kinetics of two pesticides in natural waters by ozonation and ozone combined with hydrogen peroxide. Water Res. 45(8), 2517-2526.

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.

Di Iaconi, C. (2012) Biological treatment and ozone oxidation: Integration or coupling? Bioresource Technology 106(0), 63-68.

ERCB (2012) Alberta's Energy Reserves 2011 and Supply/Demand Outlook 2012-2021. Government of Alberta, C., AB, Canada, 2012 (ed).

Frank, R.A., Fischer, K., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van der Kraak, G. and Solomon, K.R. (2009) Effect of Carboxylic Acid Content on the Acute Toxicity of Oil Sands Naphthenic Acids. Environ. Sci. Technol. 43(2), 266-271.

Fernando, J.B. (2003) Chemical Degradation Methods for Wastes and Pollutants, CRC Press.

Gamal El-Din, M., Fu, H.J., Wang, N., Chelme-Ayala, P., Perez-Estrada, L., Drzewicz, P., Martin, J.W., Zubot, W. and Smith, D.W. (2011) Naphthenic acids speciation and removal during petroleum-coke

adsorption and ozonation of oil sands process-affected water. Sci. Total Environ. 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 Res. 45(18), 5849-5857.

Glaze, W.H., Kang, J.-W. and Chapin, D.H. (1987) The Chemistry of Water Treatment Processes Involving Ozone, Hydrogen Peroxide and Ultraviolet Radiation. Ozone: Science & Engineering 9(4), 335-352.

Gosselin, P., Hrudey, S.E., Naeth, M.A., Plourde, A., Therrien, R., Van Der Kraak and G., X., Z., (2010) Environmental and Health Impacts of Canada's oil Sands Industry. Available at. Royal Society of Canada, Ottawa. http://www.rsc.ca/expertpanels_reports.php. .

Gottschalk, C., Libra, J. and Sau, A. (2010) Ozonation of Water and Waste Water : A Practical Guide to Understanding Ozone and its Applications, Wiley-VCH.

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

Han, X.M., 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.

He, Y., Wiseman, S.B., Zhang, X., Hecker, M., Jones, P.D., El-Din, M.G., Martin, J.W. and Giesy, J.P. (2010) Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line. Chemosphere 80(5), 578-584.

Headley, J.V. and McMartin, D.W. (2004) A review of the occurrence and fate of naphthenic acids in aquatic environments. J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng. 39(8), 1989-2010.

Islam, M., Dong, T., McPhedran, K., Sheng, Z., Zhang, Y., Liu, Y. and Gamal El-Din, M. (2014a) Impact of ozonation pre-treatment of oil sands process-affected water on the operational performance of a GAC-fluidized bed biofilm reactor. Biodegradation 25(6), 811-823.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and Gamal El-Din, M. (2014b) Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Sci. Total Environ. 493, 282-290.

Jagadevan, S., Graham, N.J. and Thompson, I.P. (2013) Treatment of waste metalworking fluid by a hybrid ozone-biological process. Journal of Hazardous Materials 244–245, 394-402.

Jones, D., Scarlett, A.G., West, C.E. and Rowland, S.J. (2011) Toxicity of Individual Naphthenic Acids to Vibrio fischeri. Environ. Sci. Technol. 45(22), 9776-9782.

Katsoyiannis, I.A., Canonica, S. and von Gunten, U. (2011) Efficiency and energy requirements for the transformation of organic micropollutants by ozone, O_3/H_2O_2 and UV/H_2O_2. Water Res. 45(13), 3811-3822.

Kelly, E.N., Schindler, D.W., Hodson, P.V., Short, J.W., Radmanovich, R. and Nielsen, C.C. (2010) Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. Proceedings of the National Academy of Sciences 107(37), 16178-16183.

Klamerth, N., Malato, S., Agüera, A. and Fernández-Alba, A. (2013) Photo-Fenton and modified photo-Fenton at neutral pH for the treatment of emerging contaminants in wastewater treatment plant effluents: A comparison. Water Research 47(2), 833-840.

Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and Gamal El-Din, M. (2015) Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. Sci. Total Environ. 506–507(0), 66-75.

Lee, Y., Gerrity, D., Lee, M., Bogeat, A.E., Salhi, E., Gamage, S., Trenholm, R.A., Wert, E.C., Snyder, S.A. and Von Gunten, U. (2013) Prediction of micropollutant elimination during ozonation of municipal wastewater effluents: Use of kinetic and water specific information. Environ. Sci. Technol. 47(11), 5872-5881.

Lee, Y., Kovalova, L., McArdell, C.S. and von Gunten, U. (2014) Prediction of micropollutant elimination during ozonation of a hospital wastewater effluent. Water Res. 64(0), 134-148.

Martin, J.W., Barri, T., Han, X.M., Fedorak, P.M., El-Din, M.G., Perez, L., Scott, A.C. and Jiang, J.T. (2010) Ozonation of Oil Sands Process-Affected Water Accelerates Microbial Bioremediation. Environ. Sci. Technol. 44(21), 8350-8356.

McGivney W.T. and Kawamura S. (2008) Cost estimating manual for water treatment facilities, John Wiley & Sons, Inc.

McQueen AD, Kinley CM, Hendrikse M, Gaspari DP, Calomeni AJ, Iwinski KJ, Castle JW, Haakensen MC, Peru KM, Headley JV, Rodgers Jr JH: A risk-based approach for identifying constituents of concern in oil sands process-affected water from the Athabasca Oil Sands region. Chemosphere 2017a;173:340-350.

McQueen AD, Hendrikse M, Gaspari DP, Kinley CM, Rodgers Jr JH, Castle JW: Performance of a hybrid pilot-scale constructed wetland system for treating oil sands process-affected water from the Athabasca oil sands. Ecological Engineering 2017b;102:152-165.

Moloney, M.G. (2009) Structure and Reactivity in Organic chemistry.

Nothe, T., Fahlenkamp, H. and von Sonntag, C. (2009) Ozonation of Wastewater: Rate of Ozone Consumption and Hydroxyl Radical Yield. Environ. Sci. & Technol. 43(15), 5990-5995.

Nyakas, A., Han, J., Peru, K.M., Headley, J.V. and Borchers, C.H. (2013) Comprehensive analysis of oil sands processed water by direct-infusion fourier-transform ion cyclotron resonance mass spectrometry with and without offline UHPLC sample prefractionation. Environ. Sci. Technol. 47(9), 4471-4479.

Oh, B.-T., Seo, Y.-S., Sudhakar, D., Choe, J.-H., Lee, S.-M., Park, Y.-J. and Cho, M. (2014) Oxidative degradation of endotoxin by advanced oxidation process (O3/H2O2 & UV/H2O2). J. of Hazardous Mat. 279(0), 105-110.

Paillard, H., Brunet, R. and Dore, M. (1988) Conditions optimales d'application du systeme oxydant ozone-peroxyde d'hydrogene. Water Res. 22(1), 91-103.

Pereira, A.S., Islam, M.D.S., Gamal El-Din, M. 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 Commun. Mass Spectrom. 27(21), 2317-2326.

Perez-Estrada, L.A., Han, X.M., Drzewicz, P., El-Din, M.G., Fedorak, P.M. and Martin, J.W. (2011) Structure-reactivity of naphthenic acids in the ozonation process. Environ. Sci. Technol. 45(17), 7431-7437.

Pisarenko, A.N., Stanford, B.D., Yan, D., Gerrity, D. and Snyder, S.A. (2012) Effects of ozone and ozone/peroxide on trace organic contaminants and

NDMA in drinking water and water reuse applications. Water Res. 46(2), 316-326.

Pocostales, J.P., Sein, M.M., Knolle, W., von Sonntag, C. and Schmidt, T.C. (2010) Degradation of Ozone-Refractory Organic Phosphates in Wastewater by Ozone and Ozone/Hydrogen Peroxide (Peroxone): The Role of Ozone Consumption by Dissolved Organic Matter. Environ. Sci. Technol. 44(21), 8248-8253.

Rosenfeldt, E.J., Linden, K.G., Canonica, S. and von Gunten, U. (2006) Comparison of the efficiency of OH radical formation during ozonation and the advanced oxidation processes O_3/H_2O_2 and UV/H_2O_2. Water Res. 40(20), 3695-3704.

Rowland, S.J., West, C.E., Jones, D., Scarlett, A.G., Frank, R.A. and Hewitt, L.M. (2011) Steroidal Aromatic 'naphthenic acids' in oil sands process-affected water: structural comparisons with environmental Estrogens. Environ. Sci. & Technol. 45(22), 9806-9815.

Sharma, J.R.; Najafi M.; and Qasim S.R., Preliminary cost estimation models for construction, operation, and maintenance of water treatment plants. J. of Infrastructure Systems, 2013, 19(4), 451-464.

Shu, Z., Li, C., Belosevic, M., Bolton, J.R. and El-Din, M.G. (2014) Application of a Solar UV/Chlorine Advanced Oxidation Process to Oil Sands Process-Affected Water Remediation. Environ. Sci. Technol. 48(16), 9692-9701.

Suh, J.H. and Mohseni, M. (2004) A study on the relationship between biodegradability enhancement and oxidation of 1,4-dioxane using ozone and hydrogen peroxide. Water Research 38(10), 2596-2604.

Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M., Hagen, M., Tierney, K.B., Belosevic, M. and Gamal El-Din, M. (2014) Advanced Analytical Mass Spectrometric Techniques and Bioassays to Characterize Untreated and Ozonated Oil Sands Process-Affected Water. Environ. Sci. Technol. 48(19), 11090-11099.

Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M. and Teiser, B. (2003) Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. 37(8), 1976-1982.

Tuan Vo, D. (1978) Multicomponent analysis by synchronous luminescence spectrometry. Anal. Chem. 50(3), 396-401.

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.

Von Gunten, U. and von Sonntag, C. (2012) The Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications, IWA Publishing, 2012.

Wang, C., Klamerth, N., Messele, S.A., Singh, A., Belosevic, M. and Gamal El-Din, M. (2016) Comparison of UV/hydrogen peroxide, potassium ferrate(VI), and ozone in oxidizing the organic fraction of oil sands process-affected water (OSPW). Water Res. 100, 476-485.

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

Wu, D., Yang, Z., Wang, W., Tian, G., Xu, S. and Sims, A. (2012) Ozonation as an advanced oxidant in treatment of bamboo industry wastewater. Chemosphere 88(9), 1108-1113.

Wu, T. and Englehardt, J.D. (2015) Peroxone mineralization of chemical oxygen demand for direct potable water reuse: Kinetics and process control. Water Res. 73, 362-372.

4 FATE AND ABUNDANCE OF CLASSICAL AND HETEROATOMIC NAPTHENIC ACID SPECIES AFTER ADVANCED OXIDATION PROCESSES: INSIGHTS AND INDICATORS OF TRANSFORMATION AND DEGRADATION[3]

4.1 Introduction

Many approaches have been used to address the environmental issues and concerns associated to oil sands process-affected water (OSPW) to allow its safe release into the environment (He et al. 2012, Scott et al. 2005, Sun et al. 2014). Albeit several methods have been effectively tested to decontaminate and detoxify the OSPW (Martin et al. 2010, Quesnel et al. 2015, Wang et al. 2016), the identification of its toxic organic components (He et al. 2012, Jones et al. 2013, Morandi et al. 2015, Rowland et al. 2014) and the elucidation of their removal mechanisms need further research. The relative contributions of all constituents present in OSPW that induce toxic effects toward selected organisms are not known (Grewer et al. 2010, Jones et al. 2013, Sun et al. 2014, Thomas et al. 2009, Zhang et al. 2016). However, the acute toxicity of OSPW has been initially attributed to a group of compounds found in the OSPW acidic fraction called naphthenic acids (NAs) (Anderson et al. 2012, Garcia-Garcia et al. 2011b, Jones

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et al. 2013, Lo et al. 2006). NAs might also exist in petroleum products and refinery wastewaters (e.g., during hydraulic fracturing) (Misiti et al. 2013, Shrestha et al. 2017) which demand further treatment. It is worth to note that high levels of NAs do not have any toxicopathological effects on birds (Beck et al. 2015, Gentes et al. 2007). However, the toxicity of NAs toward bacteria, fish (He et al. 2012) among other aquatic organisms is significant and has been associated to their chemical structure (Rogers et al. 2002) (i.e., species specific (Peng et al. 2016)).

The general formula of NAs has been defined as $C_nH_{2n+Z}O_x$ where the number of carbons and the number of hydrogens lost are represented by n and Z, respectively. While the NA species differ according to the number of oxygens or x ($2 \leq x \leq 6$), the classical NAs are denoted by O_2 species at x = 2 and the oxidized NAs are the O_3, O_4, O_5, O_6 species at ($3 \leq x \leq 6$). In addition, the heteroatomic NAs (i.e., sulfur-containing and nitrogen-containing species) are designated as ($C_nH_{2n+z}SO_x$) and ($C_nH_{2n+z}NO_x$) (Nyakas et al. 2013). To relate the toxicity of NAs to their chemical structure as suggested by Rogers et al. (2002), the characteristic correlation can be linked to the carbon number and double bond equivalent (DBE) or Z number. Overall, the toxicity of NAs has been correlated to the complexity of the mixtures and their contents (Lai et al. 1996) while the toxicity of the NAs toward *Vibrio fischeri* was previously correlated with the lower molecular weight constituents (Frank et al. 2008). The influence of molecular weight and the chemical structure is not only relevant to toxicity but it also extends to the rate of biodegradation of NAs (Biryukova et al. 2007, Herman

et al. 1993, Scott et al. 2005, Smith et al. 2008) and their biotransformation (Rowland et al. 2014, West et al. 2014).

Despite the toxic effects caused by OSPW may not only be limited to NAs (He et al. 2012a, Quesnel et al. 2015) but also to other compounds in the OSPW (i.e., including those found in the OSPW inorganic fraction) (Morandi et al. 2015, Scarlett et al. 2013), the current challenges in OSPW treatment is to find cost-effective methods to degrade at least those persistent NAs (Johnson et al. 2011, Wang et al. 2015) as one of the goals for the future OSPW remediation guidelines. Recent studies on the OSPW remediation have mainly focused on the organic compounds responsible for the acute toxicity (Frank et al. 2008, Klamerth et al. 2015). Particularly, the speciation of NAs and the fate of the individual components after treatment should be monitored as indicators for better understanding of toxicity reduction. Recent studies confirmed that the O_2 species (i.e., classical NAs) are abundant in OSPW (Jones et al. 2013) while these O_2-NAs are positively associated with *Vibrio fischeri* toxicity, especially the tricyclic and bicyclic structures (Yue et al. 2016) rather than the oxidized species present in the OSPW organic fraction (Weltens et al. 2014, Yue et al. 2015). In addition, nitrogen and sulfur heteroatomic compounds have been related to toxicity (Morandi et al. 2015, Morandi et al. 2016, Quesnel et al. 2015). Therefore, the aim of OSPW remediation should remove or monitor the variations of the compounds or species associated with toxicity (Yue et al. 2016) for possible release or reuse. Eventually, this can lead to the establishment of water quality guidelines and best available technologies for OSPW remediation. While high

levels of scavengers in wastewater matrices can hinder the full mineralization and removals during advanced oxidation processes (AOPs) treatment (Keen et al. 2012, Keen et al. 2014), AOP coupled with biological processes may be required for complete OSPW remediation and detoxification.

The focus of this study was to explore the differences in distributions of different NA species for instance O_2, O_3, O_4, O_2S, O_3S, N_2Ox and others after oxidation and the susceptibility of treated water for further biodegradation after different AOPs. The objectives of the study were: i) to characterize the treated OSPW and to observe the variations in composition of NAs and other species after treatment compared to raw OSPW using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) and Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS); ii) to explore the differences between different processes in transforming NA species; iii) to elucidate the variations of water characteristics after treatments using several markers and indices, for instance mineralization, cyclicity, biodegradability enhancement as well as a toxicity marker by examining the impact of treatments toward *Vibrio fischeri*.

4.2 Materials and methods

4.2.1 Chemicals and OSPW samples

Optima grade dichloromethane (DCM), sulfuric acid (H_2SO_4) (95-98% w/w); hydrogen peroxide (H_2O_2) (30% w/w), catalase of bovine liver (1 mg has 2950 units), and tert-butyl alcohol (TBA) analytical grade product were obtained

from Fisher Scientific Company (Edmonton, AB, Canada) and used without any further purification unless otherwise stated. Ultra-dry oxygen for ozone generation and nitrogen for residual ozone purging were obtained from Praxair (Edmonton, AB, Canada).

OSPW sample was collected in 2014 from one of the oil sand tailings site in Fort McMurray, Alberta, Canada. The water sample was stored at 4°C until further use.

4.2.2 Oxidation experiments

Different ozone-based AOPs were implemented including: ozone treatments at utilized doses of 30 mg/L (O30) and 50 mg/L (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Additionally, we implemented separately same previous treatments by spiking a scavenger tert-butyl alcohol (TBA). Spiking tert-butyl alcohol (TBA) as hydroxyl radical (•OH) scavenger should suppress the •OH pathways of oxidation. Comprehensive investigation about the individual contribution of molecular ozone or •OH in transforming naphthenic acid (NA) species is currently under investigations by using several concentrations of probe compounds and is beyond the scope of this study. Semi-batch experiments were conducted in 4000 mL reactors (i.e., vacuum flask) at pH 8 and room temperature (20±1 °C). TBA was diluted to a required stock solution and was added with the required concentration in the experiments without any further treatment. Specific amount of hydrogen peroxide (H_2O_2) was added from the prepared stock solution for the peroxone experiments and the required H_2O_2

dose was added prior to ozone exposure. The residual H_2O_2 was quenched using the bovine liver catalase and the procedure can be found elsewhere (Klamerth et al. 2013). Detailed procedure for the ozonation experiments can be found in elsewhere (Islam et al. 2014).

4.2.3 Water quality analyses

The chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD_5) were measured according to the Standard Methods. Total organic carbon (TOC) measurements were performed using an Apollo 9000 TOC Combustion Analyzer (FOLIO Instruments Inc.) without filtration of any of the samples. After filtration, the soluble COD and dissolved organic carbon (DOC) measurements were conducted as per the Standard Methods (American Public Health Association 2005). Samples were filtered using a 0.45 μm nylon filter (Supelco Analytical, Bellefonte, PA, USA). To semi-quantify the NA concentrations, an Ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) system (Waters, Milford, MA) was used.

4.2.3.1 Analysis of naphthenic acids and organic species

UPLC-TOFMS was equipped with an electrospray ionization source (i.e., operated in the negative ion mode) and with several software including MassLynx ver. 4.1, TargetLynx ver. 4.1, and DriftScope ver. to analyze the data of target compounds as well as to control the system. Myristic acid-1-^{13}C of 0.4 mg/L was used as internal standard. 2 mL aliquot of treated sample was taken for analysis. The concentrations of NA species were determined as a function of carbon (n) and

Z numbers; however, double bond equivalent (DBE) was calculated and used to plot the results instead of Z. DBE calculation can be found elsewhere (Nyakas et al. 2013, Yue et al. 2015). The DBE has been used as direct index to characterize the different classes in the petrochemical compounds and to illustrate the aromaticity patterns (Mapolelo et al. 2011).

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), Bruker 9.4 T Apex-Qe FTICR-MS from (Bruker Daltonics, Billerica, MA, USA) was used to analyze the samples and estimate the differences in species before and after treatment (e.g., O_2, O_3,... , O_3S, O_4S, etc.). The Bruker system was equipped with Bruker Daltonics Data Analysis version 4.0 software to process the raw data which can generate the formulae using the "Smart formula" algorithm. Injection of samples was done through direct infusion at 2.0 μL/min flow rate to an ESI source. Each sample was pretreated using liquid-liquid extraction and dichloromethane (DCM) as the solvent. A subsample (100 mL) of treated and raw water was acidified with H_2SO_4 to pH 2 and extracted twice with 50 mL DCM. For negative ESI analysis, each fraction after drying was re-constituted in DCM (1000 mg/L) then a dilution of 500 times was made in isopropyl alcohol (IPA). A final concentration of 2 mg/L was achieved after adding 0.1% (v/v) of NH_4OH. The collection of the data range was selected between 145-2000 m/z while 10 s was kept as an ion-accumulation time in the external hexapole collision-cell of prior to injection to the ICR cell.

For both UPLC-TOF-MS and FTICR-MS analyses; duplicate samples from duplicate experiments were prepared, however, single injection was used.

An alternative to double injections; two control samples a blank as well as raw OSPW with known NA concentration were injected in duplicate to rule out any potential interference and to check the accuracy and long-term consistency for the instruments and the entire measurements. It is worth to note the superiority and reliability of these types of instruments enabled previous researchers to conduct individual analyses (Anderson et al. 2012, Hwang et al. 2013, Sun et al. 2014).

4.2.4 Toxicity assays

Acute toxicity was measured using *Vibrio fischeri* 81.9% screening test protocol with a Microtox analyser (Model 500, Azur Environmental, Carlsbad, U.S.A.) (Chelme-Ayala et al. 2011, Islam et al. 2014). The luminosity higher than 50% reflects the threshold for acute toxicity toward *V. fischeri*. The % of luminescence inhibition was monitored during incubation while the decrease in luminosity is a marker for toxicity reduction (Chelme-Ayala et al. 2011, Shu et al. 2014). Statistical analyses for *V. fischeri* data, via one-way ANOVA and the Tukey Pairwise comparisons were conducted using MiniTab 17 Software (version Minitab® 17.3.1).

4.3 Results and discussion

4.3.1 Variations and abundance of species

4.3.1.1 Classical and oxidized naphthenic acids (NAs)

Figures 4.1 a and 4.1b depict the results of UPLC-TOFMS and FTICR-MS, respectively, for the abundances of O_2, O_3, O_4, O_5 and O_6 species of NAs after oxidation treatments compared to raw OSPW, while Table 4.1 summarizes the percent abundance of the individual species as well as oxidized NAs for the two analytical methods (UPLC-TOFMS and FTICR-MS). Our results indicated that the percentage of oxidized species O_3, O_4, O_5 and O_6 increased after oxidation while the O_2 species decreased (Figures 4.1a, 4.1b). Overall, the results show similarity between the P(1:2) and O50 treatments, where the abundances of O_3, O_4, O_5 and O_6 were almost in the same levels for all the treatments (e.g., percentages of O_3, O_4, O_5 and O_6 for P(1:2) were 32%, 27%, 15% and 7%, respectively).

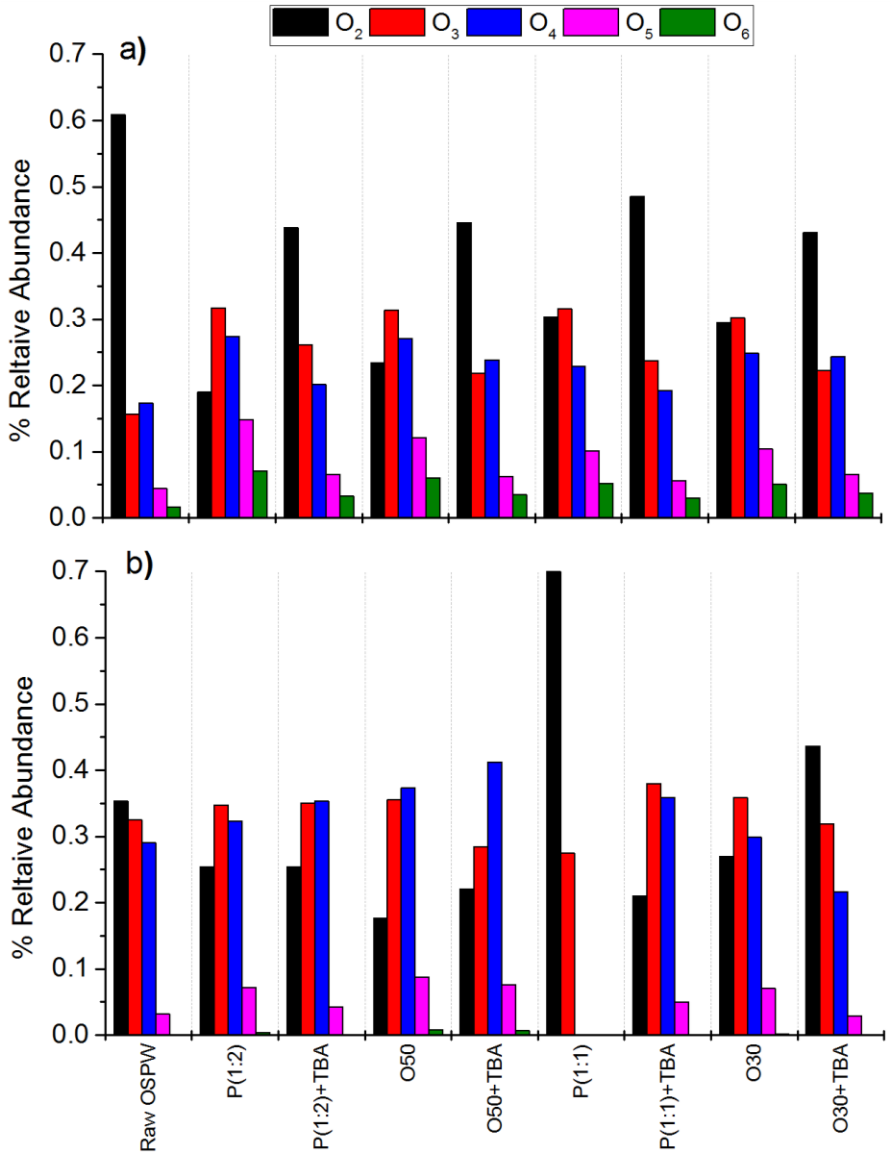

Figure 4.1. (%) Relative abundance of NA species ($2 \leq x \leq 6$) or (O_2, O_3, O_4, O_5 and O_6) species for raw and treated OSPWs at different conditions with and without TBA (i.e., 30 mg/L ozone, 30 mg/L ozone +TBA, peroxone (1:1), peroxone (1:1) +TBA, 50 mg/L ozone, 50 mg/L ozone + TBA, peroxone (1:2) and peroxone (1:2) + TBA) measured by; a) UPLC-TOFMS; b) FTICR-MS. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Note: 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

Table 4.1. The % abundance of NA species of raw and advanced oxidation-treated OSPW samples with and without TBA.

UPLC-TOFMS

Species (%)	Treatment Conditions								
	Raw	**P(1:2)+ TBA**	**P(1:2)**	**O50+ TBA**	**O50**	**P(1:1)+ TBA**	**P(1:1)**	**O30+ TBA**	**O30**
	Relative Abundance								
O_2-NAs	61	44	19	45	24	48	30	43	30
O_3-NAs	16	26	32	22	31	24	32	22	30
O_4-NAs	17	20	27	24	27	19	23	24	25
O_5-NAs	4	7	15	6	12	6	10	7	10
O_6-NAs	2	3	7	3	6	3	5	4	5
Oxidized NAs	= O_3-NAs + O_4-NAs + O_5-NAs + O_6-NAs								
Oxidized NAs	39	56	81	55	76	52	70	57	70

FTICR-MS

Species (%)	Treatment Conditions								
	Raw	**P(1:2)+ TBA**	**P(1:2)**	**O50+ TBA**	**O50**	**P(1:1)+ TBA**	**P(1:1)**	**O30+ TBA**	**O30**
	Relative Abundance								
O_2-NAs	35	25.4	25.4	22	18	21	72.5	43.5	27
O_3-NAs	33	35	35	29	35	38	27.5	32	36
O_4-NAs	29	35.3	32	41	37	36	0	22	30
O_5-NAs	3	4.3	7.2	7.5	9	5	0	2.5	7
O_6-NAs	0	0	0.4	0.5	1	0	0	0	0
Oxidized NAs	= O_3-NAs + O_4-NAs + O_5-NAs + O_6-NAs								
Oxidized NAs	65	74.6	74.6	78	82	79	27.5	56.5	73

Note: Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

As reported in previous studies (Sun et al., 2014; Wang et al., 2016), a correlation was observed between the results by UPLC-TOFMS and those obtained by FTICR-MS (Figure 4.2). The discrepancies between the two analytical methods can be due to sample preparation. Liquid-liquid extraction using dichloromethane (DCM) as the solvent was required for FTICR-MS, while no sample pretreatment was needed for UPLC-TOFMS. It has been reported that the solvent used in the extraction method highly impacts the recovery. Headley et al. (2013) found less recovery of O_2 species in DCM compared to hexane when using selective solvent extraction and Orbitrap MS. Though similar trends between HPLC-TOFMS and FTICR-MS have been noted, few discrepancies due to the differences in ionization efficiencies have also been reported (Headley et al. 2009). It is worth to note that even the two analytical methods (UPLC-TOFMS and FTICR-MS) are considered higher resolution methods (Sun et al. 2014); the FTICR-MS has relatively higher mass resolution (1 order of magnitude higher) than UPLC-TOFMS that allows to identify the N and S species from the different O_x species (Headley et al. 2013).

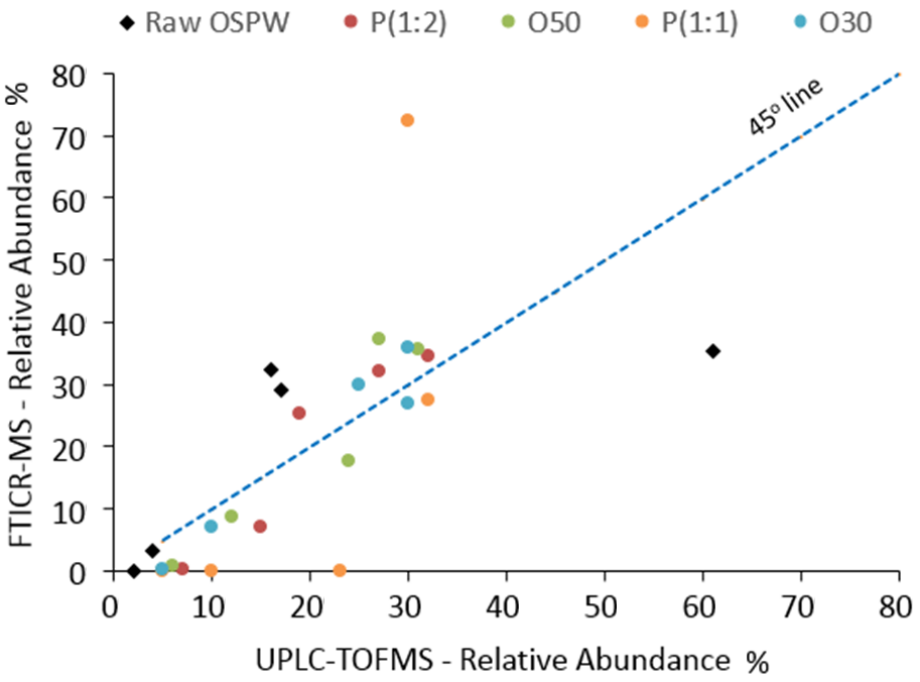

Figure 4.2. Correlation between the relative abundance of UPLC-TOFMS and FTICR-MS of all O_x species at ($2 \leq x \leq 6$). Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3).

Our results also show that the transformation of O_2 species to other oxidized species was partially inhibited after spiking TBA. Despite the role of the molecular ozone in the transformation of O_2 species cannot be ignored, the contribution of •OH can be considered significant. For instance, in both P(1:2)+TBA and O50+TBA, the transformation of O_2 species was reduced and their abundance reached 44-45% compared to 19% and 24% in P(1:2) and O50, respectively, and 61% in raw OSPW (Table 4.1). In contrast, the abundance of O_3, O_4, O_5, and O_6 species in P(1:2)+TBA remained unchanged or slightly increased compared to raw OSPW (Table S1). In summary, the O_3, O_4, O_5 and O_6 species are hydroxylated NAs (Martin et al. 2010) while the O_2 species were transformed

to other species through different pathways. It is worth to note that high abundance of oxidized species ($3 \leq x \leq 6$) was previously reported as marker for the groundwater and surface water samples (Ahad et al. 2013).

The O_2:O_4 ratio was previously proposed to track OSPW as a diagnostic potential for water characterization (Ahad et al. 2013, Frank et al. 2014a,b). Although the applicability of this ratio to identify the water sources and the impact of OSPW to background water is controversial (Yi et al. 2014), it is still a useful marker to assess the treatment effectiveness. Our study showed a decrease in the ratio after all treatments. The O_2:O_4 ratio calculated for both analytical instruments at all treatment conditions are illustrated in Table S2. For instance, the ratio decreased from 3.59 in raw OSPW to 0.7 and 0.89 in P(1:2) and O50, respectively (UPLCTOF-MS; Table S2). The addition of TBA to either treatments increased the ratio to 1.87 and 2.17 in P(1:2) and O50, respectively. Same observations were made with the O_2:O_3 ratio. Previous studies reported O_2:O_4 ratio in river waters and wells to be around 0.57, 1.04 and 0.84 while our findings exhibit a decreasing trend for this ratio after oxidation, becoming close to that reported in natural waters (Table 4.2) (Frank et al. 2014a).

Table 4.2. The ratios of species in raw and advanced oxidation-treated OSPW samples versus natural water.

UPLC-TOFMS

	Treatment Conditions						
Ratio	**Raw**	**P(1:2)**	**O50**	**P(1:1)**	**O30**	**Natural water** [a]	**Wells** [b]
O_2:O_4	3.59	0.70	0.89	1.30	1.2	0.4,0.57, 0.92, 1.04	0.84

FTICR-MS

	Treatment Conditions						
Ratio	**Raw**	**P(1:2)**	**O50**	**P(1:1)**	**O30**	**Natural water** [a,c]	**Wells**[b]
O_2S:O_3S:O_4S	2.7:4.8:2.1	0:1.4:0.5	0:1.8:1.9	0:2.1:0.7	0:1.7:1.5	1:12:2 or 1:3:1	
Normalizing ratio with O_3S at 1							
O_2S:O_3S:O_4S	0.57:1:0.44	0:1:0.36	0:1:1.06	0:1:0.33	0:1:0.88	0.08:1:0.17 or 0.33:1:0.33	
O_2:O_4	1.22	0.70	0.47	0.79	0.90	0.4,0.57, 0.92, 1.04	0.84

Notes: Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L is the initial concentration of H_2O_2. H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

[a] River waters sources from Athabasca river and Ells river (Frank et al. 2014).

[b] Monitoring well source (Frank et al. 2014).

[c] River waters sources from Athabasca River and Gregoire Lake (Headley et al. 2011).

4.3.1.2 Sulphur and nitrogen species

As shown in Figure 4.3, a decrease in abundance of O_2S, O_3S and O_4S species as measured by FTICR-MS after all treatments can be observed, except for P(1:1). Conversely, by spiking TBA, the abundance increased again and become similar to that of raw OSPW due to the inhibition of one of the significant oxidation pathways (i.e., •OH) (Figure 4.3). Our findings suggest the change in the distribution of the species by selective transformation from one species to another after oxidation. These findings agree with previous study about the hydroxylation of the O_2S to O_3S and other forms (Bressler and Fedorak 2001, Kropp et al. 1997) (Figure 4.3). The ratio between O_2S:O_3S:O_4S measured by FTICR-MS was previously suggested as an OSPW diagnostic marker in surface waters (Frank et al. 2014a, Headley et al. 2011) and reported as 2:5:4 for OSPW while the natural water (i.e., Athabasca River water) displays approximate a ratio of 1:12:2 and 1:3:1 (Headley et al. 2011). Interestingly, our results for all treatments showed ratios close to those in natural water, with the highest similarity for peroxone treatments. For instance, the ratio changed from 2.7:4.8:2.1 in raw OSPW to 0:1.4:0.5 and 0:1.8:1.9 in P(1:2) and O50, respectively. The O_2S:O_3S:O_4S ratio calculated for FTICR-MS results for all treatment conditions are illustrated in Table 4.2. Additionally, the low abundance of O_2S in groundwater and surface water samples was reported as a characteristic marker (Ahad et al. 2013). These markers might reveal minor change in the characteristics of treated OSPW compared to natural water sources.

Similarly, the abundance of N_2O_x species decreased after ozone treatments. In contrast, peroxone treatments were not effective in reducing these species, even it did increase in one of the peroxone conditions (i.e., slight decrease in P(1:2) and increase in P(1:1)). Spiking TBA with ozone treatments did suppress •OH that led to an increase again in the abundance of N_2O_x species (Figure 4.2). The reason of the effective reduction of N_2Ox species in ozone treatments compared to an increase after the P(1:1) and marginal reduction in the P(1:2) can be attributed due to two possibilities. The first possibility is the low generation of hydrophilic moieties through oxidation reactions which are less reactive with •OH (Keen et al. 2014). In this case, the molecular ozone is more preferable. The second possibility is the reaction of ozone with ammonia that generates different intermediates. These intermediates can be hydroxylamine (H_2NOH) and hyponitrous acid (HNO and its dimer). Specifically, HNO might decay to N_2O due to its instability and contribute to the increase in the N_2O_x (von Gunten and von Sonntag 2012). The decay of HNO can be only terminated by ozone which might be the case in the ozone treatments (von Gunten and von Sonntag 2012).

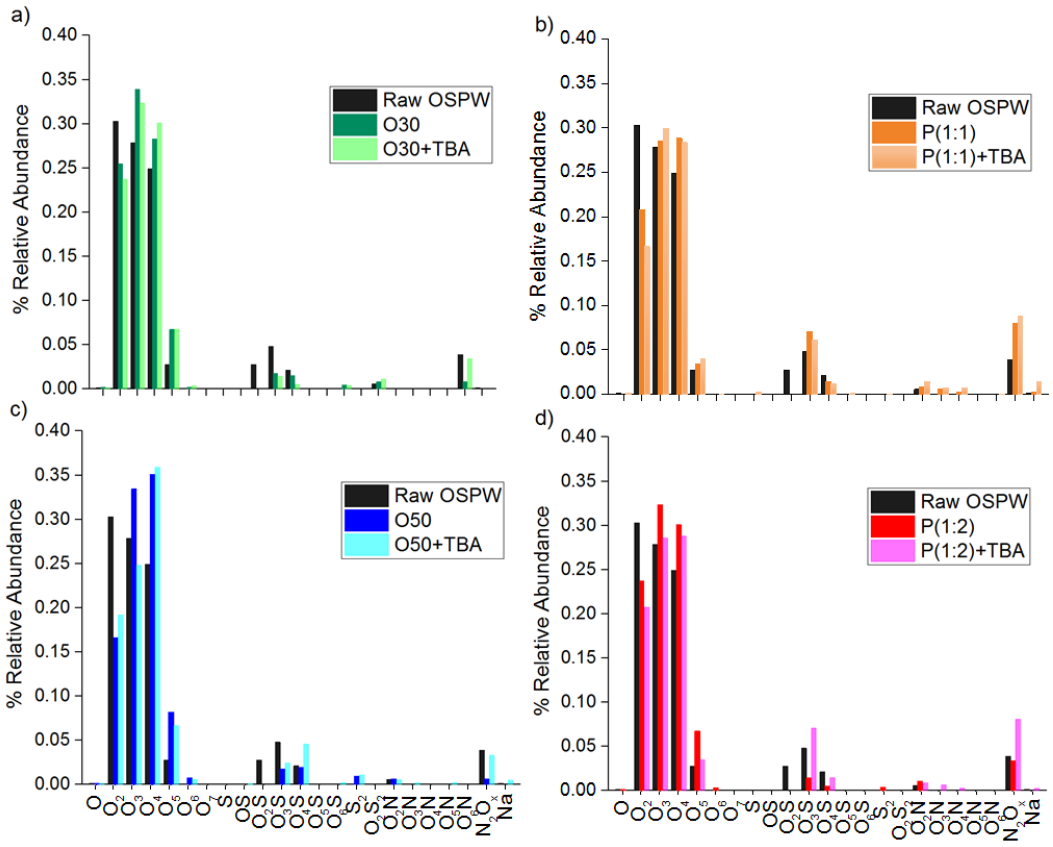

Figure 4.3. (%) Relative abundance of the different species for raw OSPW as well as treated OSPW at different conditions with and without TBA using FTICR-MS; a) 30 mg/L ozone and 30 mg/L ozone + TBA; b) peroxone (1:1) and peroxone (1:1) + TBA; c) 50 mg/L ozone and 50 mg/L ozone + TBA; and d) peroxone (1:2) and peroxone (1:2) + TBA. Note: 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

4.3.2 Variations of the organic compound characteristics (mineralization, biodegradability, cyclicity, and molecular weight)

Although our study focused on ozone-based processes, the multi-barrier treatment train for OSPW might encompass different processes. Therefore, we monitored other indices beside the NA degradation, such as biodegradability,

composition, and water characteristics (e.g., mineralization, cyclicity and molecular weight) in addition to parameters such as chemical oxygen demand (COD), total organic carbon (TOC), biochemical oxygen demand (BOD_5), and biodegradability index (BOD_5/COD). These parameters are potential markers for the treatment capability and efficiency as well as useful guide for the selection of pre- and post-treatments.

4.3.2.1 Mineralization and biodegradability enhancement

The OSPW contains recalcitrant organic matter that may be transformed, not mineralized, from parent compounds to by-products or intermediates (Martin et al. 2010). Our findings showed relatively low removals of COD and TOC for all treatments (e.g., COD removal: 24% and 14% in P(1:2) and O50, respectively). Similarly, the DOC showed no decrease after OSPW treatment (Table 4.3). Previously, partial COD reduction of 22% was reported by Gamal El-Din et al. (2011) using high ozone dose of 150 mg/L and similar to our results at P(1:2) using low ozone dose of 50 mg/L. The limited removals of COD and TOC can be attributed to the stability of the DOC after being oxidized (von Gunten and von Sonntag 2012). It has been reported that the peroxone process can be effectively enhanced through increasing ozone decay rate by adding higher ozone doses or by keeping the normalized DOC-specific ozone doses in mg O_3/mg DOC ≥ 1 (von Gunten and von Sonntag 2012). In our study, the treatments using 30 and 50 mg/L ozone have ratios of 0.7 and 1.2, respectively (Table 4.3). Clearly, even at the higher ratio, the DOC is not being impacted by the ozonation process,

indicating a low or minimal degree of mineralization (Nothe et al. 2009, von Gunten and von Sonntag 2012).

Despite the lack of complete mineralization, all oxidation treatments increased the BOD_5 concentrations which indicate the potential for further treatment of OSPW using biological processes (Table 4.3 and Figure 4.4). The BOD_5/COD ratio as an index for the change in the biodegradability increased in all treatments. For instance, the maximum BOD_5/COD ratio increased from 0.06 in raw OSPW to 0.11 and 0.08 in P(1:2) and O50 treated samples, respectively (Figure 4.4). Our results are consistent to previous study that showed a positive impact of ozonation on the growth of microbial population (Martin et al. 2010). As well, it was reported that 80 mg/L (Wang et al. 2013) and 148 mg/L (Gamal El-Din et al. 2011) of ozone increased the OSPW biodegradability by increasing the BOD_5/COD ratio from 0.01 to 0.02 (Wang et al. 2013) and 0.13 (Gamal El-Din et al. 2011), respectively.

Table 4.3. Selected parameters for raw and treated OSPWs after ozone and peroxone treatments.

Parameter	Raw	Ozone (dose)		Peroxone (Ratio)	
		O30	O50	P(1:1)	P(1:2)
	Concentrations (mg/L)				
COD	216± 2.1	179.2±2.3	186.3±1.8	200.3±4.1	163.4±3.6
TOC	60.3± 0.2	59.7± 1.6	60.2± 0.4	58± 1.6	48.9± 1.4
BOD_5	13.5±0.5	17.9±0.7	18.2±0.2	18±0.4	17.8±0.04
DOC	39.9±1	41±2.1	40±3.2	44.2± 0.8	40.4± 0.5
mg O_3/mg DOC	-	0.7	1.2	0.7	1.2

Note: Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

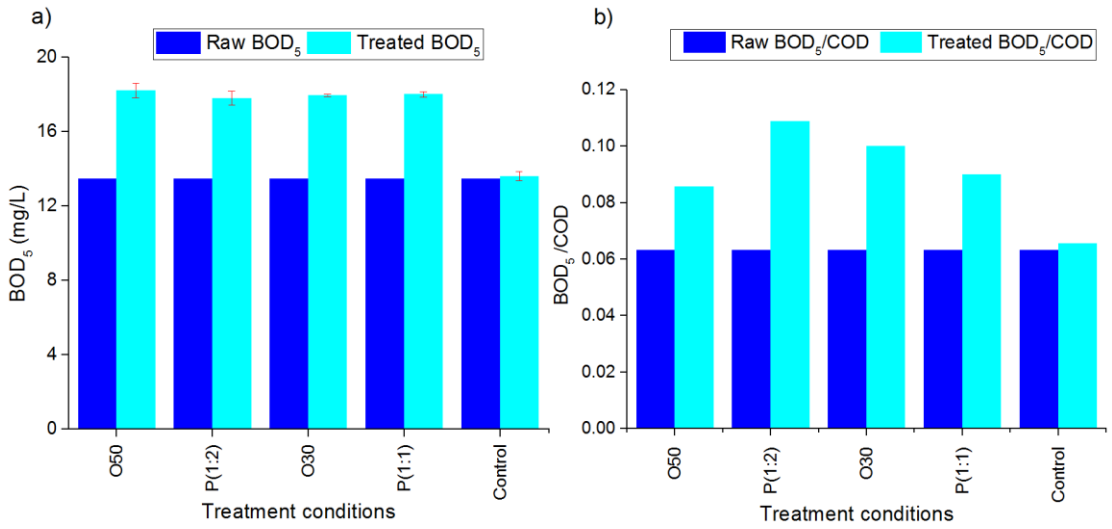

Figure 4.4. Effect of treatment conditions on the biodegradability of organic compounds in OSPW indicated by BOD_5 and BOD_5/COD ratio. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L is the initial concentration of H_2O_2, H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

The A/C ratio (acyclic to cyclic NAs) or (DBE=1/∑ DBE 2, 3 and 4) of O_2 species was previously recommended as a biomarker for the degree of biodegradation in crude oil (Fafet et al. 2008, Kim et al. 2005) and an indicator for the differences in samples' compositions such as oil sands, mature fine tailings, and tailing sands (Noah et al. 2015). However, the authors suggested more studies to examine the broader applicability of the A/C ratio as an index. Our preliminary investigations showed minimal change in the A/C ratio (data not shown). It is worth to note that the half-life of *in situ* NA biodegradation can last 13 years (Han et al. 2009); however, ozone accelerates the biodegradation by decreasing this half-life to few days (Xue et al. 2016). The current BOD_5/COD ratio presented in this study can reveal the positive influence of oxidation treatments in enhancing the OSPW biodegradability. However, the influence can be considered minimal due to the limited increase of BOD_5/COD (i.e., increase from 0.06 in raw OSPW to 0.11 in treated OSPW compared to 0.4 in biodegradable waste), and low reduction in COD and limited change in BOD_5. The rationale for having the oxidation as preliminary step prior to biological processes is due to the formation of more easily biodegradable compounds (e.g., aldehydes and carboxylic acids) after oxidation. It is important to note that assessing the biodegradability enhancement should be implemented through different parameters (Tembhekar et al. 2015). Implementing different biodegradability indices and their correlations in addition to changes of microbial populations and biodegradation kinetics might be beneficial to understand the changes of the OSPW recalcitrant organic compounds and to reach the optimum

treatment performance. Therefore, we suggest more comprehensive studies to assess the applicability of all different biodegradability indicators (e.g., half-life), especially in recalcitrant matrices.

While our study focused on chemical treatments only, the microbial populations and their activities are out of scope of this paper and further research is warranted. Alternatively, we studied the changes of molecular weights and cyclization after AOPs as they can affect the biodegradation rates (Whitby 2010) and the toxicity (Jones et al. 2011).

4.3.2.2 Variations of molecular weight (carbon only) and cyclicity

Carbon number (n) cannot differentiate between high and low molecular weights NAs. However, the impact of the molecular weight on the toxicity was previously reported where the increase of toxicity toward *Vibrio fischeri* is accounted with an increase in carbon number (Jones et al. 2011). Similarly, high molecular weight NAs are less readily biodegradable compared to lower ones (Whitby 2010). The high molecular weight NAs were previously denoted from n = 16 and 22 as reported by Sohrabi et al. (2013) though the authors of the study used n = 15 as margin to show the difference in degradation. Jones et al. (2011) also referred to relative toxicity at n=10-14 while the insolubility of n≥15 pentayclic acids inhibited their toxic effects toward *Vibrio fischeri*. Here, we used same criteria and we divided the n of O_2 species into two groups: lower and greater than 15 as illustrated in Figure 4.5. The high molecular weight NAs from n = 15-26 decreased from 66% to 44%, 39%, 48%, and 34% at O30, O50, P(1:1)

and P(1:2), respectively. In contrast, the low molecular weight NAs from n = 7-14 increased from 34% to 56%, 61%, 52%, and 66% at O30, O50, P(1:1) and P(1:2), respectively. Suppressing the •OH pathway by TBA reduced the former observation. For instance, the abundance of NAs with n = 15-26 at P(1:2) increased from 34% to 53% while the abundance of NAs with n= 7-14 decreased from 66% to 47% to become closer to the abundance in raw OSPW. Therefore, we can confirm the susceptibility of increasing biodegradation following an effective decrease of higher molecular weight NAs (i.e., higher carbon) after AOP treatment.

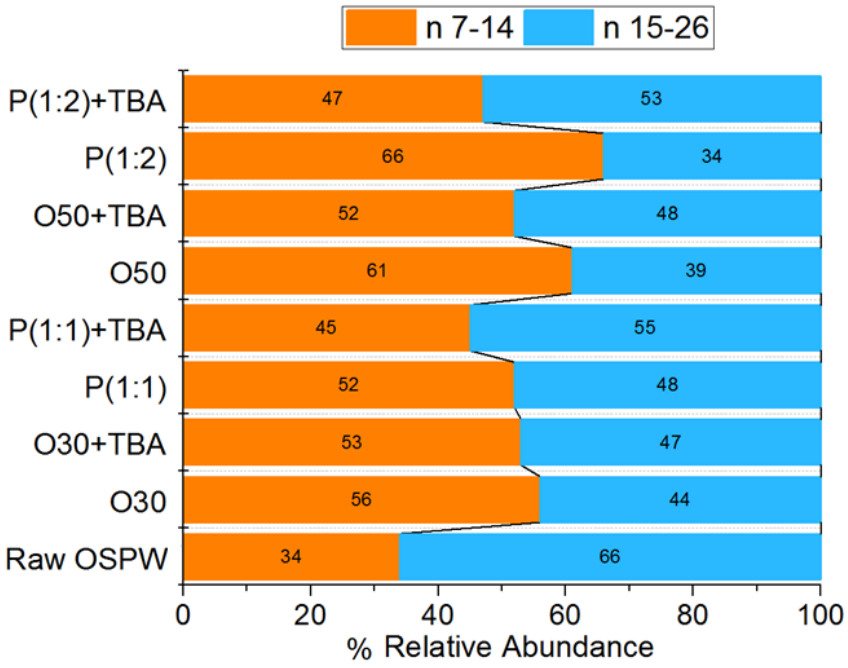

Figure 4.5. Variations in the carbon number (n) of the O_2-NAs after different treatment conditions. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Note: 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

The species were also categorized into three large subgroups based on DBE. The relative abundance of the DBE (1-2), (3-6) and (7-10) of total NAs (sum of classical and oxidized NAs) was 4%, 63% and 33% respectively, in raw OSPW. These abundances changed to 19%, 61% and 20%, respectively, in P(1:2) indicating the increase of monocyclic (or classes with no rings and 1 ring), marginal decrease in bicyclic acids, tricyclic, tetracyclic acids and pentacyclic (or classes with number of rings = 2-5), and moderate decrease in higher cyclic structure (6-9 rings). The two ozone treatments reported same relative abundance, while the abundance for P(1:2) was relatively higher than that of P(1:1) at DBE 3-6. The addition of scavenger to eliminate the •OH pathway showed almost minimal differences in ozone treatments; even the change was marginal in P(1:2). These results agree with the dominance of the 2-3 rings in biodegraded oils due to their bioresistance to transformation (Kim et al. 2005, Liao et al. 2012) as well as the non-abundance of monocyclic acids in raw OSPW (Martin et al. 2008).

To grasp more insights, we categorized the DBE into five small sub groups, Figures. 4.6a and 4.6b show the changes in the DBE (1-2, 3-4 , 5-6 , 7-8 and 9-10) in total NA and O_2-NA species, respectively. For total NAs (Fig. 4.6a), the oxidation treatments increased the classes of DBE 3-4 and moderately DBE 1-2 as well as DBE 7-8, while it decreased the classes of DBE 5-6. Although the increase of tricyclic acids or DBE 4 was reported as an indicator of biodegradation increase (Jaffé and Gallardo 1993), those tricyclic acids are also characterized by their resistance to further biodegradation (Jaffé and Gallardo 1993). Regarding the O_2 species, DBE 3-4 increased from 50% to 71%, 72%,

64%, and 58% at O30, O50, P(1:1) and P(1:2), respectively. In conjunction with O_2 species distributions, the DBE 9-10 decreased in all treatments. Previously, the tetracyclic NAs and higher cyclic NAs showed unchanged concentrations and decrease in biodegradation rate after 28 days (Han et al. 2008). The significant influence of cyclization on the persistence in biodegradation was reported previously where the preferential biodegradation occurs with the less DBE structures (Han et al. 2008). In summary, after different oxidation treatments, most of the higher molecular weight and higher cyclic (i.e., higher DBE) species were degraded or transformed to lower one as shown in Figures 4.6a and 4.6b. For instance, DBE 7-8 in O_2 species decreased from 21% to 9% in P(1:2) and DBE 5-6 in total NAs decreased from 59 to 20% and 27% in P(1:2) and O50, respectively. Therefore, these former attributes can show the compatibility of the oxidation treatments with biodegradation either as pre- or post-treatment.

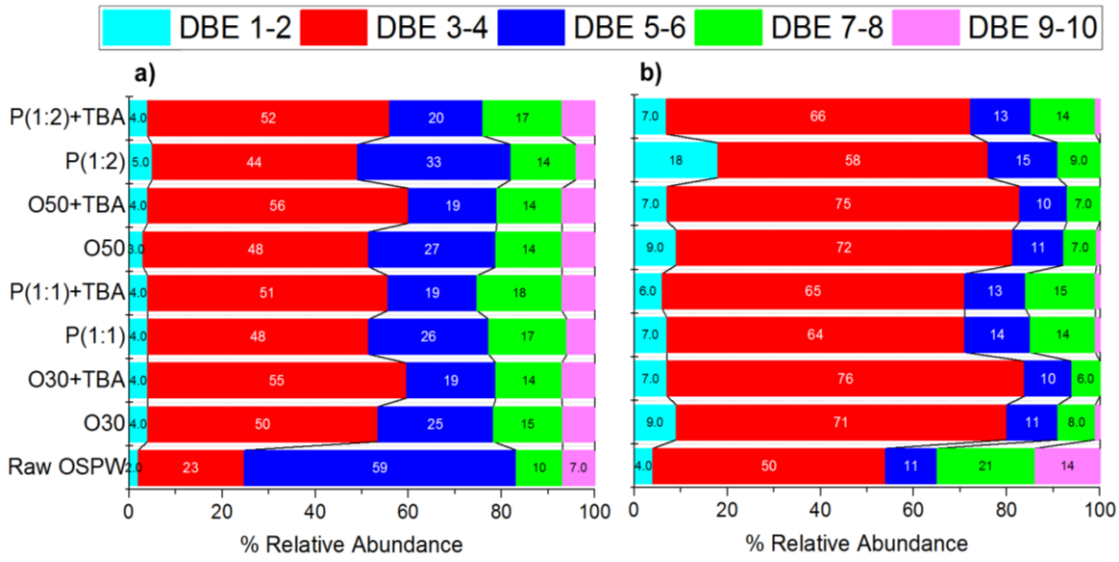

Figure 4.6. Variations of NA abundances with respect to DBE groups after different treatment conditions; a) total NA (sum of classical and oxidized NAs) and b) O_2 species. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Note: 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

The O_2-NA degradation can be attributed to molecular ozone, •OH and organic radicals. The increase of some classes for instance DBE 3-4 while using TBA refer to the possibility of major contribution from molecular ozone and other radical pathways. To have a complete assessment about the variations in classes of O_2-NAs before and after oxidation, we plotted each individual DBE with relative abundance for the eight treatment conditions as shown in Figure 4.7. The highest abundance can be observed for the DBEs of 3 and 4. The DBE 3 and DBE 4 were 28% and 22%, respectively, from the total NAs in the raw OSPW. However, the range of abundance for 5-10 DBE was slightly less and ranged from

5-10%. The increase of DBE 1-5 can be observed after treatments while decrease in DBE 6-10 was also occurring. As mentioned earlier, the higher DBE or higher molecular weights with higher cyclic are transformed to lower molecular weight and lower cyclic compounds after oxidation. Interestingly, the DBE =1 is produced after the oxidation treatments as it is not observed in the raw OSPW. Compared to initial raw OSPW distributions, spiking TBA did not change the variations in DBE 5, 7, and 8 after ozone treatments, while it decreased the transformation at same DBE with peroxone treatments. This finding can refer to the different pathways other than •OH pathway (von Gunten and von Sonntag 2012) in degrading O_2-NAs while the •OH pathway was minimized or reduced in peroxone but not during ozonation.

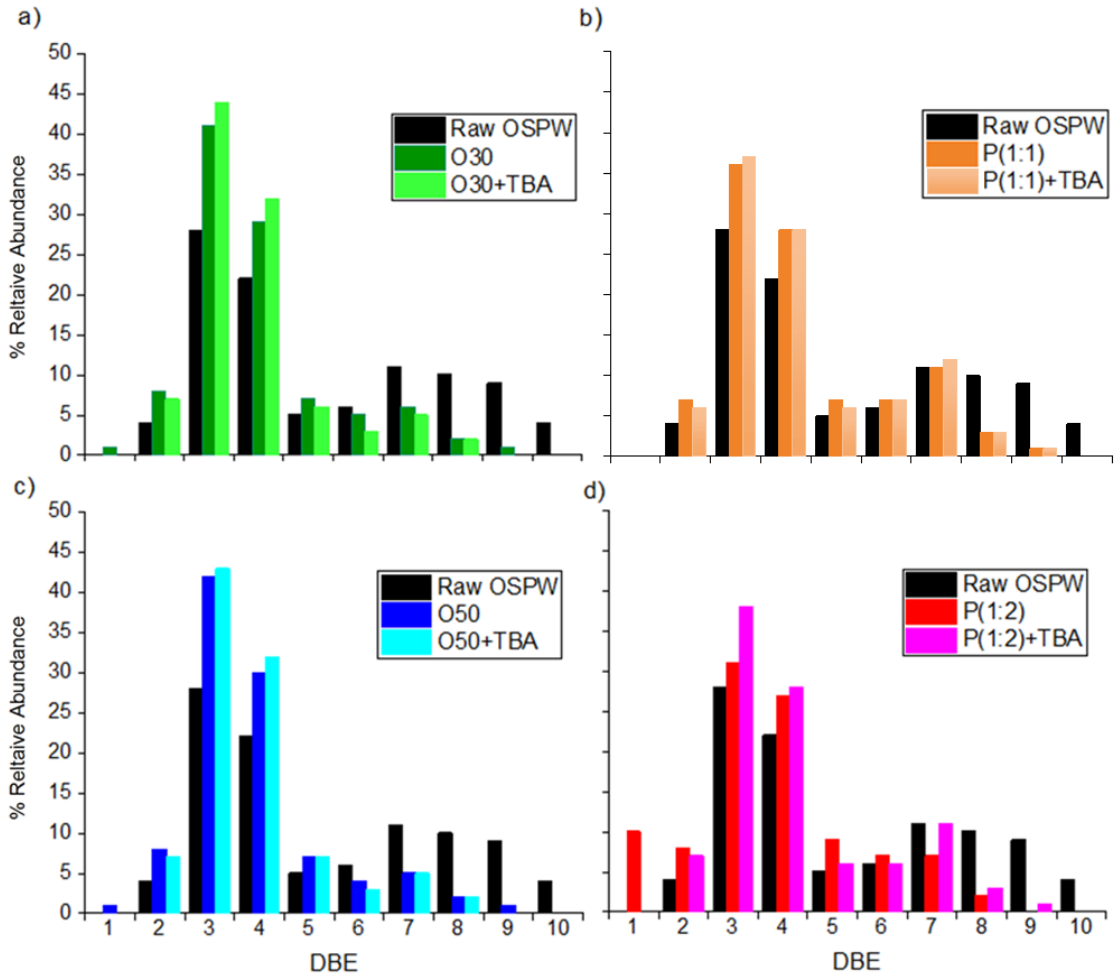

Figure 4.7. (%) Relative abundance of NA species ($2 \leq x \leq 6$) measured using UPLC-TOFMS for raw and treated OSPWs at different conditions; a) 30 mg/L ozone and 30 mg/L ozone +TBA; b) peroxone (1:1) and peroxone (1:1) +TBA; c) 50 mg/L ozone and 50 mg/L ozone + TBA; and d) peroxone (1:2) and peroxone (1:2) + TBA. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Note: 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

As shown in Figures 4.8 and 4.9, the abundance of O_3S and O_4S species with high DBE and high n decreased, while low DBE at 1, 2 and 3 with different n increased after oxidation. The increase of DBE=1, 2 and 3 is expected as they are transformed products from the larger DBE after ozone and peroxone treatments. The addition of TBA did result in reverse increase of some DBE (e.g., 5, 6, and 7) (see Figures 4.8e, g, i). In this respect, the abundance of O_3S species is plotted with respect to DBE only in Figure 4.10. Specifically, the DBE 3 and 4 in the O_3S species corresponded to the highest abundance in all samples. The increase in abundance of this classes was observed after all treatments, except P(1:2) which was almost similar to raw OSPW. Interestingly, the addition of TBA decreased these classes after treatments. This finding is confirming the production of these classes after oxidation. Similarly, the increase of these species was reported after biodegradation treatment (Yue et al. 2016). However, it is worth to note that the compounds of O_3S at DBE 4 and n=17-19 was previously reported to contribute to the toxicity of OSPW (Quesnel et al. 2015).

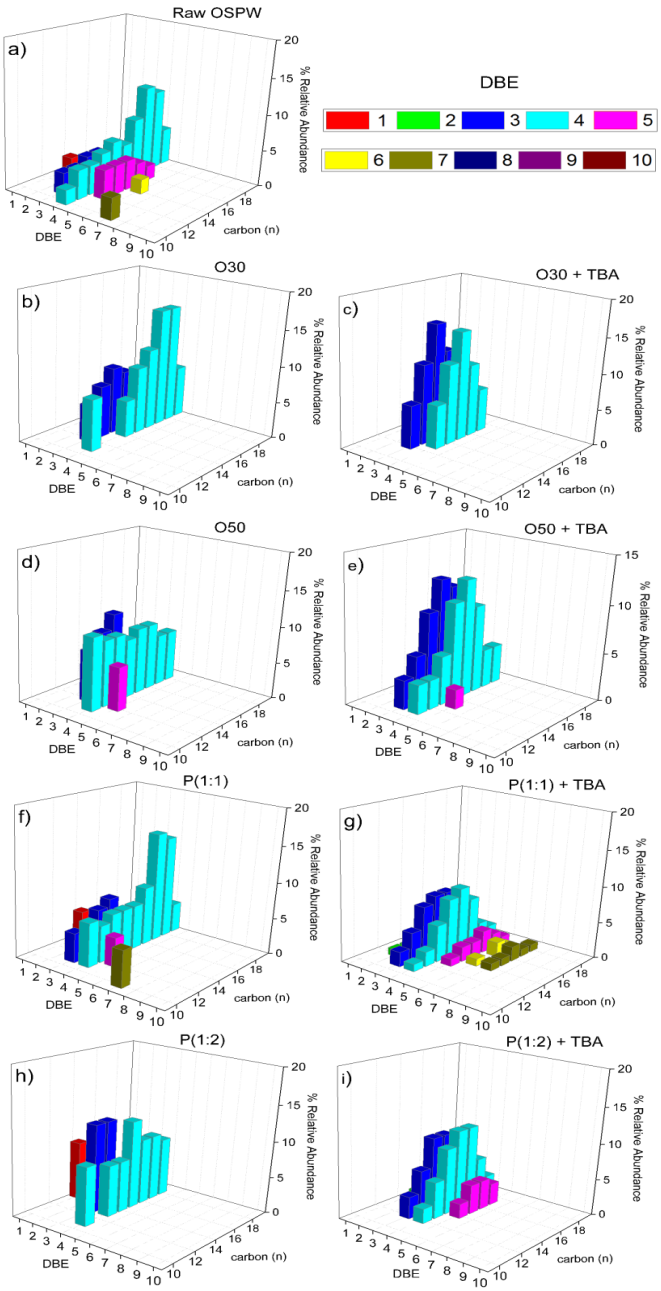

Figure 4.8. (%) Relative abundance of O_3S species for raw and treated OSPWs at different conditions using FTICR-MS; a) raw OSPW; b) 30 mg/L ozone; c) 30 mg/L ozone + TBA; d) 50 mg/L ozone; e) 50 mg/L ozone + TBA; f) peroxone (1:1); g) peroxone (1:1) + TBA; h) peroxone (1:2); and i) peroxone (1:2) + TBA. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L

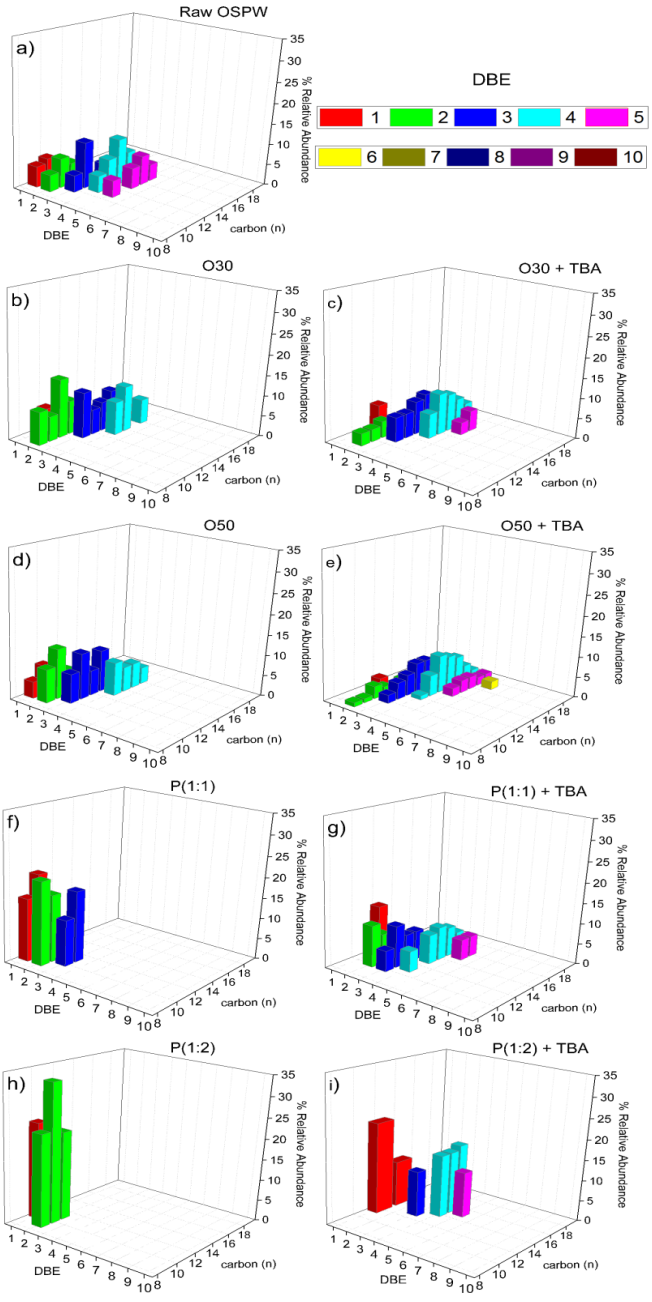

Figure 4.9. (%) Relative abundance of O_4S species for raw and treated OSPWs at different conditions using FTICR-MS; a) raw OSPW; b) 30 mg/L ozone; c) 30 mg/L ozone + TBA; d) 50 mg/L ozone; e) 50 mg/L ozone + TBA; f) peroxone (1:1); g) peroxone (1:1) + TBA; h) peroxone (1:2); and i) peroxone (1:2) + TBA. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

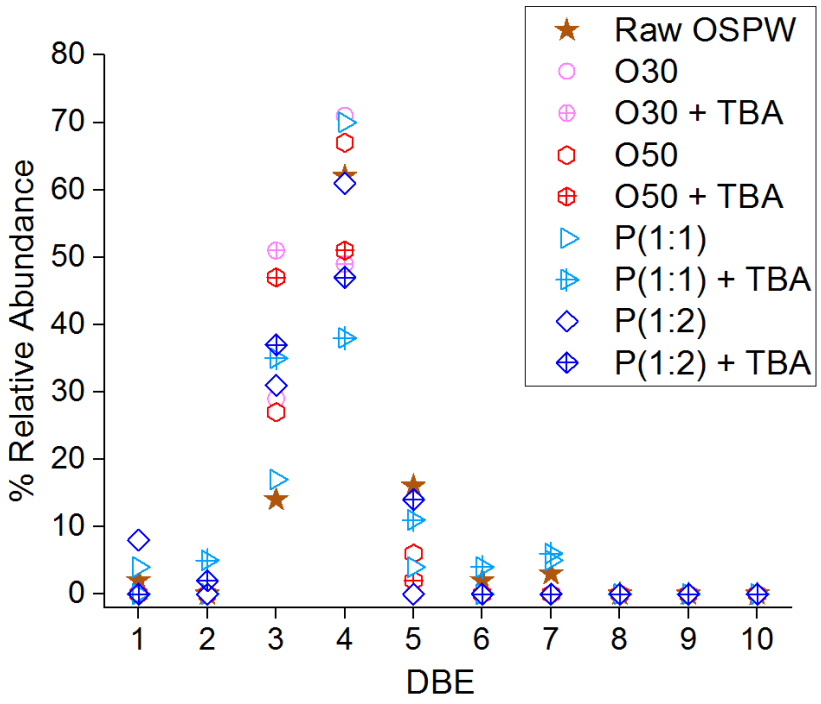

Figure 4.10. Abundance of O_3S species with DBE. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

4.3.3 Toxicity assessment and markers

The toxicity of the treated samples toward *Vibrio fischeri* was assessed based on the inhibition of the bacteria luminescence. The inhibition of *V. fischeri* by raw OSPW was 51% while all treatments resulted in significant reductions in inhibition ($p<0.05$) (Figure 4.11). The P(1:2) leads to the highest reduction in toxicity with an inhibition value of 25.4%, while being statistically similar to the 30 and 50 mg/L ozone treatments. The P(1:1) treatment was similar to the ozone-

only treatments, while being statistically lower than the (1:2) peroxone treatment. Similarly, residual toxicity has been reported after different treatments even when achieving high NA removals (He et al. 2012, Wang et al. 2013). Garcia et al. (2011a) suggested that reasonable and not complete reduction of NA levels in the organic fraction of OSPW can be significant enough to ameliorate the immunotoxic properties of OSPW in different toxicological bioassays. Additionally, recent study reported that the acute OSPW toxicity can be attributed to the non-acidic species while it is not limited to entire NAs only, but correlated with specific species including O_2^- as the most potent (Morandi et al. 2015). Therefore, singling out individual classes that contribute to OSPW toxicity is demanding (West et al. 2014).

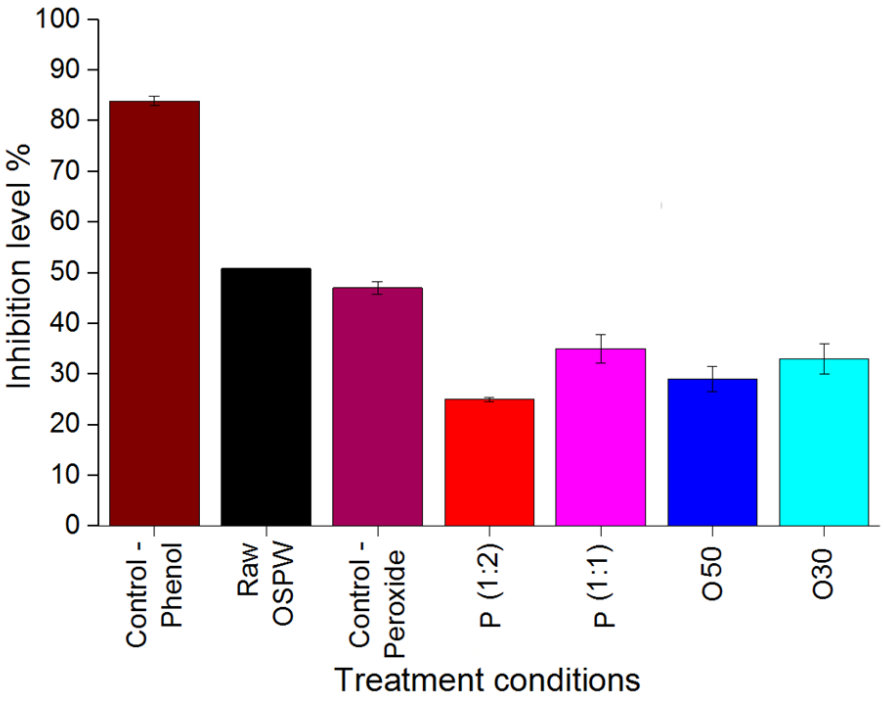

Figure 4.11. Toxicity toward *Vibrio fischeri* after various treatments; treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Note: 20 mg/L is the initial concentration of H_2O_2, utilized concentration of H_2O_2 in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

In the current study, the residual toxicity after oxidation treatments is discussed in regards to the following aspects. The first aspect is the creation of by-products (i.e., lack of complete mineralization of compounds) which might contribute to the OSPW toxicity similar to their parent compounds, especially in their protonated form (Klamerth et al. 2015, Sun et al. 2014). Our earlier observations showed very minimal DOC removal that can be due to the continuous generation of by-products (Lamsal et al. 2011). Despite the intermediates generated after AOP oxidation can be more hydrophilic (Klymenko et al. 2010), they might possess residual toxicity with specific mode of actions.

Unfortunately, the Microtox^{TM} assay is a good screening tool; however, it could not specify which mode of action is responsible for toxicity. The generic narcotic toxicity mode is relevant to hydrophobicity (Frank et al. 2009, Tollefsen et al. 2012). Zhang et al. (2015) examined the bioconcentration potential of compounds in OSPW (e.g., NAs) and confirmed the variability of hydrophobicity in terms of partition coefficient with carbon and DBE. It is worthy to note that the increase of the carboxylic content could concurrently decrease the hydrophobicity which leads to less bioaccumulation of the NAs in the cells (Frank et al. 2008, Whitby 2010). In addition, the contribution toward toxic effects could decrease by decreasing the alkyl carbon number while decreasing hydrophobicity (Scarlett et al. 2011). Unfortunately, the O_2 species at n=11-14 which was previously associated with toxicity increased in all oxidation treatments.

The second aspect is relevant to the change in abundance of species and compounds before and after treatments. It can be hypothesized that residual toxicity is correlated to these residual species after oxidation. Our focus in the coming section is to highlight the changes of the species that have been previously associated with toxicity (Yue et al. 2015) in addition to the compounds that were not originally observed in raw OSPW and generated as by-products. Here, we selected these compounds as toxicity markers to show deep insights about their recalcitrance and their level of removals.

In a previous study, toxicity was positively correlated with O_2 species only and not with O_3 and O_4 (Yue et al. 2015). The classes that have been previously associated with toxicity are the tricyclic and the bicyclic structures in O_2-NAs.

Although increasing the tricyclic structure (i.e., n=15-18 and DBE=4) is an indication for increasing the level of biodegradation, they are characterized by their resistance to further biodegradation (Jaffé and Gallardo 1993) as well as by their acute toxicity (Yue et al. 2015). Similarly, O_2-NAs with DBE = 3 and n = 14-17 which represent the bicyclic structures are characterized by their acute toxicity (Yue et al. 2015). The abundances of the n = 15-18 at DBE = 4 and n = 14-17 at DBE = 3 in raw OSPW contribute to 12 and 18%, respectively, with total proportion of 30% from the O_2 species as illustrated in Figure 4.12. Interestingly, these classes increased after all oxidation treatments to 38%, 37%, and 34%, at O30, O50, P(1:1), respectively. They decreased, however, to 24% at P(1:2). This former finding in addition to the lowest abundance (34%) of the high carbon classes (i.e., n=15-26) at P(1:2) compared to the other treatments can justify and confirm the best reduction in toxicity towards *V. fischeri* at P(1:2). Similarly, the residual toxicity necessitates the identification of other compounds inside the water matrix that might possess toxic effects.

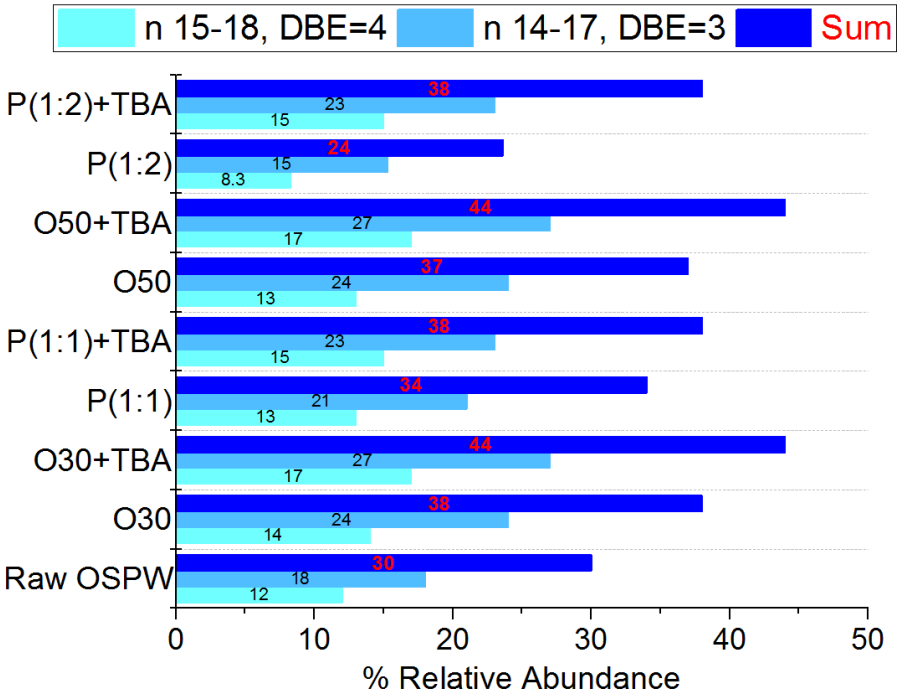

Figure 4.12. Abundance of the n 15-18 at DBE = 4 and n 14-17 at DBE = 3. Treatments are denoted as 30 mg/L ozone (O30) and 50 mg/L ozone (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L and in P(1:1) = 6.9 mg/L.

Another example of compounds that were associated with toxicity and still have significant residual concentration after treatments are $C_{15}H_{24}O_2$ and $C_{16}H_{26}O_2$ at DBE=4 (Yue et al. 2015) as shown in Figure 4.13. Additionally, same study correlated O_2-NAs with n =17 at DBE = 6 and n = 14-17 at DBE = 3 with toxicity. Slight residual abundance can be observed after oxidation at DBE = 3. Likewise, compounds have been generated after oxidation and might contribute to toxicity (e.g. $C_9H_{12}O_2$ at DBE = 4) are shown in Figure 4.13.

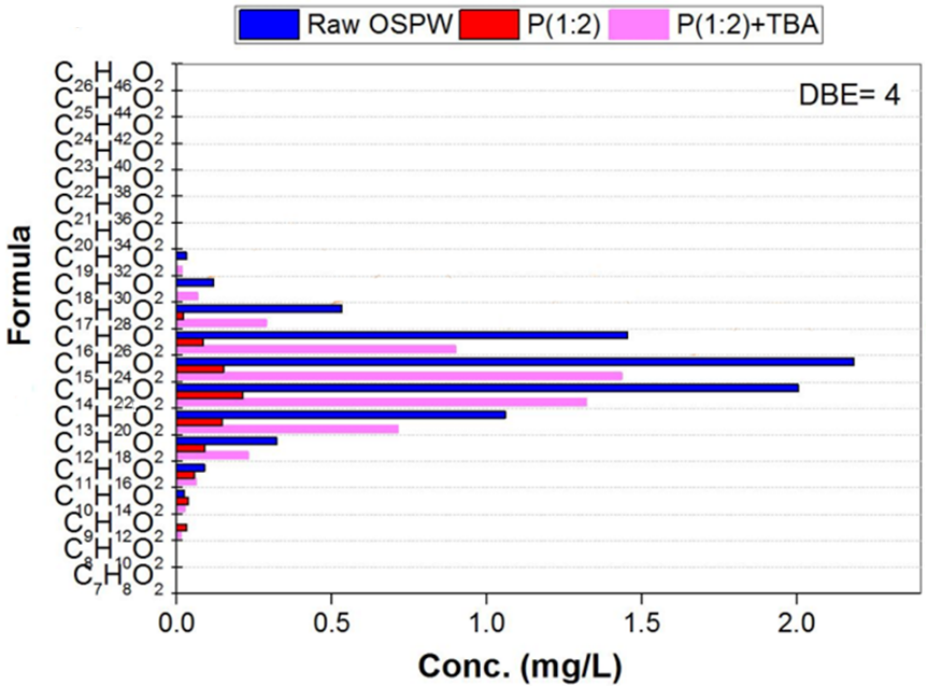

Figure 4.13. Concentrations of $C_{15}H_{24}O_2$, $C_{16}H_{26}O_2$ and $C_9H_{12}O_2$ at DBE = 4 after treatment compared to raw OSPW. Peroxone treatment at molar ratio (1:2) denoted as P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). Note: 20 mg/L and 25 mM are the initial concentrations of H_2O_2 and TBA, respectively, and H_2O_2 concentration utilized in P(1:2) = 10.2 mg/L.

As illustrated in Figure 4.3, the O_2S species were significantly removed after all treatments while O_3S and O_4S slightly decreased. However, low concentrations of these compounds might be concern (West et al. 2014). Recently, the toxicity of the O_3S at DBE = 4 was confirmed especially at n = 17-19 (Quesnel et al. 2015, Yue et al. 2015). The current findings showed an increase in these classes after oxidation (Figure 4.10). Specifically, the increase in their abundance was observed in some treatments for instance 39% and 33% at O30 and P(1:1), respectively, compared to 28% in raw OSPW.

4.4 Conclusions

In this study, we provided new insights in the variations of species and compounds after AOP treatments. The differences in compositions of the OSPW samples before and after treatments indicated the generation of some compounds and classes that were not present in raw OSPW. The compatibility of the AOPs with biological processes is warranted to be efficiently evaluated using different approaches (i.e., either oxidation coupled with biological processes as a pre- or post-treatment) and using suggested markers (e.g., O_2:O_4, O_2S:O_3S:O_4S, BOD_5/COD, and toxicity markers) with a corresponding assessment of toxicological properties of OSPW treated samples. Oxidized species of NAs as well as classes with small n and less cyclicity (i.e., increase of 1-5 DBE and decrease in 6-10) are susceptible to biodegradation and they are leftovers after the AOP treatment, probably contributing to the residual toxicity. Although the levels of biodegradation increased by generating tricyclic (i.e., n = 15-18 and DBE = 4) and bicyclic structures of O_2-NAs, these compounds can further hinder biodegradation and possess residual toxicity. Similarly, while the entire O_3S species decreased after oxidation, the species with DBE = 4 increased. Therefore, with the aid of the suggested indicators/markers, more research is warranted to investigate the levels of removals of O_2 species and other specific compounds that can be accomplished through AOPs, to reach significant reduction in toxicity with minimum costs. This study introduced useful indicators to evaluate the treatment performance that would allow selecting the best multi-barrier approaches and establishing guidelines in terms of species reductions.

4.5 References

Ahad, J.M.E., Pakdel, H., Savard, M.M., Calderhead, A.I., Gammon, P.R., Rivera, A., Peru, K.M. and Headley, J.V. (2013) Characterization and quantification of mining-related "naphthenic acids" in groundwater near a major oil sands tailings pond. Environ. Sci. & Technol. 47(10), 5023-5030.

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

Beck, E.M., Smits, J.E.G. and St Clair, C.C. (2015) Evidence of low toxicity of oil sands process-affected water to birds invites re-evaluation of avian protection strategies. Conservation Physiology 3(1).

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

Bressler, D.C. and Fedorak, P.M. (2001) Identification of disulfides from the biodegradation of dibenzothiophene. Applied and Environmental Microbiology 67(11), 5084-5093.

Chelme-Ayala, P., El-Din, M.G., Smith, D.W. and Adams, C.D. (2011) Oxidation kinetics of two pesticides in natural waters by ozonation and ozone combined with hydrogen peroxide. Water Res. 45(8), 2517-2526.

Fafet, A., Kergall, F., Da Silva, M. and Behar, F. (2008) Characterization of acidic compounds in biodegraded oils. Organic Geochemistry 39(8), 1235-1242.

Frank, R.A., Fischer, K., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van der Kraak, G. and Solomon, K.R. (2009) Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environ. Sci. Technol. 43(2), 266-271.

Frank, R.A., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van Der Kraak, G. and Solomon, K.R. (2008) Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72(9), 1309-1314.

Frank, R.A., Roy, J.W., Bickerton, G., Rowland, S.J., Headley, J.V., Scarlett, A.G., West, C.E., Peru, K.M., Parrott, J.L., Conly, F.M. and Hewitt, L.M. (2014a) Profiling oil sands mixtures from industrial developments and natural groundwaters for source identification. Environ. Sci. & Technol. 48(5), 2660-2670.

Frank, R.A., Roy, J.W., Bickerton, G., Rowland, S.J., Headley, J.V., Scarlett, A.G., West, C.E., Peru, K.M., Parrott, J.L., Conly, F.M. and Hewitt, L.M. (2014b) Response to comment on “profiling oil sands mixtures from industrial developments and natural groundwaters for source identification”. Environ. Sci. & Technol. 48(18), 11015-11016.

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

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

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

Gentes, M.-L., Waldner, C., Papp, Z. and Smits, J.E.G. (2007) Effects of exposure to naphthenic acids in tree swallows (Tachycineta bicolor) on the athabasca oil sands, Alberta, Canada. Journal of Toxicology and Environ. Health, Part A 70(14), 1182-1190.

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

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

He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., Gamal El-Din, M., 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 Res. 46(19), 6359-6368.

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 ultrahigh-resolution mass spectrometry. Rapid Communications in Mass Spectrometry 23(4), 515-522.

Headley, J.V., Barrow, M.P., Peru, K.M., Fahlman, B., Frank, R.A., Bickerton, G., McMaster, M.E., Parrott, J. and Hewitt, L.M. (2011) Preliminary fingerprinting of Athabasca oil sands polar organics in environmental samples using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun. Mass Spectrom. 25(13), 1899-1909.

Headley, J.V., Peru, K.M., Fahlman, B., Colodey, A. and McMartin, D.W. (2013) Selective solvent extraction and characterization of the acid extractable fraction of Athabasca oils sands process waters by Orbitrap mass spectrometry. International Journal of Mass Spectrometry 345–347, 104-108.

Herman, D.C., Fedorak, P.M. and Costerton, J.W. (1993) Biodegradation of cycloalkane carboxylic-acids in oil sand tailings. Canadian Journal of Microbiology 39(6), 576-580.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and Gamal El-Din, M. (2014) Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Sci. Total Environ. 493, 282-290.

Jaffé, R. and Gallardo, M.T. (1993) Application of carboxylic acid biomarkers as indicators of biodegradation and migration of crude oils from the Maracaibo Basin, Western Venezuela. Organic Geochemistry 20(7), 973-984.

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. Isme Journal 5(3), 486-496.

Jones, D., Scarlett, A.G., West, C.E., Frank, R.A., Gieleciak, R., Hager, D., Pureveen, J., Tegelaar, E. and Rowland, S.J. (2013) Elemental and

spectroscopic characterization of fractions of an acidic extract of oil sands process water. Chemosphere 93(9), 1655-1664.

Jones, D., Scarlett, A.G., West, C.E. and Rowland, S.J. (2011) Toxicity of individual naphthenic acids to *Vibrio fischeri*. Environ. Sci. Technol. 45(22), 9776-9782.

Keen, O.S., Baik, S., Linden, K.G., Aga, D.S. and Love, N.G. (2012) Enhanced biodegradation of carbamazepine after UV/H_2O_2 advanced oxidation. Environ. Sci. & Technol. 46(11), 6222-6227.

Keen, O.S., McKay, G., Mezyk, S.P., Linden, K.G. and Rosario-Ortiz, F.L. (2014) Identifying the factors that influence the reactivity of effluent organic matter with hydroxyl radicals. Water Res. 50, 408-419.

Kim, S., Stanford, L.A., Rodgers, R.P., Marshall, A.G., Walters, C.C., Qian, K., Wenger, L.M. and Mankiewicz, P. (2005) Microbial alteration of the acidic and neutral polar NSO compounds revealed by Fourier transform ion cyclotron resonance mass spectrometry. Organic Geochemistry 36(8), 1117-1134.

Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and Gamal El-Din, M. (2015) Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. Sci. Total Environ. 506–507(0), 66-75.

Klymenko, N.A., Kozyatnyk, I.P. and Savchyna, L.A. (2010) Removing of fulvic acids by ozonation and biological active carbon filtration. Water Res. 44(18), 5316-5322.

Kropp, K.G., Andersson, J.T. and Fedorak, P.M. (1997) Biotransformations of three Dimethyldibenzothiophenes by pure and mixed bacterial cultures. Environ. Sci. & Technol. 31(5), 1547-1554.

Lai, J.W.S., Pinto, L.J., Bendell-Young, L.I., Moore, M.M. and Kiehlmann, E. (1996) Factors that affect the degradation of naphthenic acids in oil sands wastewater by indigenous microbial communities. Environ. Toxicology and Chemistry 15(9), 1482-1491.

Lamsal, R., Walsh, M.E. and Gagnon, G.A. (2011) Comparison of advanced oxidation processes for the removal of natural organic matter. Water Res. 45(10), 3263-3269.

Liao, Y., Shi, Q., Hsu, C.S., Pan, Y. and Zhang, Y. (2012) Distribution of acids and nitrogen-containing compounds in biodegraded oils of the Liaohe Basin by negative ion ESI FT-ICR MS. Organic Geochemistry 47, 51-65.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. (2006) Mass spectrometric and toxicological assays of Athabasca oil sands naphthenic acids. Water Res. 40(4), 655-664.

Martin, J.W., Barri, T., Han, X.M., Fedorak, P.M., Gamal El-Din, M., Perez, L., Scott, A.C. and Jiang, J.T. (2010) Ozonation of oil sands process-affected

water accelerates microbial bioremediation. Environ. Sci. Technol. 44(21), 8350-8356.

Martin, J.W., Han, X.M., Peru, K.M. and Headley, J.V. (2008) Comparison of high- and low-resolution electrospray ionization mass spectrometry for the analysis of naphthenic acid mixtures in oil sands process water. Rapid Commun. in Mass Spectrometry 22(12), 1919-1924.

Misiti, T., Tezel, U. and Pavlostathis, S.G. (2013) Fate and effect of naphthenic acids on oil refinery activated sludge wastewater treatment systems. Water Res. 47(1), 449-460.

Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W. and Giesy, J.P. (2015) Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. Environ. Sci. Technol. 49(20), 12395-12404.

Morandi, G.D., Zhang, K., Wiseman, S.B., Pereira, A.d.S., Martin, J.W. and Giesy, J.P. (2016) Effect of lipid partitioning on predictions of acute toxicity of oil sands process affected Water to Embryos of Fathead Minnow (Pimephales promelas). Environ. Sci. & Technol.

Noah, M., Poetz, S., Vieth-Hillebrand, A. and Wilkes, H. (2015) Detection of residual oil-sand-derived organic material in developing soils of reclamation sites by ultra-high-resolution mass spectrometry. Environ. Sci. & Technol. 49(11), 6466-6473.

Nothe, T., Fahlenkamp, H. and von Sonntag, C. (2009) Ozonation of wastewater: rate of ozone consumption and hydroxyl radical yield. Environ. Sci. & Technol. 43(15), 5990-5995.

Nyakas, A., Han, J., Peru, K.M., Headley, J.V. and Borchers, C.H. (2013) Comprehensive analysis of oil sands processed water by direct-infusion fourier-transform ion cyclotron resonance mass spectrometry with and without offline UHPLC sample prefractionation. Environ. Sci. Technol. 47(9), 4471-4479.

Peng, H., Sun, J., Alharbi, H.A., Jones, P.D., Giesy, J.P. and Wiseman, S.B. (2016) Peroxisome proliferator-activated receptor γ is a sensitive target for oil sands process-affected water: effects on adipogenesis and identification of ligands. Environ. Sci. & Technol.

Quesnel, D.M., Oldenburg, T.B.P., Larter, S.R., Gieg, L.M. and Chua, G. (2015) Biostimulation of oil sands process-affected water with phosphate yields removal of sulfur-containing organics and detoxification. Environ. Sci. Technol. 49(21), 13012-13020.

Rogers, V.V., Liber, K. and MacKinnon, M.D. (2002) Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48(5), 519-527.

Rowland, S.J., Pereira, A.S., Martin, J.W., Scarlett, A.G., West, C.E., Lengger, S.K., Wilde, M.J., Pureveen, J., Tegelaar, E.W., Frank, R.A. and Hewitt, L.M. (2014) Mass spectral characterization of a polar, esterified fraction of

an organic extract of an oil sands process water. Rapid Commun. in Mass Spectrometry 28(21), 2352-2362.

Scarlett, A.G., Clough, R., West, C., Lewis, C.A., Booth, A.M. and Rowland, S.J. (2011) Alkylnaphthalenes: priority pollutants or minor contributors to the poor health of marine mussels? Environ. Sci. & Technol. 45(14), 6160-6166.

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 93(2), 415-420.

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. Environ. Sci. & Technol. 39(21), 8388-8394.

Shrestha, N., Chilkoor, G., Wilder, J., Gadhamshetty, V. and Stone, J.J. (2017) Potential water resource impacts of hydraulic fracturing from unconventional oil production in the Bakken shale. Water Research.

Shu, Z., Li, C., Belosevic, M., Bolton, J.R. and Gamal El-Din, M. (2014) Application of a solar UV/chlorine advanced oxidation process to oil sands process-affected water remediation. Environ. Sci. Technol. 48(16), 9692-9701.

Smith, B.E., Lewis, C.A., Belt, S.T., Whitby, C. and Rowland, S.J. (2008) Effects of alkyl chain branching on the biotransformation of naphthenic acids. Environ. Sci. & Technol. 42(24), 9323-9328.

Sohrabi, V., Ross, M.S., Martin, J.W. and Barker, J.F. (2013) Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. Chemosphere 93(11), 2698-2703.

Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M., Hagen, M., Tierney, K.B., Belosevic, M. and Gamal El-Din, M. (2014) Advanced analytical mass spectrometric techniques and bioassays to characterize untreated and ozonated oil sands process-affected water. Environ. Sci. Technol. 48(19), 11090-11099.

Tembhekar, P., Padoley, K., Chandra, T., Malik, S., Sharma, A., Gupta, S., Pandey, R. and Mudliar, S. (2015) Environmental Waste Management, pp. 299-339, CRC Press.

Thomas, K.V., Langford, K., Petersen, K., Smith, A.J. and Tollefsen, K.E. (2009) Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in north sea offshore produced water discharges. Environ. Sci. & Technol. 43(21), 8066-8071.

Tollefsen, K.E., Petersen, K. and Rowland, S.J. (2012) Toxicity of synthetic naphthenic acids and mixtures of these to fish liver cells. Environ. Sci. & Technol. 46(9), 5143-5150.

Von Gunten, U. and von Sonntag, C. (2012) The chemistry of ozone in water and wastewater treatment: From basic principles to applications, IWA Publishing, 2012.

Wang, B., Wan, Y., Gao, Y., Zheng, G., Yang, M., Wu, S. and Hu, J. (2015) Occurrences and behaviors of naphthenic acids in a petroleum refinery wastewater treatment plant. Environ. Sci. Technol. 49(9), 5796-5804.

Wang, C., Huang, R., Klamerth, N., Chelme-Ayala, P. and Gamal El-Din, M. (2016) Positive and negative electrospray ionization analyses of the organic fractions in raw and oxidized oil sands process-affected water. Chemosphere 165, 239-247.

Wang, C., Klamerth, N., Messele, S.A., Singh, A., Belosevic, M. and Gamal El-Din, M. (2016) Comparison of UV/hydrogen peroxide, potassium ferrate(VI), and ozone in oxidizing the organic fraction of oil sands process-affected water (OSPW). Water Res. 100, 476-485.

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. Environ. Sci. Technol. 47(12), 6518-6526.

Weltens, R., Deprez, K. and Michiels, L. (2014) Validation of microtox as a first screening tool for waste classification. Waste Manag. 34(12), 2427-2433.

West, C.E., Scarlett, A.G., Tonkin, A., O'Carroll-Fitzpatrick, D., Pureveen, J., Tegelaar, E., Gieleciak, R., Hager, D., Petersen, K., Tollefsen, K.-E. and Rowland, S.J. (2014) Diaromatic sulphur-containing 'naphthenic' acids in process waters. Water Res. 51, 206-215.

Whitby, C. (2010) Microbial naphthenic acid degradation. Advances in Applied Microbiology, Vol 70 70, 93-125.

Xue, J., Zhang, Y., Liu, Y. and Gamal El-Din, M. (2016) Treatment of raw and ozonated oil sands process-affected water under decoupled denitrifying anoxic and nitrifying aerobic conditions: a comparative study. Biodegradation 27(4), 247-264.

Yi, Y., Gibson, J., Birks, J., Han, J. and Borchers, C.H. (2014) Comment on "Profiling oil sands mixtures from industrial developments and natural groundwaters for source identification". Environ. Sci. & Technol. 48(18), 11013-11014.

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J. (2015) Toxicity and composition profiles of solid phase extracts of oil sands process-affected water. Sci. Total Environ. 538, 573-582.

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J.A. (2016) Biodegradation and detoxification of naphthenic acids in oil sands process affected waters. Sci. Total Environ. 572, 273-279.

Zhang, K., Pereira, A.D.S. and Martin, J.W. (2015) Estimates of octanol-water partitioning for thousands of dissolved organic species in oil sands process-affected water. Environ. Sci. & Technol. 49(14), 8907-8913.

Zhang, K., Wiseman, S., Giesy, J.P. and Martin, J.W. (2016) Bioconcentration of dissolved organic compounds from oil sands process-affected water by Medaka (*Oryzias Latipes*): importance of partitioning to phospholipids. Environ. Sci. & Technol. 50(12), 6574-6582.

5 OSPW REMEDIATION BY ADVANCED OXIDATION PROCESSES: OXIDATION KINETICS AND TOXICITY VARIATIONS[4]

5.1 Introduction

The Athabasca oil sands in northern Canada represent the second largest reserve worldwide (Barrow et al. 2010). The extraction of bitumen from oil sands is based on a hot water alkaline extraction process. The generated water, commonly referred to as oil sands process-affected water (OSPW), is stored in tailings containment structures. Reclamation efforts are required to permit the eventual safe integration of the OSPW into the environment. The corresponding decrease of OSPW toxicity with classical naphthenic acids (NAs) reduction is not always correlated (Barrow et al. 2010) that warrants more research to identify the constituents of concern in OSPW (McQueen et al. 2017).

Advanced oxidation processes (AOPs) have been proven to be effective methods for the degradation of organic contaminants in OSPW (Anderson et al. 2011, Pereira et al. 2013, Wang et al. 2013). In particular, ozone and ozone with hydrogen peroxide (peroxone) have been effectively implemented in the oxidation of several refractory compounds in different water matrices. During the ozonation

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processes, both direct and indirect pathways should be significant at pH 7, therefore, both mechanisms should influence the removal of organic compounds (Gottschalk et al. 2010). To predict the impact of several factors on the oxidation processes (Islam et al. 2014) and to grasp more understanding about the mechanisms of removal and the performance (Aghaeinejad-Meybodi et al. 2015), the development of kinetic models will be crucial in the design of the multi-barrier approaches or the treatment trains (Chelme-Ayala et al. 2011). Different models have been developed to monitor the removal efficiency of contaminants in water such as R_{ct} model for O_3 (Elovitz and von Gunten 1999) and $R_{OH,UV}$ model for UV/H_2O_2 (Rosenfeldt and Linden 2007). However, some models are limited and applicable for a certain conditions and certain characteristics of water. Thus, kinetic data are required to initially assess the AOPs, to explore its extent for the degradation of any contaminant, and to optimize the treatment processes in pilot studies (Jin et al. 2012). As O_3 is considered one of the significant emerging technologies in OSPW (El-Din et al. 2011, Martin et al. 2010, Perez-Estrada et al. 2011a, Scott et al. 2008), therefore, the assessment, upgrading and optimization of this technology are warranted. Up till now, few studies have examined the kinetics of ozonation in OSPW (Islam et al. 2014) with more focus on model compounds (Perez-Estrada et al. 2011b) with no reported studies about peroxone. This work can be considered the first comprehensive study to attempt to strengthen this topic and fill the research gap. The main focus of this study is to highlight the differences and similarities between the different peroxone conditions and ozone with different mild and small oxidants doses. Additionally, the reaction kinetics of OSPW NA species was assessed in both O_3 and O_3/H_2O_2 processes. The effect of

the treatment on the degradation efficiency of the O_x-NAs (sum of classical NAs and oxidized NAs) and other species as well as the toxicity effects of the treated water on goldfish primary kidney macrophages (PKMs) function were evaluated. The reaction rate constants for the O_2-NAs and other species were also determined.

5.2 Materials and methods

5.2.1 Raw OSPW and chemicals reagents

OSPW sample was collected in 2014 from one of the oil sand tailings site in Fort McMurray, Alberta, Canada. The water sample was stored at 4°C until further use. Sulfuric acid (H_2SO_4) (95-98% w/w, optima grade dichloromethane (DCM); hydrogen peroxide (H_2O_2) (30% w/w), and catalase of bovine liver (1 mg has 2950 units) were purchased from Fisher Scientific Company (Edmonton, AB, Canada). From Praxair (Edmonton, AB, Canada), ultra-dry oxygen was obtained for the generation of ozone and purging residual ozone.

5.2.2 Oxidation experiments

Different ozone-based AOPs were implemented including: ozone treatments at doses of 30 mg/L (O30) and 50 mg/L (O50); peroxone treatments at different molar ratios: P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3); and P(1:2) (20 mg/L H_2O_2: 50 mg/L O_3). A peroxone (hydrogen peroxide/ozone; H_2O_2:O_3) process using mild-ozone doses of 30 and 50 mg/L was investigated at different H_2O_2:O_3 molar ratios (1:1, 1:2 and 1:3): peroxone (1:1) [20 mg/L H_2O_2 and 30 mg/L utilized O_3 dose], peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose],

peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose] and peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose]. Both utilized ozone doses (30 and 50 mg/L) were applied alone in other treatments to be compared to the peroxone treatments. Ozonation and peroxone experiments were performed separately in a semi batch reactor (4000 mL) at the natural pH of OSPW (8.2 ± 0.1). Detailed procedure for the ozonation experiments can be found in chapter 3 and Appendix A.

5.2.3 Analysis of naphthenic acids and organic species

The NA concentrations was semi-quantified using an ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) system (Waters, Milford, MA) as described in previous chapters.

5.2.4 Toxicity assays using goldfish primary kidney macrophage (PKM)

The isolation and cultivation procedures of primary kidney macrophages (PKM) have been previously described (Neumann et al. 1998, Neumann et al. 2000, Shu et al. 2014). Briefly, goldfish were anesthetized using tricaine methanesulfonate (TMS,40 mg/L solution; Aqua Life, Syndel Laboratories Ltd, Nanaimo BC Canada) and killed by spinal dislocation. Complete medium (C-MGFL-15) was used (supplemented with 10% newborn calf serum (NCS: Hyclone, Loan, UT) and 5% carp serum) for the isolation and cultivation of PKMs. Six day old PKMs were used for nitric oxide bioassay. The 1.5 x 10^5 PKMs were seeded into 96-well plates in 50 µL of complete C-MGFL-15 and exposed to test samples for 18 hrs. After exposure to test samples, PKM were

treated with heat-killed *A. salmonicida* (3.6 * 10^7cfu/well) and incubated at 20^{o}C for 72 h. The negative and positive controls were exposed to 50 µL of C-MGFL-15 and heat killed *A. salmonicida,* respectively. Nitrite production was determined using Griess reaction by adding 1% sulfanilamide and 0.1% N-naphthylethylenediamine to supernatants from the treated cells and nitrite levels were determined calorimetrically at 540 nm and a nitrite standard curve.

5.3 Results and discussion

5.3.1 Degradation

Figure 5.1 depicts the percentage of degradation for the different treatment conditions for the O_2-NAs and O_x-NAs (sum of classical NAs and oxidized NAs) while the percent values of the O_2-NAs, oxidized NAs and O_x-NAs degradations are illustrated in Table 5.1. The order of the O_2-NAs degradation effectiveness from the highest to the lowest was P(1:2) 20+50> O50>O30>P(1:1) 20+30> P(1:3) 10+50> P(1:1) 11+20 with degradation percentage of 86%, 84%, 78%, 76%, 61%, and 47%, respectively. Similar order of the O_x-NAs degradation can be observed as in O_2-NAs, except the O30 and P(1:1) 20+30 (they have same O_x-NAs degradation). The change with time of the O_2-NAs and other oxidized species after the different treatment conditions is provided in Appendix A (Figures A4–A9).

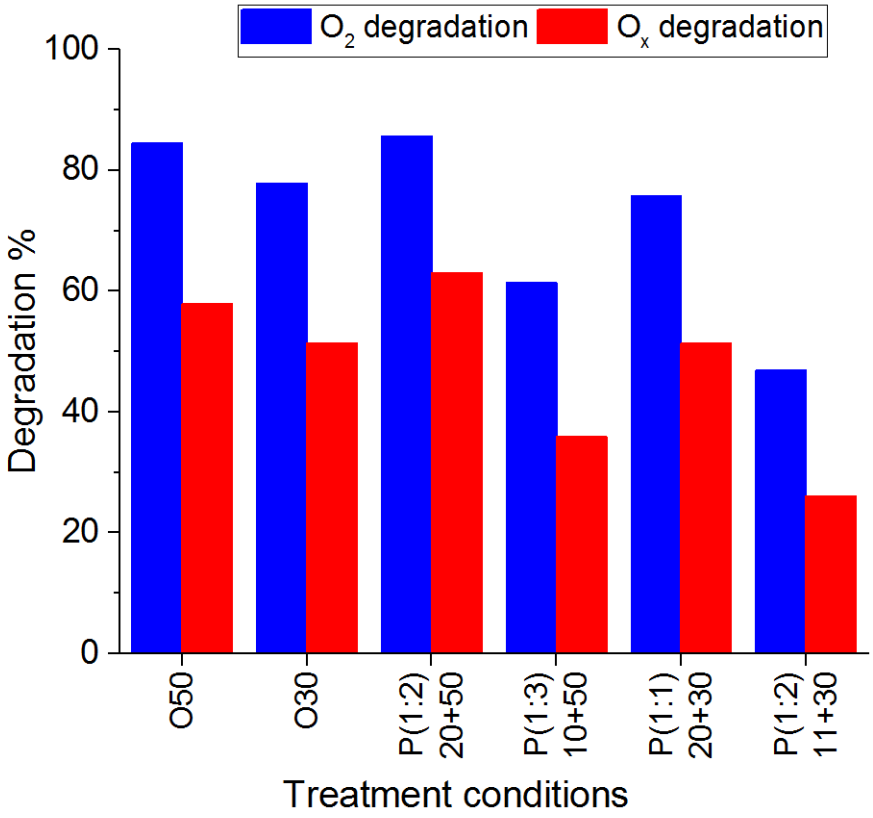

Figure 5.1. Degradation % of the O_2-NA and O_x-NA at different treatment conditions; Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] (P(1:3) 10+50) ; 50 mg/L utilized O_3 dose (O50); Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] (P(1:2) 20+50); and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose] (P(1:2) 11+30).

Table 5.1. Degradation % of the NA species

	O_2-NAs		Oxidized NAs		O_x-NAs or Total-NAs	
Initial conc. of Raw OSPW (mg/L)	33.87		35.74		69.61	
Treatment	**Final conc. (mg/L)**	**Degradation %**	**Final conc. (mg/L)**	**Degradation %**	**Final conc. (mg/L)**	**Degradation %**
O50	5.3	84%	23.1	33%	28.3	59%
O30	7.5	78%	26.4	26%	33.9	51%
P(1:2) 20+50	4.9	86%	20.7	42%	25.5	63%
P(1:1) 20+30	8.2	76%	25.7	28%	33.9	51%
P(1:3) 10+50	13.1	61%	31.6	12%	44.7	36%
P(1:2) 11+30	17.9	47%	33.5	6%	51.5	26%

Notes:

- O_x-NAs or Total-NAs = (O_2-NAs + O_3-NAs + O_4-NAs + O_5-NAs+O_6-NAs).
- Oxidized NAs = (O_3-NAs + O_4-NAs + O_5-NAs + O_6-NAs).
- All ozone doses are utilized O_3 dose.
- Different treatment conditions are designated as follows: 50 mg/L ozone and 30 mg/L ozone (O30) (O50); peroxone treatments at different molar ratios: P(1:2) 20+50 (20 mg/L H_2O_2: 50 mg/L O_3); P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3) ; P(1:3) 10+50 (10 mg/L H_2O_2: 50 mg/L O_3) ; and P(1:2) 11+30 (11 mg/L H_2O_2: 30 mg/L O_3). 10, 11 and 20 mg/L are the initial concentrations of H_2O_2. Residual H_2O_2 concentration in P(1:2) 20+50= 14.8±0.15 mg/L; P(1:1) = 16.4±0.07 mg/L ; P(1:3) 10+50 = 4.71mg/L ; and in P(1:2) 11+30 = 6.92 mg/L.

Figure 5.2 shows the change of the carbon (n) distribution with time while Table 5.2 illustrates the evolution of the degradation of the four treatment conditions with time. The coming discussion highlighted only those three peroxone treatment conditions and the 50 mg/L ozone as a reference and control. With respect to the n number, it can be observed similar pattern in all conditions along the time. Additionally, the fast kinetics and fast evolution of O_2-NAs or rapid changes in concentration was observed at the ozone treatments compared to peroxone treatment conditions. Interestingly, the decrease in the concertation of all n was almost the same. Though uniform reduction can be observed in most treatment conditions, at P(1:3) 10+50, at $12 \leq n \leq 15$ the concentration decreased dramatically after 1 minute then increased again with time.

This can be attributed to the degradation of these classes accompanying with a production of species with lower molecular weights. The carbon distribution in Figures 5.3 to 5.6 depicts the change of carbon distributions in the oxidized NAs at $3< x <6$. These figures can reveal the significance of n=14-16 in all NA species. In brief, the oxidized NAs especially at x=5 and x= 6 were increased with time (i.e., their concentrations increase with time compared to those in raw OSPW). The generation of oxidation by-products was previously reported (Sun et al. 2014).

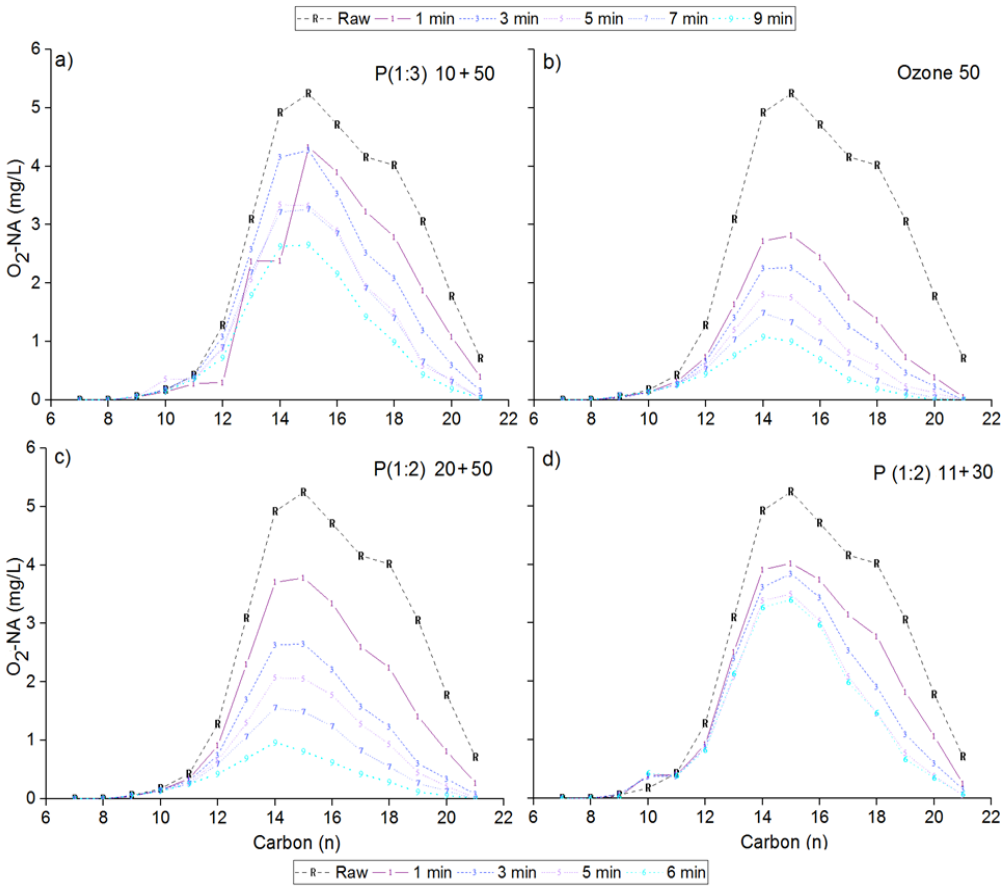

Figure 5.2. Change of the O_2-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to carbon number and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

Table 5.2. Percent degradation of the NA species with time.

Time (min)	Treatment	O_2-NAs	O_3-NAs	O_4-NAs	Oxidized NAs	O_x-NAs or Total-NAs
1	P(1:2) 20+50	35%	-1%	21%	11%	23%
3	P(1:2) 20+50	58%	8%	32%	18%	38%
5	P(1:2) 20+50	67%	13%	36%	21%	43%
7	P(1:2) 20+50	76%	28%	44%	31%	53%
9	P(1:2) 20+50	86%	43%	54%	42%	63%
1	O50	55%	10%	29%	18%	36%
3	O50	65%	17%	34%	23%	43%
5	O50	74%	23%	36%	25%	49%
7	O50	79%	28%	38%	27%	53%
9	O50	84%	35%	43%	33%	59%
1	P(1:3) 10+50	32%	7%	15%	9%	20%
3	P(1:3) 10+50	33%	-17%	4%	-9%	11%
5	P(1:3) 10+50	48%	0%	20%	7%	27%
7	P(1:3) 10+50	49%	-6%	13%	0%	24%
9	P(1:3) 10+50	61%	10%	24%	12%	36%

Time (min)	Treatment	O_2-NAs	O_3-NAs	O_4-NAs	Oxidized NAs	O_x-NAs or Total-NAs
1	P(1:2) 11+30	26%	1%	11%	4%	15%
3	P(1:2) 11+30	37%	0%	15%	5%	21%
5	P(1:2) 11+30	46%	3%	19%	8%	26%
6	P(1:2) 11+30	47%	-3%	17%	6%	26%

Notes:

- O_x-NAs or Total-NAs = (O_2-NAs + O_3-NAs + O_4-NAs + O_5-NAs+O_6-NAs).
- Oxidized NAs = (O_3-NAs + O_4-NAs + O_5-NAs + O_6-NAs).
- All ozone doses are utilized O_3 dose.
- Different treatment conditions are designated as follows: 50 mg/L ozone and 30 mg/L ozone (O30) (O50); peroxone treatments at different molar ratios: P(1:2) 20+50 (20 mg/L H_2O_2: 50 mg/L O_3); P(1:1) (20 mg/L H_2O_2: 30 mg/L O_3) ; P(1:3) 10+50 (10 mg/L H_2O_2: 50 mg/L O_3) ; and P(1:2) 11+30 (11 mg/L H_2O_2: 30 mg/L O_3). 10, 11 and 20 mg/L are the initial concentrations of H_2O_2. Residual H_2O_2 concentration in P(1:2) 20+50= 14.8±0.15 mg/L; P(1:1) = 16.4±0.07 mg/L ; P(1:3) 10+50 = 4.71mg/L ; and in P(1:2) 11+30 = 6.92 mg/L.

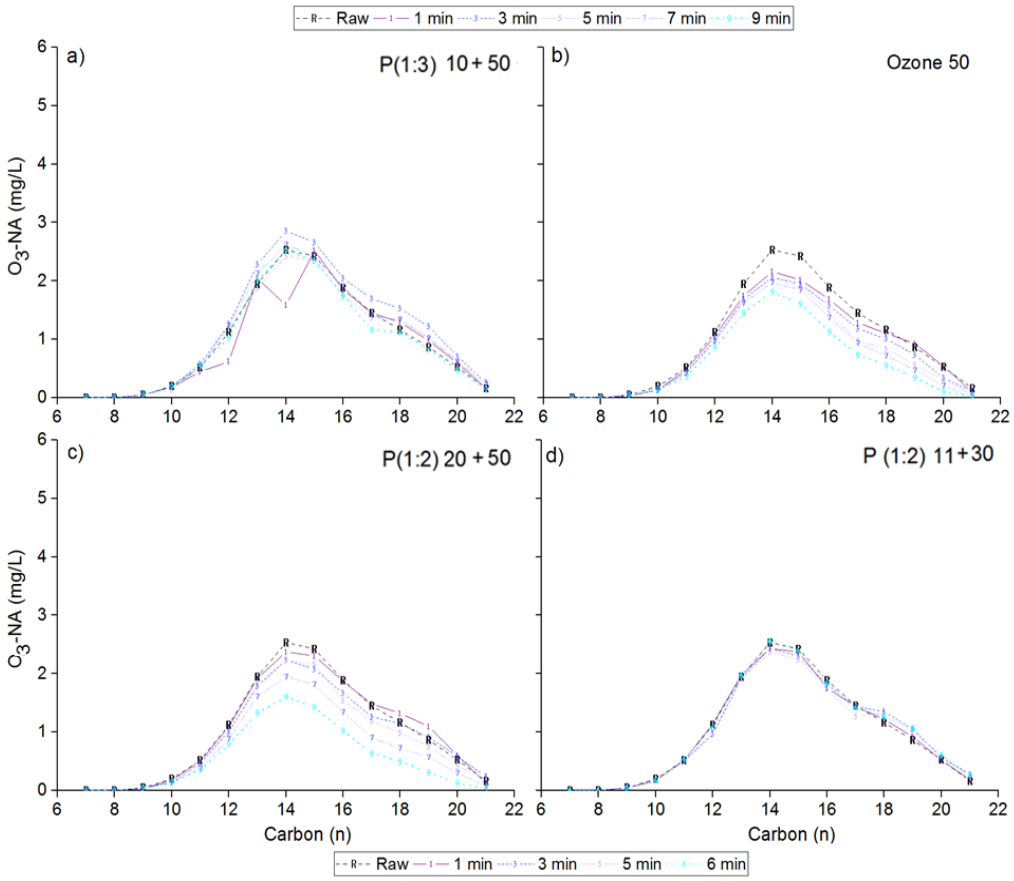

Figure 5.3. Change of the O_3-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to carbon number and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

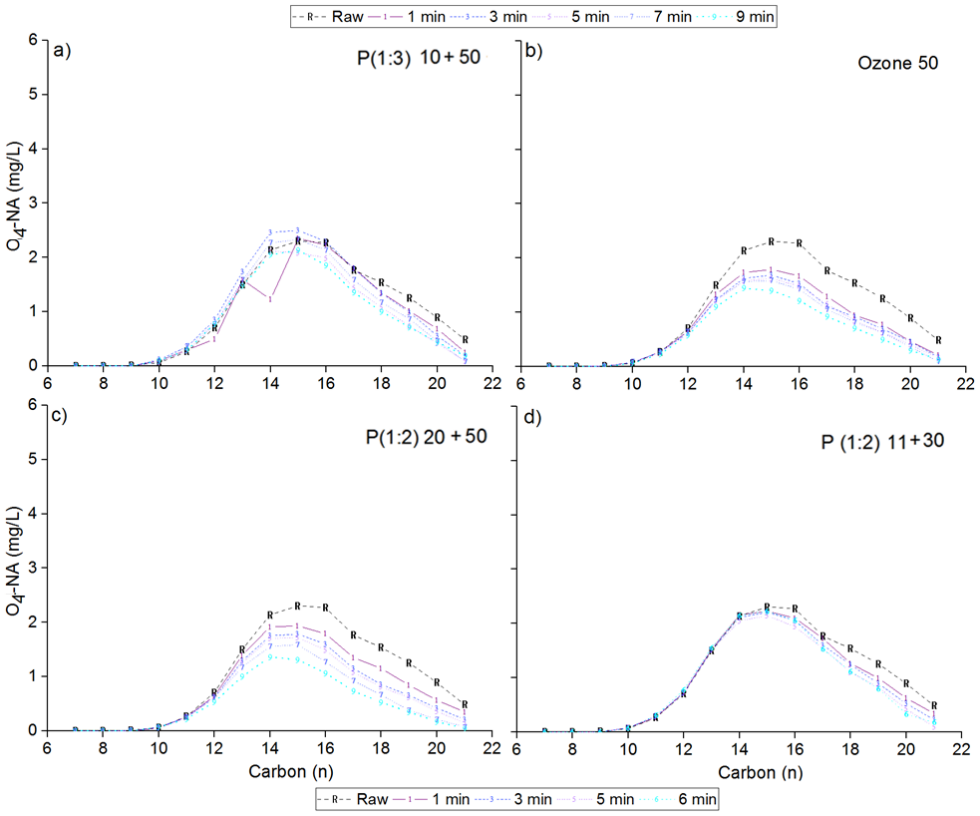

Figure 5.4. Change of the O_4-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to carbon number and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

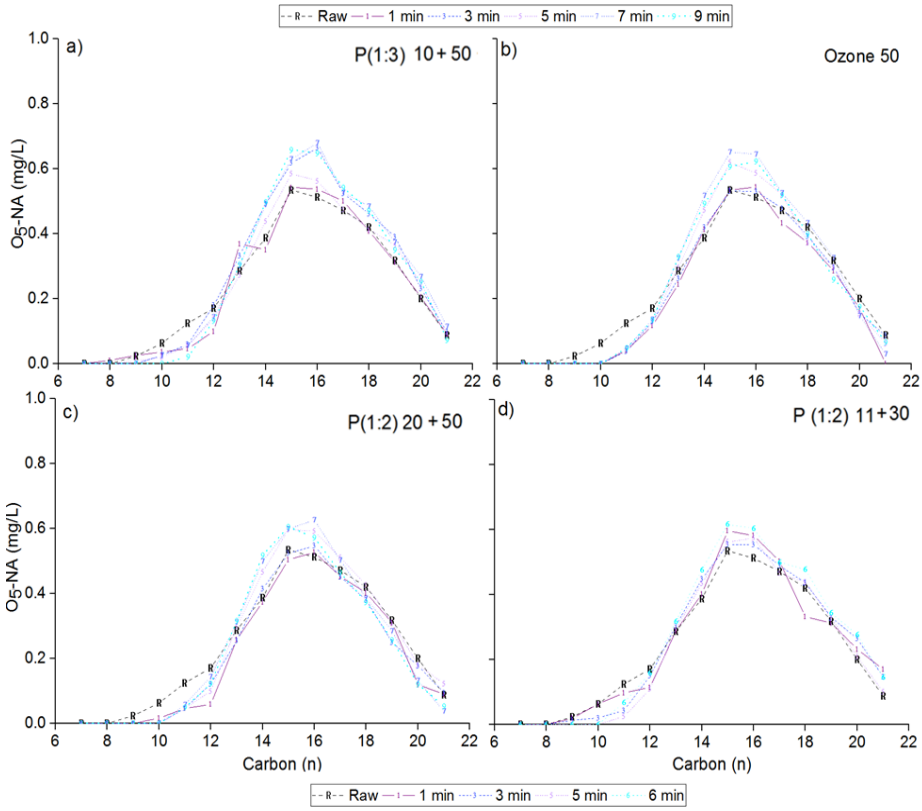

Figure 5.5. Change of the O_5-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to carbon number and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

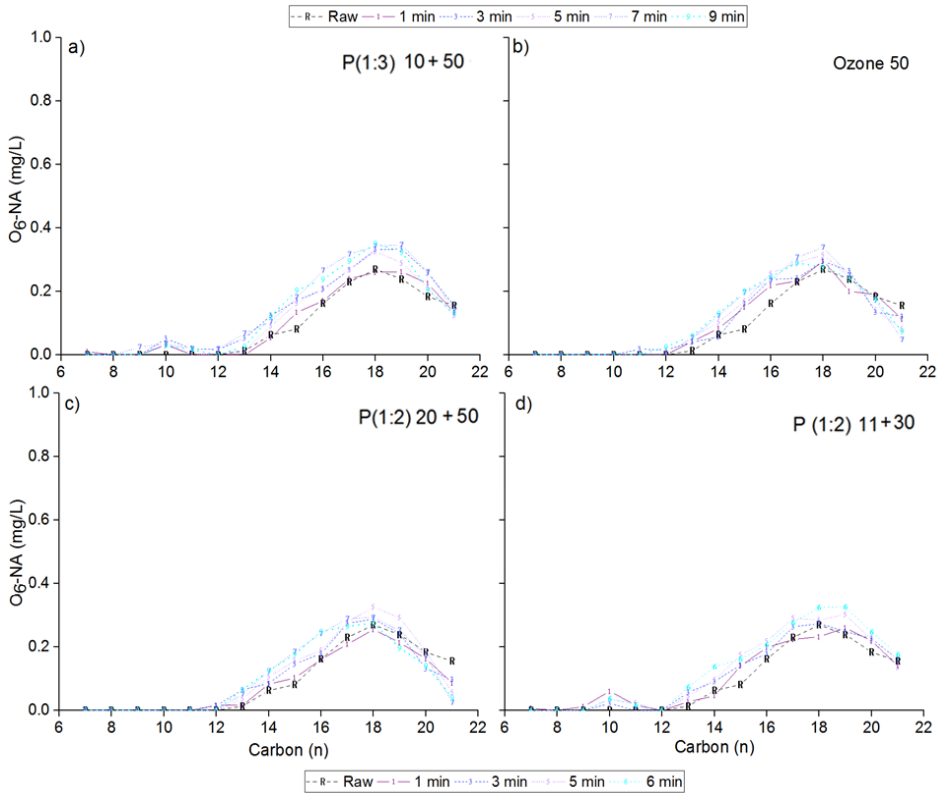

Figure 5.6. Change of the O_6-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to carbon number and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

With respect to the DBE, it can be observed the uniform reduction of the DBE distribution with time in most of the treatments (Figure 5.7). However, in P(1:3) 10+50 some DBE did fluctuate and increased again for instance DBE= 3-5. These types of classes showed their recalcitrance in the previous chapters with the possibility of their generation during oxidation with time. Interestingly, these classes were further degraded and decreased after the minute 5 along until the end of treatment. As illustrated in Figures 5.8-5.11, the change of DBE distributions/patterns in the oxidized NAs at $3< x <6$ was almost uniform with time and most of the distributions were decreasing with time. Nevertheless, exceptions about the increase in O_5 and O_6 with time can suggest the similar findings in n about the production of these species. It is worth to note that the low removal or low degradation of the entire O_x-NAs in some treatments (i.e., (1:3) 10+50 and P(1:1) 11+20) are the reflection of the generation and increase of O_5 and O_6 (Figures 5.10 a,d and 5.11 a,d). On the other hand, the (1:2) peroxone 20+50 treatment is effective in degrading these produced species which explains their better performance and has the best removal of total O_2-NAs, oxidized NAs and O_x-NAs compared to the remaining conditions (Figure 5.11d).

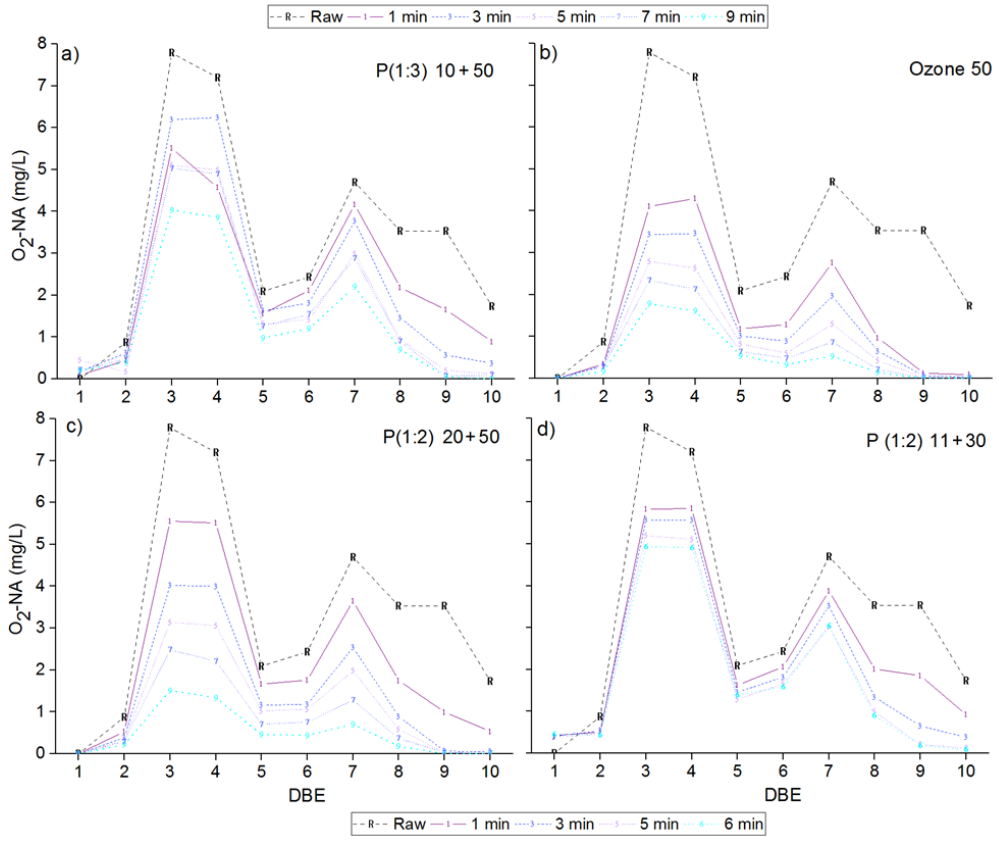

Figure 5.7. Change of the O_2-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to double bond equivalent (DBE) and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

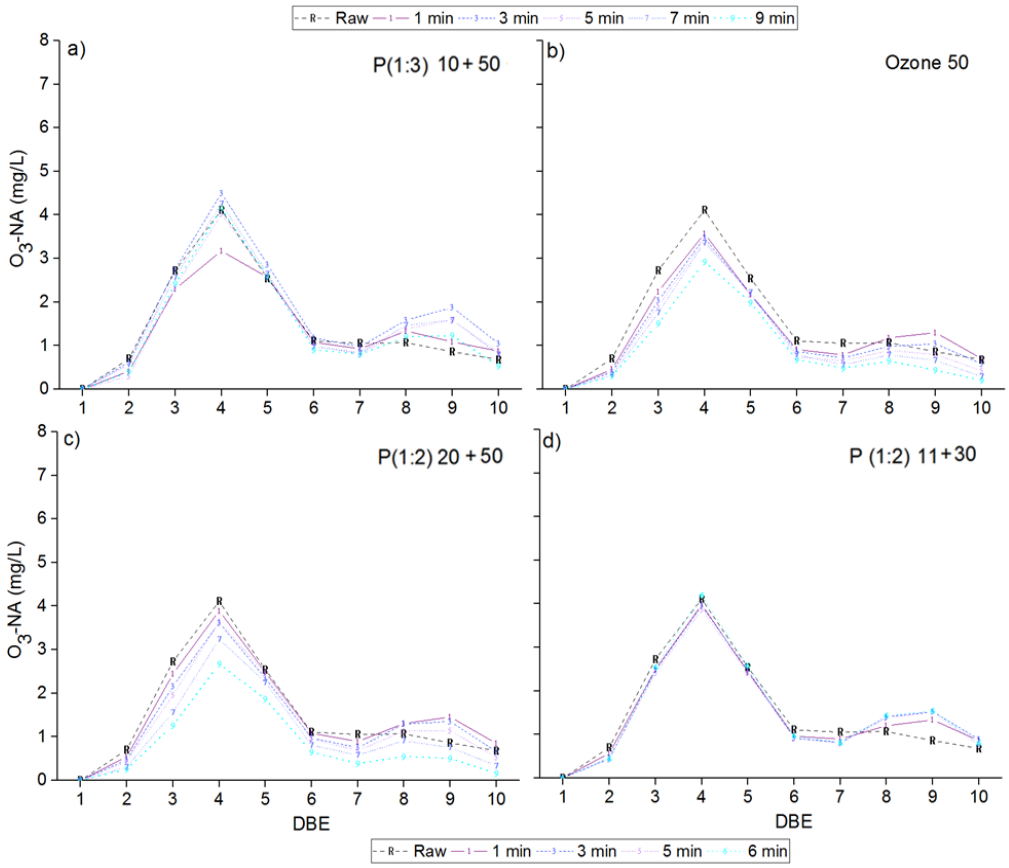

Figure 5.8. Change of the O_3-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to double bond equivalent (DBE) and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

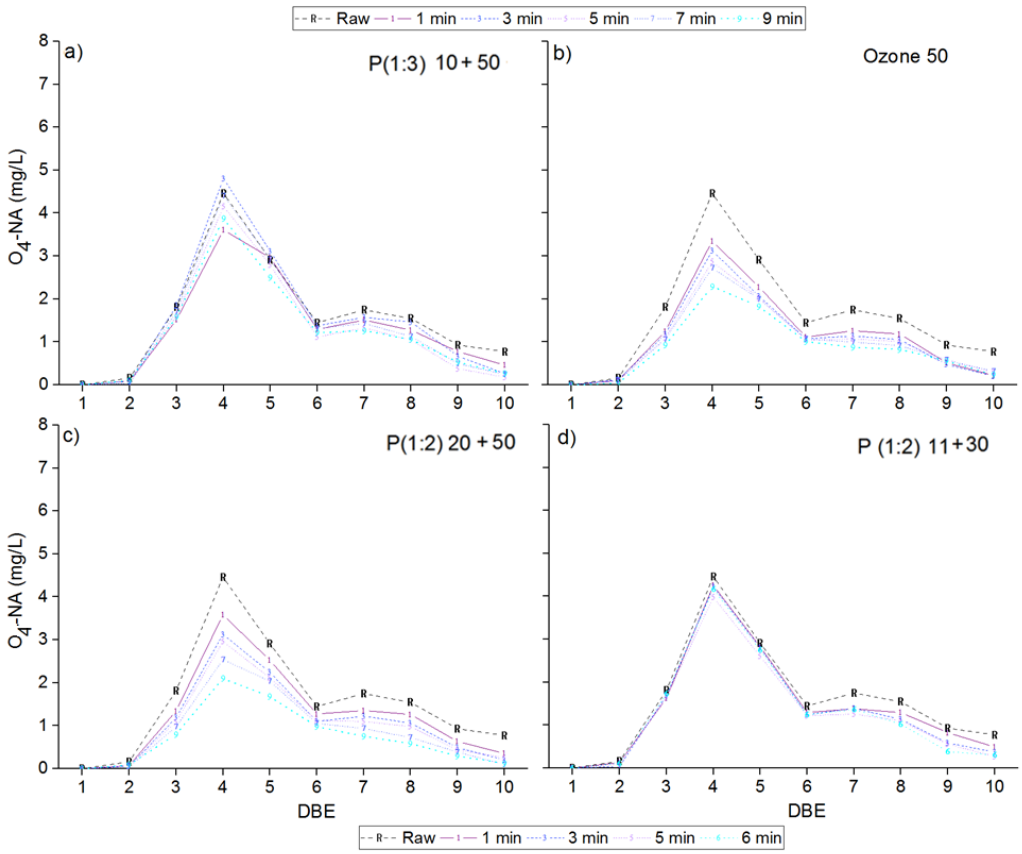

Figure 5.9. Change of the O_4-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to double bond equivalent (DBE) and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

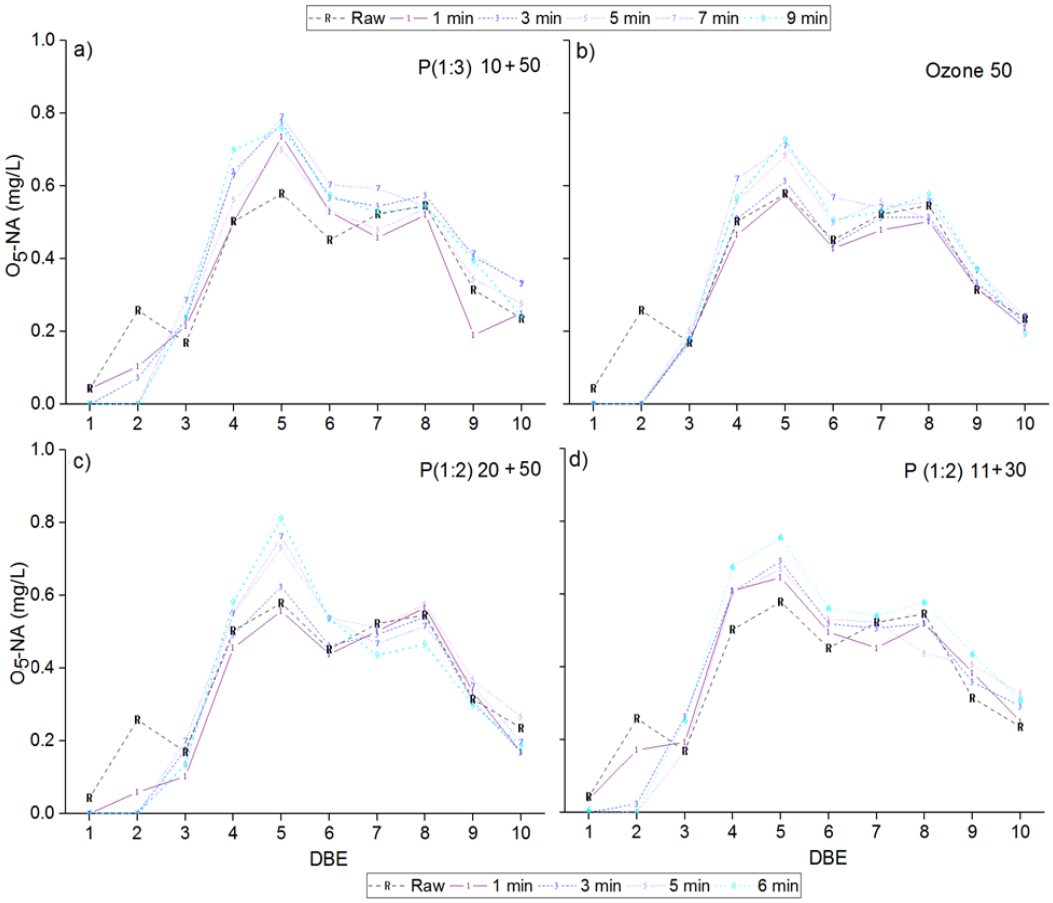

Figure 5.10. Change of the O_5-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to double bond equivalent (DBE) and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

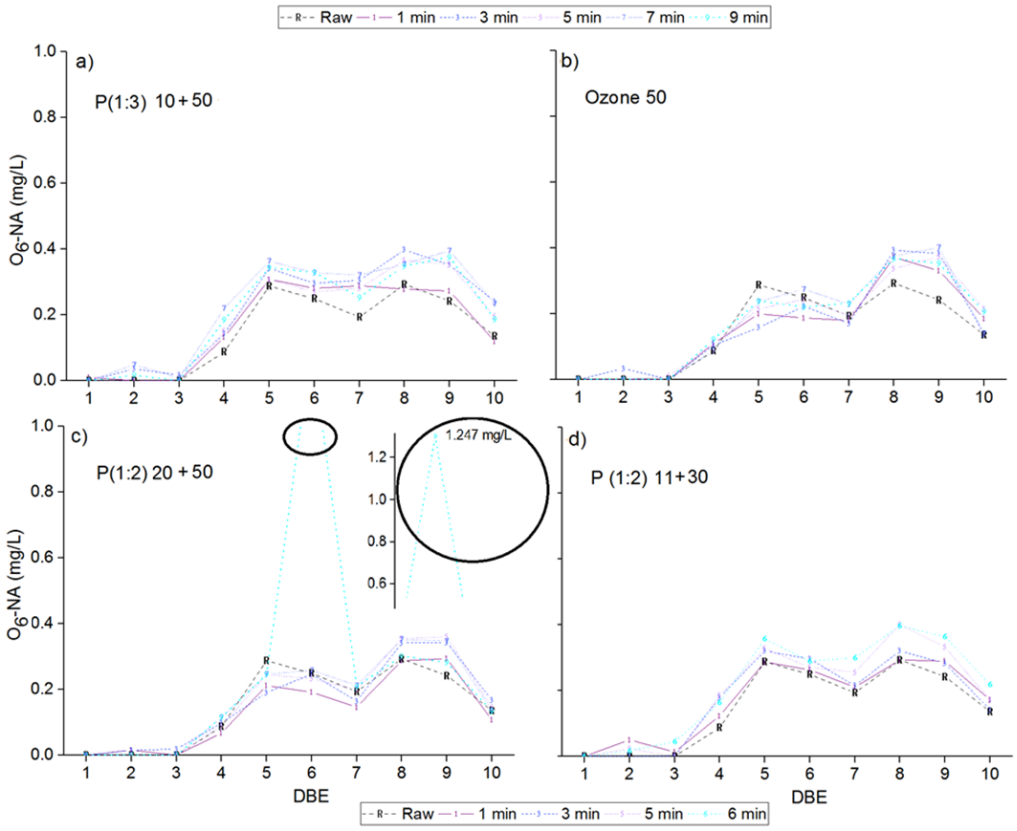

Figure 5.11. Change of the O_6-NA concentrations from raw OSPW, 1 minute, 3 minutes, 5 minutes, 7 minutes, and end of the treatment at 6 or 9 minutes with regards to double bond equivalent (DBE) and treatment ; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

5.3.2 Kinetics

The reaction rate constant values were obtained from the experiments at the different treatment conditions. It is worth to note that the reaction proceeds with linear rate and only depends on the reactant concentration; therefore, it can be assumed to be first-order reaction. Plotting the data for the different treatment conditions using the zero, first order and second order reveals the best fitting of the data to the first rather than second order. Additionally, it was previously assumed that the kinetics of the commercial and OSPW NAs to be second order and first order reactions, respectively (Perez-Estrada et al. 2011b) while the pseudo-first-order reaction was suggested for the structure reactivity of OPSW for both O_2 and oxidized NAs (Islam et al. 2014). The overall pseudo first-order kinetic rate constant (k) can be estimated by two different methods, first using the integral rate law and second with linear regression method. Both methods gave almost similar and close values; however, the linear regression k was slightly lower than the other method. A semi-log plot versus time is illustrated in Figure 5.12 while k values estimated and determined by both the linear regression models (i.e., $\ln C = \ln C_o - kt$) and the experimental calculations for the different treatment conditions are illustrated in Table 5.3.

It is worth to note that the O_2-NAs were the most abundant in the OSPW (Jones et al. 2013) and the recent studies correlated and associated the *Vibrio fischeri* toxicity with O_2-NAs rather than the oxidized species specifically the tricyclic and bicyclic structures of O_2-NAs (Yue et al. 2015). Thus, most of the coming discussions are deeply built upon the O_2-NAs only. As mentioned earlier

in the first chapter, the oxidized species are degraded and produced at the same time which has been reflected in the stable concentrations with time with very small reaction rate constants or k as illustrated in Figure 5.12.

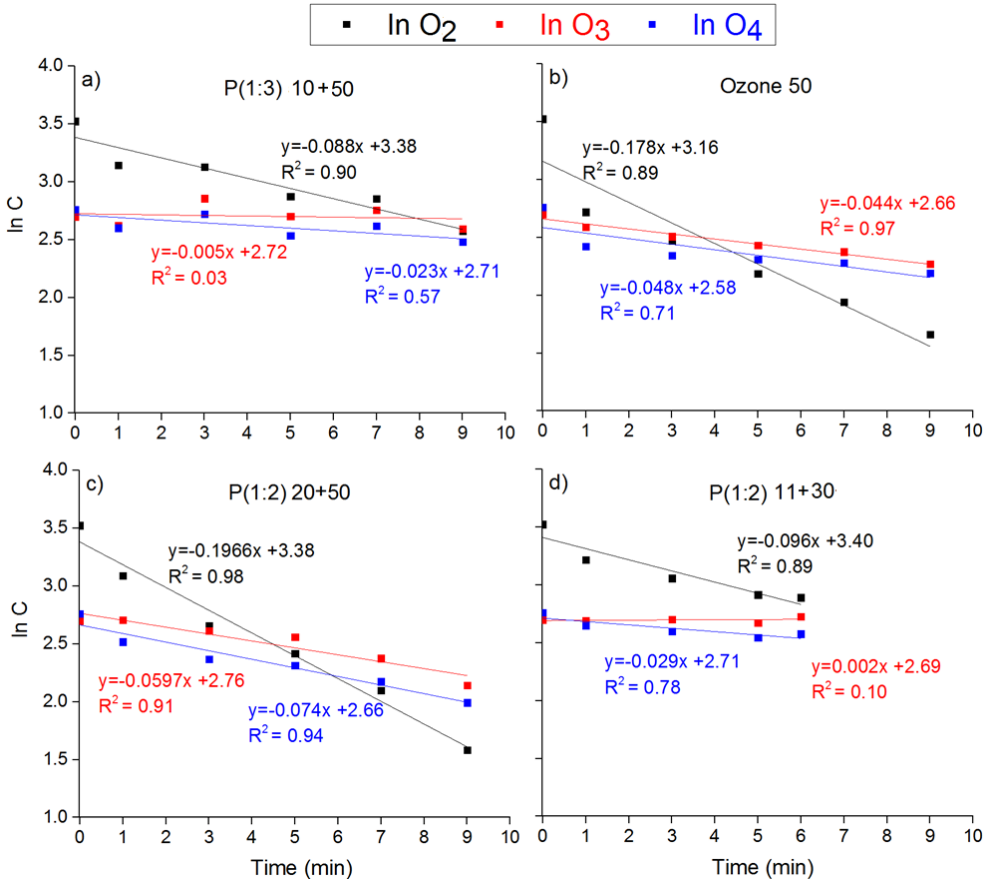

Figure 5.12. Semi-log plot of O_2-NA, O_3-NA and O_4-NA concentrations from with regards to time at different treatment conditions; a) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and d) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

Table 5.3. Reaction rate constants for O_2-NAs.

Treatment conditions	Experimental calculations	Linear regression model		
	k (min^{-1})	Equation	k (min^{-1})	R^2
O50	**-0.210**	y = -0.1802x + 3.1622	-0.180	0.8925
P(1:2) 20+50	**-0.216**	y = -0.1966x + 3.3800	-0.197	0.9766
P(1:3) 10+50	**-0.101**	y = -0.0851x + 3.3764	-0.085	0.8892
P(1:1) 20+30	**-0.236**	y = -0.2154x + 3.4042	-0.215	0.9665
O30	**-0.251**	y = -0.2154x + 3.4042	-0.215	0.8611
P(1:2) 11+30	**-0.106**	y = -0.0966x + 3.4092	-0.097	0.8919
Experimental calculations				
Lines and description	P(1:2) 20+50 k (min^{-1})	Time/Period		
a (average) long period	**-0.216**	0-9 min		
b (initial average) short period	-0.434	0-1 min		
c (end average) short period	-0.258	7-9 min		
Lines and description	O50 k (min^{-1})	Time/Period		
a (average) long period	**-0.210**	0-9 min		
b (initial average) short period	-0.804	0-1 min		
c (end average) short period	-0.156	7-9 min		
Lines and description	P(1:3) 10+50 k (min^{-1})	Time/Period		
a (average) long period	**-0.101**	0-9 min		
b (initial average) short period	-0.380	0-1 min		
c (end average) short period	-0.121	7-9 min		
Lines and description	P(1:2) 11+30 k (min^{-1})	Time/Period		
a (average) long period	**-0.106**	0-6 min		
b (initial average) short period	-0.306	0-1 min		
c (end average) short period	-0.028	5-6 min		

For O_2-NAs O30 and P(1:1) 20+30 were the highest with 0.251 and 0.236 min^{-1}, respectively. The order from the highest value to the lowest value of k are as follows: O30>P(1:1) 20+30> P(1:2) 20+50>O50> P(1:2) 11+30> P(1:3) 10+50, while the corresponding rate constants are 0.251 min^{-1} >0.236 min^{-1} >0.216 min^{-1} >0.210 min^{-1} >0.106 min^{-1} >0.101 min^{-1}, respectively (Table 5.3). Figure 5.12 displays the ln of the O_2-NAs and the oxidized O_3 and O_4 species with time at the four treatment conditions. The k values estimated for O_2-NAs oxidation based on the linear regression model were 0.215 min^{-1}=0.215 min^{-1} >0.197 min^{-1} >0.180 min^{-1} >0.097 min^{-1} >0.086 min^{-1} for O30=P(1:1) 20+30> P(1:2) 20+50>O50> P(1:2) 11+30> P(1:3) 10+50, respectively (Table 5.3).

For P(1:2) 20+50, the O_2-NAs at n=14 had the lowest reaction rate constant while at n=16 had the highest. For O50, n=14, k= 0.168 min^{-1}, n=15, K= 0.184 min^{-1}, n=16, k= 0.21 min^{-1}. These results agree with previous studies that reported the increases of the reactivity with increasing carbon number, while higher n showed the highest degradation (Perez-Estrada et al. 2011b). The k values were 0.225>0.207>0.181 min^{-1} at n=16, n=15, and n=14, respectively (data not shown). With respect to double bond equivalent (DBE), for instance at P(1:2) 20+50, increasing the DBE increases the k as follows: at DBE=3, k= 0.182 min^{-1}, DBE=4, k =0.187 min^{-1}, DBE=5, k=0.167 min^{-1}, DBE=6, k= 0.189 min^{-1}, DBE=7, k= 0.21 min^{-1}, DBE=8, k= 0.33 min^{-1}, and DBE=9, k= 0.65 min^{-1} (Table 5.4).

Table 5.4. Reaction rate constants at different DBE values.

DBE	Treatment conditions	k (min^{-1})	Equation	R^2
3	O50	-0.164	y = 0.3380x	0.5366
	P(1:2) 20+50	-0.182	y = 0.8032x	0.6803
	P(1:3) 10+50	-0.073	y = 1.9554x	0.7332
	P(1:2) 11+30	-0.076	y = 1.7151x	0.7511
4	O50	-0.166	y = 0.3392x	0.9690
	P(1:2) 20+50	-0.187	y = 1.1884x	0.9982
	P(1:3) 10+50	-0.069	y = 3.4164x	0.9628
	P(1:2) 11+30	-0.064	y = 0.8136x	0.9985
5	O50	-0.149	y = 0.3079x	0.9849
	P(1:2) 20+50	-0.169	y = 1.2310x	0.9777
	P(1:3) 10+50	-0.084	y = 3.9538x	0.9641
	P(1:2) 11+30	-0.068	y = 0.788x	0.9969
6	O50	-0.222	y = 0.3025x	0.7342
	P(1:2) 20+50	-0.190	y = 1.1369x	0.9313
	P(1:3) 10+50	-0.078	y = 2.8994x	0.5832
	P(1:2) 11+30	-0.071	y = 0.7033x	0.9828
7	O50	-0.243	y = 0.1719x	0.5923
	P(1:2) 20+50	-0.210	y = 0.7431x	0.8132
	P(1:3) 10+50	-0.084	y = 3.6397x	0.8131
	P(1:2) 11+30	-0.072	y = 0.7228x	0.9858
8	O50	-0.358	y = 0.1665x	0.908
	P(1:2) 20+50	-0.330	y = 0.7216x	0.936
	P(1:3) 10+50	-0.179	y = 4.2739x	0.992
	P(1:2) 11+30	-0.228	y = 0.7310x	0.9979
9	O50	-0.652	y = 0.1715x	0.8321
	P(1:2) 20+50	-0.652	y = 0.7976x	0.7887
	P(1:3) 10+50	-0.469	y = 4.6213x	0.9293
	P(1:2) 11+30	-0.487	y = 0.7516x	0.9928

Similarly, with regard to n at P(1:3) 10+50 , the O_2-NAs at n=16 had the highest reaction rate constant (0.086>0.075>0.069 min^{-1} at n=16, 15, and 14, respectively) which agreed with the findings reported by Perez-Estrada et al. (Perez-Estrada et al. 2011b) and former conditions.

To grasp more insights about the changes of the kinetics with respect to DBE, Figure 5.13 displays the influence of the DBE on the k at the different treatment conditions. The increase of the reaction rate constant is observed with increase the DBE from 7-9; however, the DBE from 3-6 had almost similar values. Table 5.4 shows the reaction rate constant values with the change of the DBE for all treatment conditions. Although the k values had same trend in all conditions, the k values for the P(1:2) 20+50 and O50 were very similar and high while for the other conditions P(1:3) 10+50 and P(1:2) 11+30 the k values were very similar and lower. For instance at P(1:3) 10+50, the values of k increase with increasing DBE from 3 to 9 as follows: DBE=3, k= 0.073 min^{-1}, DBE=4, k=0.069 min^{-1}, DBE=5, k=0.084 min^{-1}, DBE=6, k= 0.078 min^{-1}, DBE=7, k= 0.084 min^{-1}, DBE=8, k= 0.179 min^{-1}, and DBE=9, k= 0.46 min^{-1} (Table 5.4).

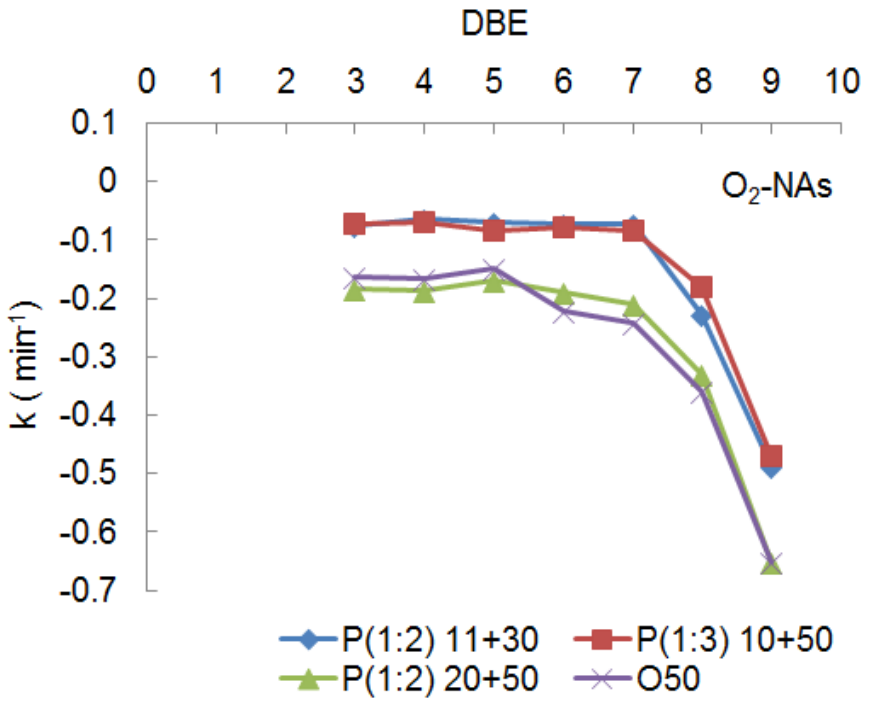

Figure 5.13. Change of the reaction rate constant in min^{-1} with the DBE at different treatment conditions.

5.3.3 Correlations and relative performance in degrading specific classes

With regards to removing and targeting specific structures of O_2-NAs with changes in DBE (2-8) and n (9-16); the correlation between the residual concentrations of each two treatments are shown in Tables 5.5 and 5.6, respectively. For P(1:2) 11+30 and O50, it can be observed that the range of the residual concentrations in O50 were 0.33 time of those reported using P(1:2) 11+30 at DBE=2-5, while the range decreased by increasing the DBE from 5-8 to 0.17. Similar trend was observed for the n numbers while the range decrease from 0.60 at n=9 to 0.26 at n=16.

For P(1:2) 20+50 and O50, it can be observed that the range of the residual concentrations in O50 were 0.7-0.8 times the concentrations by using P(1:2) 20+50 at DBE=6-8 while the range increased at DBE 3-5 to 1.2. These results confirm the effectiveness of P(1:2) 20+50 on O50, especially the most recalcitrant DBE =2-5. Within n=9-16, the range of the residual concentrations in O50 were 1.1 of the P(1:2) 20+50 which confirms the better performance of P(1:2) 20+50 compared to O50.

The preliminary results confirmed the feasibility of adding H_2O_2 with low dose to enhance the ozonation process while targeting specific structures. For instance, the P(1:2) 20+50 and P(1:3) 10+50, always show better effect on the degradation, in particular with low oxidant dose 30 mg/L ozone and 11 mg/L H_2O_2. The range of the of the residual concentrations in P(1:3) 10+50 was 0.7-0.86 of the P(1:2) 11+30. Figure 5.14 displays some examples for the correlation between some treatments at n=14 and DBE=1-10.

Table 5.5. Correlation between the treatment conditions with respect to DBE.

DBE	x	y	Equation	R^2
2	P(1:2) 11+30	O50	y = 0.3380x	0.5366
3	P(1:2) 11+30	O50	y = 0.3392x	0.9690
4	P(1:2) 11+30	O50	y = 0.3079x	0.9849
5	P(1:2) 11+30	O50	y = 0.3025x	0.7342
6	P(1:2) 11+30	O50	y = 0.1719x	0.5923
7	P(1:2) 11+30	O50	y = 0.1665x	0.9080
8	P(1:2) 11+30	O50	y = 0.1715x	0.8321
2	P(1:2) 20+50	O50	y = 0.8032x	0.6803
3	P(1:2) 20+50	O50	y = 1.1884x	0.9982
4	P(1:2) 20+50	O50	y = 1.231x	0.9777
5	P(1:2) 20+50	O50	y = 1.1369x	0.9313
6	P(1:2) 20+50	O50	y = 0.7431x	0.8132
7	P(1:2) 20+50	O50	y = 0.7216x	0.9360
8	P(1:2) 20+50	O50	y = 0.7976x	0.7887
2	P(1:2) 20+50	P(1:2) 11+30	y = 1.9554x	0.7332
3	P(1:2) 20+50	P(1:2) 11+30	y = 3.4164x	0.9628
4	P(1:2) 20+50	P(1:2) 11+30	y = 3.9538x	0.9641
5	P(1:2) 20+50	P(1:2) 11+30	y = 2.8994x	0.5832
6	P(1:2) 20+50	P(1:2) 11+30	y = 3.6397x	0.8131
7	P(1:2) 20+50	P(1:2) 11+30	y = 4.2739x	0.9920
8	P(1:2) 20+50	P(1:2) 11+30	y = 4.6213x	0.9293
2	P(1:2) 11+30	P(1:3) 10+50	y = 0.8608x	0.9780
3	P(1:2) 11+30	P(1:3) 10+50	y = 0.8136x	0.9985
4	P(1:2) 11+30	P(1:3) 10+50	y = 0.788x	0.9969
5	P(1:2) 11+30	P(1:3) 10+50	y = 0.7033x	0.9828
6	P(1:2) 11+30	P(1:3) 10+50	y = 0.7228x	0.9858
7	P(1:2) 11+30	P(1:3) 10+50	y = 0.731x	0.9979
8	P(1:2) 11+30	P(1:3) 10+50	y = 0.7516x	0.9928
2	P(1:2) 20+50	P(1:3) 10+50	y = 1.7151x	0.7511
3	P(1:2) 20+50	P(1:3) 10+50	y = 2.7886x	0.9698
4	P(1:2) 20+50	P(1:3) 10+50	y = 3.1282x	0.9714
5	P(1:2) 20+50	P(1:3) 10+50	y = 2.0448x	0.5771
6	P(1:2) 20+50	P(1:3) 10+50	y = 2.7092x	0.869
7	P(1:2) 20+50	P(1:3) 10+50	y = 3.1291x	0.9941
8	P(1:2) 20+50	P(1:3) 10+50	y = 3.4992x	0.9397

Table 5.6. Correlation between the treatment conditions with respect to carbon number (n).

n	x	y	Equation	R^2
9	P(1:2) 11+30	O50	y = 0.6053x	N/A
10	P(1:2) 11+30	O50	y = 0.0921x	N/A
11	P(1:2) 11+30	O50	y = 0.6036x	0.9179
12	P(1:2) 11+30	O50	y = 0.5011x	0.9869
13	P(1:2) 11+30	O50	y = 0.3403x	0.9852
14	P(1:2) 11+30	O50	y = 0.3162x	0.9750
15	P(1:2) 11+30	O50	y = 0.3107x	0.9950
16	P(1:2) 11+30	O50	y = 0.2575x	0.9315
9	P(1:2) 20+50	O50	y = 1.1619x	0.9454
10	P(1:2) 20+50	O50	y = 1.0786x	0.9748
11	P(1:2) 20+50	O50	y = 1.0239x	0.9962
12	P(1:2) 20+50	O50	y = 1.0941x	0.9841
13	P(1:2) 20+50	O50	y = 1.1623x	0.9915
14	P(1:2) 20+50	O50	y = 1.1692x	0.9908
15	P(1:2) 20+50	O50	y = 1.2978x	0.9718
16	P(1:2) 20+50	O50	y = 1.1308x	0.9679
9	P(1:2) 20+50	P(1:2) 11+30	y = 0.5072x	0.1933
10	P(1:2) 20+50	P(1:2) 11+30	y = 1.2744x	N/A
11	P(1:2) 20+50	P(1:2) 11+30	y = 1.6003x	0.908
12	P(1:2) 20+50	P(1:2) 11+30	y = 2.1587x	0.969
13	P(1:2) 20+50	P(1:2) 11+30	y = 3.3704x	0.9711
14	P(1:2) 20+50	P(1:2) 11+30	y = 3.6479x	0.9798
15	P(1:2) 20+50	P(1:2) 11+30	y = 4.1833x	0.9797
16	P(1:2) 20+50	P(1:2) 11+30	y = 3.7256x	0.9175
9	P(1:2) 11+30	P(1:3) 10+50	y = 1.0595x	0.4309
10	P(1:2) 11+30	P(1:3) 10+50	y = 0.1092x	N/A
11	P(1:2) 11+30	P(1:3) 10+50	y = 0.8874x	0.9445
12	P(1:2) 11+30	P(1:3) 10+50	y = 0.8523x	0.9983
13	P(1:2) 11+30	P(1:3) 10+50	y = 0.8352x	0.9995
14	P(1:2) 11+30	P(1:3) 10+50	y = 0.803x	0.9985
15	P(1:2) 11+30	P(1:3) 10+50	y = 0.7905x	0.9987
16	P(1:2) 11+30	P(1:3) 10+50	y = 0.7314x	0.9994

n	x	y	Equation	R^2
9	P(1:2) 20+50	P(1:3) 10+50	y = 1.1411x	0.591
10	P(1:2) 20+50	P(1:3) 10+50	y = 1.1778x	0.8729
11	P(1:2) 20+50	P(1:3) 10+50	y = 1.437x	0.8851
12	P(1:2) 20+50	P(1:3) 10+50	y = 1.8456x	0.9744
13	P(1:2) 20+50	P(1:3) 10+50	y = 2.821x	0.9762
14	P(1:2) 20+50	P(1:3) 10+50	y = 2.9251x	0.9736
15	P(1:2) 20+50	P(1:3) 10+50	y = 3.3104x	0.9818
16	P(1:2) 20+50	P(1:3) 10+50	y = 3.1527x	0.9693

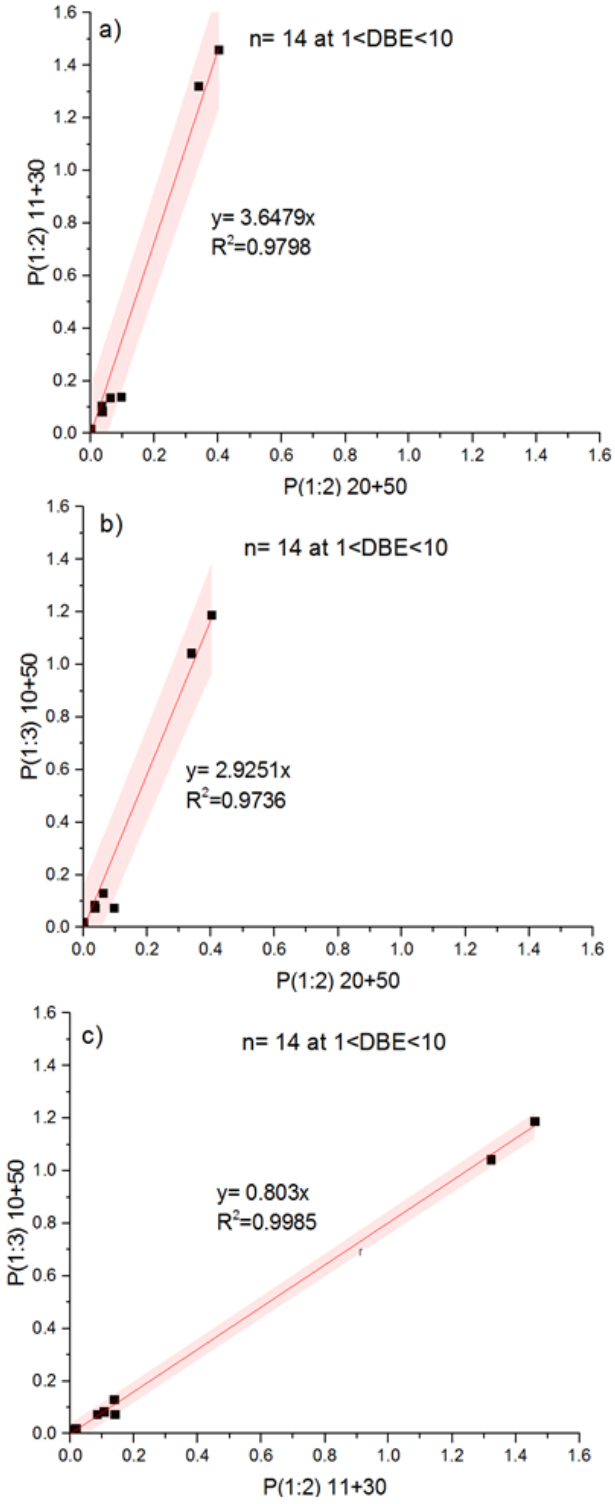

Figure 5.14. Correlation between the different treatment conditions at n=14 and DBE=1-10.

5.3.4 Metal Removal

The trace/heavy metals in OSPW samples might reveal toxicity (Allen 2008, Wang et al. 2015) or low toxicological effects (Beck et al. 2015). The elements of interest that might have toxicity impact are arsenic (As), chromium (Cr), nickel (Ni), zinc (Zn), lead (Pb), copper (Cu) and cadmium (Cd) (Allen 2008, Beck et al. 2015, Hagen et al. 2014). In addition, chromium existence might produce teratogenic effects (Beck et al. 2015). In particular, the chromium hexavalent compounds have been confirmed to be highly toxic on the inhibition of estrogen receptors (Guével et al. 2000). Figure 5.15 shows the removal of the Cr hexavalent in terms of total Cr in mg/L while Table 5.7 shows the removal of Ni and Cr in all treatment conditions. The peroxone treatments did achieve similar % removal with a range of 75.2% to 88.4% compared to O50 of 92%. Fortunately, Ni was removed with 84.1% at P(1:2) 20+50 compared to very low removal in P(1:2) 11+30. Most of the remaining metals still have some traces after treatment; however, an enhanced decrease can be seen in Cr and slight decrease in Ni with some conditions. It was reported the convenience of the new biotic ligand model to examine the metal toxicity; however, more research is need to investigate the metal toxicity for OSPW (Hagen 2013).

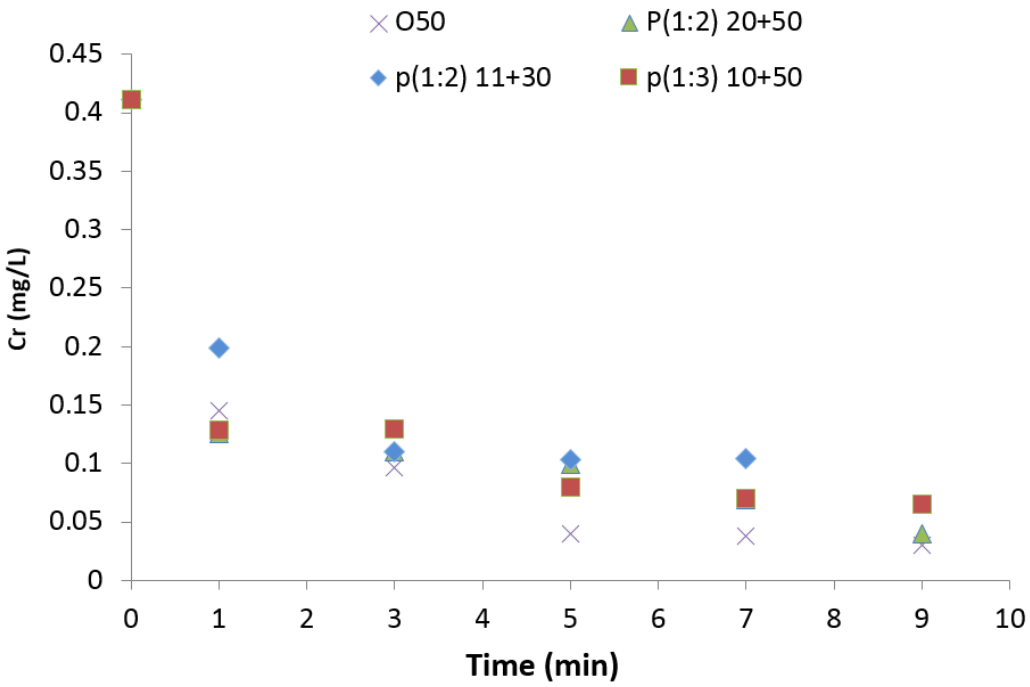

Figure 5.15. Change of the chromium (Cr) in mg/L with time at the different treatment conditions.

Table 5.7. Percent removal of nickel (Ni) and chromium (Cr).

Initial conc. in (mg/L) of Raw OSPW	**Ni**		**Cr**	
	1.30		0.378	
Treatment	**Final conc. (mg/L)**	**Degradation %**	**Final conc. (mg/L)**	**Degradation %**
O50	0.96	26%	0.03±0.03	92%
P(1:2) 20+50	0.20	84.1%	0.04±0.04	88.4%
P(1:3) 10+50	0.82	36.6%	0.07±0.03	83%
P(1:2) 11+30	1.29	0.8%	0.10±0.05	75.2%

5.3.5 Species detected in positive ionization mode

The species detected in positive ionization mode were previously correlated with toxicity (Morandi et al. 2015). Unfortunately, most of the positive species were increased after the oxidation, except slight decrease in the relative abundance of the O_2^+ from 100% in raw to 72% after P(1:2) 20+50. All variations of the positive species measured in the UPLC-TOFMS are described in Table 5.8. Due to limited scope of the current study, a parallel report is undergoing for analysis other analysis such as FTICR-MS.

Table 5.8. Variations of the relative abundance of the different species in the positive mode using UPLC-TOFMS; the raw abundance is 100%; a value lower than 100 % means decrease and removal, otherwise it means the species has been generated and increased.

	Relative Abundance		**Relative Abundance**	
Treatment /Species	**O_2^+**	**O_4^+**	**O_3^+**	**O_5^+**
O50	84%	118%	107%	150%
P(1:2) 20+50	72%	137%	93%	137%
P(1:3) 10+50	125%	146%	178%	215%
P(1:2) 11+30	126%	130%	191%	179%

5.3.6 Toxicity assays

The toxicological effects of all treatment conditions were investigated towards the goldfish primary kidney macrophages (PKMs) using an indicative to evaluate either the enhancement or impairment of PKM antimicrobial response. The nitrite production for all samples was measured and compared to control samples through assessing the ability of PKM to generate a nitric oxide response. The high functional activity of PKMs is observed by high production of nitrite which can be attributed as low toxicity effects from the sample. Figure 5.16 depicts the nitrite production by primary PKMs exposed to medium control. Compared to nitrite production of raw OSPW (i.e., 7.9 ± 2.2 μM), all treatment conditions did reduce the toxicity with significant differences. From the lowest nitrite production to the highest, the order was P(1:2) 20+50<=O50< P(1:3) 10+50< P(1:2) 11+30 with nitrite production of 16.3 ± 1.8 μM <16.5 ± 0.9 μM <21.5.3 ± 1.9 μM <38.1± 2.1 μM, respectively. The significant reduction of toxicity corresponding to the highest nitrite production was accomplished in P(1:2) 11+30. Previous studies using same indicative showed slight reduction with high ozone dose (i.e., 96 mg/L ozone) (Wang et al. 2016). The authors reported 26.5 ± 2.7 μM nitrite at 96 mg/L ozone compared to 9.2 ± 1.2 μM in raw OSPW which confirms the significance of peroxone treatment approach for OSPW remediation.

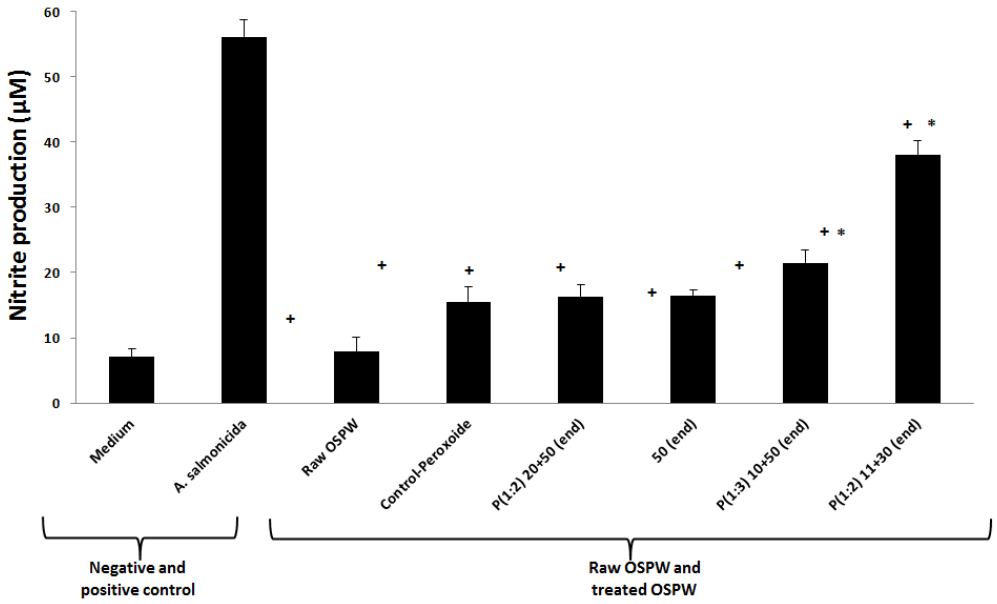

Figure 5.16. Nitrite production by primary kidney macrophages (PKMs) exposed to medium control, raw OSPW and treated OSPW using 50 mg/L utilized O_3 dose; peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose]; peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and e) peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

5.4 Conclusions

The research described here highlighted the different peroxone conditions that can be applied with different ratios and still compete with higher ozone doses. The effective treatment in O_2-NAs degradation was observed at P(1:2) 20+50, followed by O50, O30, P(1:1) 20+30, P(1:3) 10+50, and P(1:2)11+30 with percent degradations of 86%, 84%, 78%, 76%, 61%, and 47%, respectively. Similar trend was observed in the degradation of O_x-NAs. As well, in the current study, the evolution of the NA degradation with time and the reaction rate

constant were estimated based on the experimental calculations, the integral rate law as well as linear regression models. The increase of the DBE increased the reaction rate constant, specifically at DBE = 7-9 with similar values at DBE =3-6. In terms of toxicity, the highest production of nitrite which can be attributed as the lowest toxicity effects from the sample on the PKMs was observed in P(1:2) 11+30 warranting more research for the conditions of low oxidants doses even lowest removal. These findings specify that the complete removal of acidic species (NAs) in OSPW might not be a specific treatment goal. However, reducing the NA concentrations of 40-60% with limited oxidant doses (economically or minimal doses) can be significant in reducing the toxicity input from these species. These findings indicate that the entire OSPW matrix, including the inorganic species, non-acidic species (i.e., not NAs and detected species in positive ionization mode) and byproducts formed during oxidation might have significant impacts on the OSPW toxicity.

5.5 References

Aghaeinejad-Meybodi, A., Ebadi, A., Shafiei, S., Khataee, A.R. and Rostampour, M. (2015) Modeling and optimization of antidepressant drug Fluoxetine removal in aqueous media by ozone/H_2O_2 process: Comparison of central composite design and artificial neural network approaches. Journal of the Taiwan Institute of Chemical Engineers 48(0), 40-48.

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. (2011) Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to *Chironomus dilutus*. Environ. Sci. Technol. 46(1), 486-493.

Barrow, M.P., Witt, M., Headley, J.V. and Peru, K.M. (2010) Athabasca oil sands process water: characterization by atmospheric pressure photoionization and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Analytical Chemistry 82(9), 3727-3735.

Beck, E.M., Smits, J.E.G. and St Clair, C.C. (2015) Evidence of low toxicity of oil sands process-affected water to birds invites re-evaluation of avian protection strategies. Conservation Physiology 3(1).

Chelme-Ayala, P., El-Din, M.G., Smith, D.W. and Adams, C.D. (2011) Oxidation kinetics of two pesticides in natural waters by ozonation and ozone combined with hydrogen peroxide. Water Res. 45(8), 2517-2526.

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

Elovitz, M.S. and von Gunten, U. (1999) Hydroxyl radical/ozone ratios during ozonation processes. I. The R_{ct} concept. Ozone: Sci. & Engin. 21(3), 239-260.

Gottschalk, C., Libra, J. and Sau, A. (2010) Ozonation of Water and waste water : A practical guide to understanding ozone and its applications, Wiley-VCH.

Guével, R.L., Petit, F.G., Goff, P.L., Métivier, R., Valotaire, Y. and Pakdel, F. (2000) Inhibition of rainbow trout (Oncorhynchus Mykiss) estrogen receptor activity by cadmium. Biology of Reproduction 63(1), 259-266.

Hagen, M.O. (2013) Analysis of goldfish innate immunity following exposure to oil sands process affected water , Dissertation , University of Alberta, Edmonton, Alberta, Canada.

Hagen, M.O., Katzenback, B.A., Islam, M.D.S., Gamal El-Din, M. and Belosevic, M. (2014) The Analysis of Goldfish (Carassius auratus L.) Innate Immune

Responses After Acute and Subchronic Exposures to Oil Sands Process-Affected Water. Toxicological Sciences 138(1), 59-68.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and Gamal El-Din, M. (2014) Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Sci. Total Environ. 493, 282-290.

Jin, X., Peldszus, S. and Huck, P.M. (2012) Reaction kinetics of selected micropollutants in ozonation and advanced oxidation processes. Water Research 46(19), 6519-6530.

Jones, D., Scarlett, A.G., West, C.E., Frank, R.A., Gieleciak, R., Hager, D., Pureveen, J., Tegelaar, E. and Rowland, S.J. (2013) Elemental and spectroscopic characterization of fractions of an acidic extract of oil sands process water. Chemosphere 93(9), 1655-1664.

Martin, J.W., Barri, T., Han, X.M., Fedorak, P.M., El-Din, M.G., Perez, L., Scott, A.C. and Jiang, J.T. (2010) Ozonation of Oil Sands Process-Affected Water Accelerates Microbial Bioremediation. Environ. Sci. Technol.44(21), 8350-8356.

McQueen, A.D., Kinley, C.M., Hendrikse, M., Gaspari, D.P., Calomeni, A.J., Iwinski, K.J., Castle, J.W., Haakensen, M.C., Peru, K.M., Headley, J.V. and Rodgers Jr, J.H. (2017) A risk-based approach for identifying constituents of concern in oil sands process-affected water from the Athabasca Oil Sands region. Chemosphere 173, 340-350.

Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W. and Giesy, J.P. (2015) Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. Environ. Sci. Technol. 49(20), 12395-12404.

Pereira, A.S., Islam, M.D.S., Gamal El-Din, M. 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 Commun. Mass Spectrom. 27(21), 2317-2326.

Perez-Estrada, L.A., Han, X.M., Drzewicz, P., El-Din, M.G., Fedorak, P.M. and Martin, J.W. (2011) Structure-reactivity of naphthenic acids in the ozonation process. Environ. Sci. Technol. 45(17), 7431-7437.

Rosenfeldt, E.J. and Linden, K.G. (2007) The R-OH,R-UV concept to characterize and the model UV/H_2O_2 process in natural waters. Environ. Sci. Technol. 41(7), 2548-2553.

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.

Sun, N., Chelme-Ayala, P., Klamerth, N., McPhedran, K.N., Islam, M.S., Perez-Estrada, L., Drzewicz, P., Blunt, B.J., Reichert, M., Hagen, M., Tierney, K.B., Belosevic, M. and Gamal El-Din, M. (2014) Advanced analytical mass spectrometric techniques and bioassays to characterize untreated and

ozonated oil sands process-affected water. Environ. Sci. Technol. 48(19), 11090-11099.

Wang, C., Alpatova, A., McPhedran, K.N. and Gamal El-Din, M. (2015) Coagulation/flocculation process with polyaluminum chloride for the remediation of oil sands process-affected water: Performance and mechanism study. J. of Environ.l Management (0).

Wang, C., Klamerth, N., Messele, S.A., Singh, A., Belosevic, M. and Gamal El-Din, M. (2016) Comparison of UV/hydrogen peroxide, potassium ferrate(VI), and ozone in oxidizing the organic fraction of oil sands process-affected water (OSPW). Water Res. 100, 476-485.

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

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J. (2015) Toxicity and composition profiles of solid phase extracts of oil sands process-affected water. Sci. Total Environ. 538, 573-582.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Thesis overview

Oil sands process-affected water (OSPW) has been generated for years from the oil sands industry as a result of the extraction of the bitumen. The zero discharge policy, the vast amount of stored process water, the potential contamination of surface water and groundwater through either leaching of naphthenic acids (NAs) and other compounds of concern or filtration from the tailings ponds, as well as the different toxic impacts of OSPW to aquatic organisms; warrant the development of sustainable remediation approaches. Albeit several techniques including adsorption, coagulation and flocculation, membrane filtration, natural biodegradation by indigenous microorganisms, oxidation using solar UV-driven, and other treatment methods have been tested, the complexity of the OSPW matrix requires the application of multi-barrier approaches to allow the detoxification and decontamination of OSPW in a reasonable period of time. Additionally, a huge challenge facing the oil sands industry is the characterization of OSPW itself because hundreds of organic compounds present in OSPW cannot be easily separated. Moreover, the lack of understanding of the fate of NAs and other organic species as well as their recalcitrance has hindered the establishment of specific guidelines for OSPW discharge. The main objective of OSPW management is, therefore, to integrate the OSPW into the environment through reclamation efforts, aiming to protect both the environment and human health. To do so, attention should be given to

multi-barrier remediation approaches such as advanced oxidation processes (AOPs) combined with biological treatment. To evaluate the performance of these remediation approaches, markers and indicators for the removal of the contaminants of concerns should be employed. These markers might pave the ground to establish eventual guidelines for specific compounds/structures and distinct surrogate parameters to allow the safe return of the treated OSPW into receiving environments.

Ozonation (O_3) has shown its effectiveness in degrading OSPW NAs with partial or complete toxicity reduction to *Vibrio fischeri*. However, previous studies used high ozone doses from 20 mg/L to 360 mg/L, which negatively reduced the degradation efficiency, especially at doses higher than 100 mg/L O_3. Clearly, the optimization of the ozonation process is warranted given the high costs of ozonation, coupled with the inefficient degradation at high doses, which may limit the feasibility of its use in large-scale industrial applications. Therefore, the focus of this research was to examine the applicability of the peroxone process for OSPW treatment by introducing hydrogen peroxide (H_2O_2) in the ozonation process as an AOP. The study employed both ozone and peroxone processes at different treatment conditions. The differences in distributions of different NAs and other species after oxidation were examined and compared to raw OSPW using different analytical methods such as ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS) and Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). Moreover, the characterization of the NAs present not only in OSPW but also in groundwater

(GW) samples was investigated using two different methods, namely UPLC-TOFMS and Fourier transformation infrared (FTIR) spectroscopy, and two different extraction methods (i.e., liquid–liquid extraction (LLE) and solid-phase extraction (SPE)).

6.2 Conclusions

The main conclusions drawn from this research based on the different experiments are as follows:

1. Higher recovery of O_x-NAs and NAFCs/ or AEF in SPE was achieved compared to LLE (i.e., 1.0-1.4 fold high in SPE based on its less selectivity) in all samples, regardless the water source (OSPW or GW) and quantification methods (i.e., FTIR and UPLC-TOFMS).
2. Similar concentrations of O_2 species were observed in both LLE and SPE with higher abundance of O_2 species in LLE (e.g., in the three OSPW samples, (63.1±2.1%) in LLE compared to (58.5±3.0%) in SPE). The increase of O_2 species abundance using LLE was due to the high impact of the hydrophobicity in which the conveyance of acids from water to DCM or the solvent increased.
3. Compared with OSPW extract, higher abundance was observed in commercial Fluka NAs with lower double bound equivalent (DBE) and lower carbon number (n) for O_2 species. On the other hand,

higher abundance with higher DBE and higher n was observed in the OSPW extract.

4. A strong correlation was confirmed between the FTIR and UPLC-TOFMS that highlights the possibility of using FTIR-Fluka with LLE pre-treated samples as an alternative to the high resolution analysis (UPLC-TOFMS) and a better surrogate parameter and preliminary tool to monitor the total NA concentrations in different water matrices at different concentration levels.
5. In terms of treatment, O_x-NAs (classical (O_2-NAs) + oxidized NAs) degradation efficiency improved from 58% at 30 mg/L ozone to 59%, 63% and 76% at peroxone (1:1) or (20 mg/L H_2O_2: 30 mg/L O_3), 50 mg/L ozone, and peroxone (1:2) or (20 mg/L H_2O_2: 50 mg/L O_3), respectively.
6. While adding tert-butyl alcohol suppressed the hydroxyl radical **(**•OH) pathway and significantly reduced the degradation in all treatments, the molecular ozone contributed to 50% and 35% of the degradation of O_2-NAs and O_x-NAs, respectively.
7. Taking into consideration the combined effect of n and Z, the degradation pathway for $|-Z|\geq 10$ species in the ozone treatments through molecular ozone was significant compared to •OH.
8. The peroxone (1:2) highly reduced the fluorophore organics and toxicity toward *Vibrio fischeri.*

9. The optimum utilization of the oxidant in the degradation of O_2-NAs (mg/L) per ozone dose (mg/L) was observed in the peroxone (1:1) (20 mg/L H_2O_2: 30 mg/L O_3) (0.91) and 30 mg/L ozone treatments (0.92).
10. At specific classes, such as n = 9-11, the peroxone (1:1) (20 mg/L H_2O_2: 30 mg/L O_3) had similar or even enhanced effect on the O_2-NAs degradation compared 50 mg/L ozone.
11. Changes and similarities of the treated water characteristics with natural waters were confirmed by two markers (O_2S:O_3S:O_4S and O_2:O_4 ratios). Both ratios decreased after all treatments, for instance in peroxone (1:2), O_2S:O_3S:O_4S and O_2:O_4 decreased from 2.7:4.8:2.1 and 3.59 in raw OSPW to 0:1:0.36 and 0.7, respectively, matching closely the reported ratios in natural waters (0.08:1:0.17 and 0.92).
12. Residual toxic effects toward *Vibrio fischeri* observed after ozone and peroxone treatments, suggested that specific compounds of NAs may be partially the cause for acute toxicity (i.e., similar reduction (50%) was achieved in both toxicity and abundance in O_2 species with carbon 15-26) and/or generated by-products (e.g., O_3S classes at double bond equivalent (DBE) = 4 and $C_9H_{12}O_2$ at DBE = 4).
13. The peroxone (1:2) treatment slightly enhanced the biodegradability and reduced the chemical oxygen demand (COD)

compared to ozone, suggesting the possibility of using combined OSPW remediation approaches (i.e., peroxone coupled with biological process).

14. The entire O_3S species decreased after oxidation; however, the species associated with toxicity at DBE = 4 increased.
15. The range of the reaction rate constants for the different peroxone and ozone treatment conditions was 0.101 - 0.251 min^{-1} while the lowest and highest values belong to the peroxone (1:3) 10+50 or (10 mg/L H_2O_2: 50 mg/L O_3) and the 30 mg/L ozone or O30, respectively.
16. For all different peroxone and ozone treatment conditions, the reaction rate constant increased with the increase of the DBE, specifically at DBE = 7-9, however, similar or close values at DBE =3-6.
17. With respect to the best peroxone molar ratio and doses of oxidants: peroxone (1:2) 20+50 or (20 mg/L H_2O_2: 50 mg/L O_3) was the most effective in degrading O_2-NAs and O_x-NAs; peroxone (1:2) 11+30 or (11 mg/L H_2O_2: 30 mg/L O_3) was the most effective in the producing nitrite which can be attributed as the lowest toxicity effects from the treated water on the PKMs.

6.3 Recommendations

Based on the findings obtained in this research, the following recommendations can be proposed for future research.

- Although the focus of this study was the chemical treatment of OSPW using ozone and peroxone AOPs, the research introduced useful indicators to evaluate the treatment performance that would further allow selecting the best multi-barrier approaches and establishing guidelines in terms of species reductions. For instance, the compatibility of the oxidation treatments with biodegradation either as pre- or post-treatment should be examined as possible train using low oxidants doses. To understand the changes of the OSPW recalcitrant organic compounds and to reach the optimum treatment performance, the markers and indicators presented in this research along with the changes of microbial populations and biodegradation kinetics need to be further investigated.
- The focus of this study was real OSPW; however, to better understand the structure reactivity and mechanism of removal as well as the optimization of the peroxone remediation in OSPW, model NA compounds with different molecular structure and high oxygen number should be utilized.

- O_3 and O_3/H_2O_2 studies were conducted in semi-batch systems only. Additionally, H_2O_2 was added at the beginning of treatment with high residual concentration. Thus, spiking H_2O_2 in pulses or consecutive addition of small doses of H_2O_2 to the reactor might reduce the oxidants doses and enhance the removal efficiency. Further studies using flow-through or continuous flow reactors and introducing the H_2O_2 to the reactor are mandatory to eventually apply AOPs at large scale.
- Studies about identifying and modeling all important operational parameters and their impact on the effectiveness of the OSPW treatment using AOPs in addition to cost studies will lead to the future scale-up of these remediation approaches.
- More investigations are required to optimize the ozone and peroxone treatments by reducing the high oxidants doses and test their application at the pilot scale level as well as examine their toxicological effects of AOP treated water towards *In vitro* and *In vivo* assays. In addition, efforts should be directed to establish water quality treatment goals and guidelines for the possible release of treated OSPW into the environment.

BIBLIOGRAPHY

Afzal, A., Drzewicz, P., Perez-Estrada, L.A., Chen, Y., Martin, J.W. and El-Din, M.G. (2012) Effect of Molecular Structure on the Relative Reactivity of Naphthenic Acids in the UV/H_2O_2 Advanced Oxidation Process. Environ. Sci. Technol. 46(19), 10727-10734.

Afzal, A., Chelme-Ayala, P., Drzewicz, P., Martin, J.W. and Gamal El-Din, M. (2014) Effects of ozone and ozone/hydrogen peroxide on the degradation of model and real oil-sands-process-affected-water naphthenic acids. Ozone Sci. Eng. 37(1), 45-54.

Aghaeinejad-Meybodi, A., Ebadi, A., Shafiei, S., Khataee, A.R. and Rostampour, M. (2015) Modeling and optimization of antidepressant drug Fluoxetine removal in aqueous media by ozone/H_2O_2 process: Comparison of central composite design and artificial neural network approaches. Journal of the Taiwan Institute of Chemical Engineers 48(0), 40-48.

Ahad, J.M.E., Pakdel, H., Savard, M.M., Calderhead, A.I., Gammon, P.R., Rivera, A., Peru, K.M. and Headley, J.V. (2013) Characterization and quantification of mining-related “naphthenic acids” in groundwater near a major oil sands tailings pond. Environ. Sci. & Technol. 47(10), 5023-5030.

Allen, E.W. (2008) Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. Journal of Environ. 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. (2011) Effectiveness of Ozonation Treatment in Eliminating Toxicity of Oil Sands Process-Affected Water to Chironomus dilutus. Environ. Sci. & Technol. 46(1), 486-493.

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

Armstrong, S.A., Headley, J.V., Peru, K.M. and Germida, J.J. (2008) Phytotoxicity of oil sands naphthenic acids and dissipation from systems planted with emergent aquatic macrophytes. Journal of Environ. Sci. and Health Part A-Toxic/Hazardous Substances & Environ.Engineering 43(1), 36-42.

Barrow, M.P., Headley, J.V., Peru, K.M. and Derrick, P.J. (2004) Fourier transform ion cyclotron resonance mass spectrometry of principal components in oilsands naphthenic acids. Journal of Chromatography A 1058(1–2), 51-59.

Barrow, M.P., Witt, M., Headley, J.V. and Peru, K.M. (2010) Athabasca oil sands process water: characterization by atmospheric pressure photoionization and electrospray ionization fourier transform ion cyclotron resonance mass spectrometry. Analytical Chemistry 82(9), 3727-3735.

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

Beltrán, F.J. (2004) Ozone Reaction Kinetics for Water and Wastewater Systems, Lewis Publishers, Boca Raton, Fla.

Bes-Pía, A., Iborra-Clar, A., Mendoza-Roca, J.A., Iborra-Clar, M.I. and Alcaina-Miranda, M.I. (2004) Desalination Strategies in South Mediterranean CountriesNanofiltration of biologically treated textile effluents using ozone as a pre-treatment. Desalination 167, 387-392.

Beck, E.M., Smits, J.E.G. and St Clair, C.C. (2015) Evidence of low toxicity of oil sands process-affected water to birds invites re-evaluation of avian protection strategies. Conservation Physiology 3(1).

Bressler, D.C. and Fedorak, P.M. (2001) Identification of disulfides from the biodegradation of dibenzothiophene. Applied and Environmental Microbiology 67(11), 5084-5093.

Bowman, D.T., Slater, G.F., Warren, L.A. and McCarry, B.E. (2014) Identification of individual thiophene-, indane-, tetralin-, cyclohexane-, and adamantane-type carboxylic acids in composite tailings pore water from Alberta oil sands. Rapid Communications in Mass Spectrometry 28(19), 2075-2083.

Buffle, M.-O., Schumacher, J., Meylan, S., Jekel, M. and von Gunten, U. (2006) Ozonation and advanced oxidation of wastewater: Effect of O_3 dose, pH, DOM and HO center dot-scavengers on ozone decomposition and HO center dot generation. Ozone Sci. Eng. 28(4), 247-259.

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

Chelme-Ayala, P., El-Din, M.G., Smith, D.W. and Adams, C.D. (2011) Oxidation kinetics of two pesticides in natural waters by ozonation and ozone combined with hydrogen peroxide. Water Res. 45(8), 2517-2526.

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.

Di Iaconi, C. (2012) Biological treatment and ozone oxidation: Integration or coupling? Bioresource Technology 106(0), 63-68.

Elovitz, M.S. and von Gunten, U. (1999) Hydroxyl radical/ozone ratios during ozonation processes. I. The Rct concept. Ozone: Sci. & Engin. 21(3), 239-260.

ERCB (2012) Alberta's Energy Reserves 2011 and Supply/Demand Outlook 2012-2021. Government of Alberta, C., AB, Canada, 2012 (ed).

Energy Resources Conservation Board (2012) Energy Resources Conservation Board Tailings Management Assessment Report: Oil Sands Mining

Industry , (posted date: June 12, 2013), from Alberta Energy Regulator, Accessed online November 2016: http://osipfiles.alberta.ca/datasets/158/TailingsManagementAssessmentReport2011-2012.pdf.

Fafet, A., Kergall, F., Da Silva, M. and Behar, F. (2008) Characterization of acidic compounds in biodegraded oils. Organic Geochemistry 39(8), 1235-1242.

Frank, R.A., Fischer, K., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van der Kraak, G. and Solomon, K.R. (2009) Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environ. Sci. Technol. 43(2), 266-271.

Frank, R.A., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van Der Kraak, G. and Solomon, K.R. (2008) Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72(9), 1309-1314.

Frank, R.A., Roy, J.W., Bickerton, G., Rowland, S.J., Headley, J.V., Scarlett, A.G., West, C.E., Peru, K.M., Parrott, J.L., Conly, F.M. and Hewitt, L.M. (2014a) Profiling oil sands mixtures from industrial developments and natural groundwaters for source identification. Environ. Sci. & Technol. 48(5), 2660-2670.

Frank, R.A., Roy, J.W., Bickerton, G., Rowland, S.J., Headley, J.V., Scarlett, A.G., West, C.E., Peru, K.M., Parrott, J.L., Conly, F.M. and Hewitt, L.M.

(2014b) Response to comment on “profiling oil sands mixtures from industrial developments and natural groundwaters for source identification”. Environ. Sci. & Technol. 48(18), 11015-11016.

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

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

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

Gentes, M.-L., Waldner, C., Papp, Z. and Smits, J.E.G. (2007) Effects of exposure to naphthenic acids in tree swallows (Tachycineta bicolor) on the athabasca oil sands, Alberta, Canada. Journal of Toxicology and Environ. Health, Part A 70(14), 1182-1190.

Gosselin, P., Hrudey, S.E., Naeth, M.A., Plourde, A., Therrien, R., Van Der Kraak and G., X., Z., (2010) Environmental and Health Impacts of Canada's oil Sands Industry. Available at. Royal Society of Canada, Ottawa. http://www.rsc.ca/expertpanels_reports.php. .

Gottschalk, C., Libra, J. and Sau, A. (2010) Ozonation of Water and waste water : A practical guide to understanding ozone and its applications, Wiley-VCH.

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

Guével, R.L., Petit, F.G., Goff, P.L., Métivier, R., Valotaire, Y. and Pakdel, F. (2000) Inhibition of rainbow trout (Oncorhynchus Mykiss) estrogen receptor activity by cadmium. Biology of Reproduction 63(1), 259-266.

Hagen, M.O. (2013) Analysis of goldfish innate immunity following exposure to oil sands process affected water , Dissertation , University of Alberta, Edmonton, Alberta, Canada.

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

Hagen, M.O., Katzenback, B.A., Islam, M.D.S., Gamal El-Din, M. and Belosevic, M. (2014) The Analysis of Goldfish (Carassius auratus L.) Innate Immune

Responses After Acute and Subchronic Exposures to Oil Sands Process-Affected Water. Toxicological Sciences 138(1), 59-68.

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

He, Y., Wiseman, S.B., Zhang, X., Hecker, M., Jones, P.D., El-Din, M.G., Martin, J.W. and Giesy, J.P. (2010) Ozonation attenuates the steroidogenic disruptive effects of sediment free oil sands process water in the H295R cell line. Chemosphere 80(5), 578-584.

He, Y., Patterson, S., Wang, N., Hecker, M., Martin, J.W., Gamal El-Din, M., 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 Res. 46(19), 6359-6368.

He, Y., Wiseman, S.B., Wang, N., Perez-Estrada, L.A., El-Din, M.G., Martin, J.W. and Giesy, J.P. (2012b) Transcriptional responses of the brain-gonad-liver axis of fathead minnows exposed to untreated and ozone-treated oil sands process-affected water. Environ. Sci. & Technol. 46(17), 9701-9708.

Headley, J.V. and McMartin, D.W. (2004) A review of the occurrence and fate of naphthenic acids in aquatic environments. J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng. 39(8), 1989-2010.

Headley, J.V., Du, J.L., Peru, K.M. and McMartin, D.W. (2009a) Electrospray ionization mass spectrometry of the photodegradation of naphthenic acids mixtures irradiated with titanium dioxide. J. of Environ.l Sci. and Health Part A 44(6), 591-597.

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. (2009b) Aquatic plant-derived changes in oil sands naphthenic acid signatures determined by low-, high- and ultrahigh-resolution mass spectrometry. Rapid Commun. in Mass Spec. 23(4), 515-522.

Headley, J.V., Peru, K.M., Adenugba, A.A., Du, J.-L. and McMartin, D.W. (2010a) Dissipation of naphthenic acids mixtures by lake biofilms. J. of Environ. Sci.and Health, Part A 45(9), 1027-1036.

Headley, J.V., Peru, K.M., Mishra, S., Meda, V., Dalai, A.K., McMartin, D.W., Mapolelo, M.M., Rodgers, R.P. and Marshall, A.G. (2010c) Characterization of oil sands naphthenic acids treated with ultraviolet and microwave radiation by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun. Mass Spec. 24(21), 3121-3126.

Headley, J.V., Barrow, M.P., Peru, K.M., Fahlman, B., Frank, R.A., Bickerton, G., McMaster, M.E., Parrott, J. and Hewitt, L.M. (2011) Preliminary fingerprinting of Athabasca oil sands polar organics in environmental samples using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun. Mass Spec. 25(13), 1899-1909.

Headley, J.V., Peru, K.M., Fahlman, B., Colodey, A. and McMartin, D.W. (2013) Selective solvent extraction and characterization of the acid extractable fraction of Athabasca oils sands process waters by Orbitrap mass spectrometry. International Journal of Mass Spectrom. 345–347, 104-108.

Headley, J.V., Kumar, P., Dalai, A., Peru, K.M., Bailey, J., McMartin, D.W., Rowland, S.M., Rodgers, R.P. and Marshall, A.G. (2015) Fourier transform ion cyclotron resonance mass spectrometry characterization of treated athabasca oil sands processed waters. Energy & Fuels 29(5), 2768-2773.

Herman, D.C., Fedorak, P.M. and Costerton, J.W. (1993) Biodegradation of cycloalkane carboxylic-acids in oil sand tailings. Canadian Journal of Microbiology 39(6), 576-580.

Hindle, R., Noestheden, M., Peru, K. and Headley, J. (2013) Quantitative analysis of naphthenic acids in water by liquid chromatography-accurate mass time-of-flight mass spectrometry. J. Chroma. A 1286, 166-174.

Huang, R., McPhedran, K.N. and Gamal El-Din, M. (2015a) Ultra Performance liquid chromatography ion mobility time-of-flight mass spectrometry characterization of naphthenic acids species from oil sands process-affected water. Environ. Sci. Technol.

Huang, R., Sun, N., Chelme-Ayala, P., McPhedran, K.N., Changalov, M. and Gamal El-Din, M. (2015b) Fractionation of oil sands-process affected water using pH-dependent extractions: A study of dissociation constants for naphthenic acids species. Chemosphere 127, 291-296.

Hwang, G., Dong, T., Islam, M.S., Sheng, Z.Y., Perez-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 Technol. 130, 269-277.

Islam, M., Dong, T., McPhedran, K., Sheng, Z., Zhang, Y., Liu, Y. and Gamal El-Din, M. (2014a) Impact of ozonation pre-treatment of oil sands process-affected water on the operational performance of a GAC-fluidized bed biofilm reactor. Biodegradation 25(6), 811-823.

Islam, M.S., Moreira, J., Chelme-Ayala, P. and Gamal El-Din, M. (2014b) Prediction of naphthenic acid species degradation by kinetic and surrogate models during the ozonation of oil sands process-affected water. Sci. Total Environ. 493, 282-290.

Juhascik, M.P. and Jenkins, A.J. (2009) Comparison of liquid/liquid and solid-phase extraction for alkaline drugs. J. of Chroma. Science 47(7), 553-557.

Jaffé, R. and Gallardo, M.T. (1993) Application of carboxylic acid biomarkers as indicators of biodegradation and migration of crude oils from the Maracaibo Basin, Western Venezuela. Organic Geochemistry 20(7), 973-984.

Jagadevan, S., Graham, N.J. and Thompson, I.P. (2013) Treatment of waste metalworking fluid by a hybrid ozone-biological process. Journal of Hazardous Materials 244–245, 394-402.

Jin, X., Peldszus, S. and Huck, P.M. (2012) Reaction kinetics of selected micropollutants in ozonation and advanced oxidation processes. Water Res. 46(19), 6519-6530.

Jivraj MN, M.M., Fung B. (1995) Naphthenic acids extraction and quantitative analyses with FT-IR spectroscopy. Syncrude analytical methods manual 4th ed. Edmonton, Canada: Syncrude Canada Ltd. Research Department.

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. Isme Journal 5(3), 486-496.

Jones, D., West, C.E., Scarlett, A.G., Frank, R.A. and Rowland, S.J. (2012) Isolation and estimation of the 'aromatic' naphthenic acid content of an oil sands process-affected water extract. J. of Chroma. A 1247, 171-175.

Jones, D., Scarlett, A.G., West, C.E., Frank, R.A., Gieleciak, R., Hager, D., Pureveen, J., Tegelaar, E. and Rowland, S.J. (2013) Elemental and spectroscopic characterization of fractions of an acidic extract of oil sands process water. Chemosphere 93(9), 1655-1664.

Jones, D., Scarlett, A.G., West, C.E. and Rowland, S.J. (2011) Toxicity of individual naphthenic acids to Vibrio fischeri. Environ. Sci. & Technol. 45(22), 9776-9782.

Katsoyiannis, I.A., Canonica, S. and von Gunten, U. (2011) Efficiency and energy requirements for the transformation of organic micropollutants by ozone, O_3/H_2O_2 and UV/H_2O_2. Water Res. 45(13), 3811-3822.

Keen, O.S., Baik, S., Linden, K.G., Aga, D.S. and Love, N.G. (2012) Enhanced biodegradation of carbamazepine after UV/H_2O_2 advanced oxidation. Environ. Sci. & Technol. 46(11), 6222-6227.

Keen, O.S., McKay, G., Mezyk, S.P., Linden, K.G. and Rosario-Ortiz, F.L. (2014) Identifying the factors that influence the reactivity of effluent organic matter with hydroxyl radicals. Water Res. 50, 408-419.

Kelly, E.N., Schindler, D.W., Hodson, P.V., Short, J.W., Radmanovich, R. and Nielsen, C.C. (2010) Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. Proceedings of the National Academy of Sciences 107(37), 16178-16183.

Kim, S., Stanford, L.A., Rodgers, R.P., Marshall, A.G., Walters, C.C., Qian, K., Wenger, L.M. and Mankiewicz, P. (2005) Microbial alteration of the

acidic and neutral polar NSO compounds revealed by Fourier transform ion cyclotron resonance mass spectrometry. Organic Geochemistry 36(8), 1117-1134.

Klamerth, N., Moreira, J., Li, C., Singh, A., McPhedran, K.N., Chelme-Ayala, P., Belosevic, M. and Gamal El-Din, M. (2015) Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. Sci. Total Environ. 506–507(0), 66-75.

Klamerth, N., Malato, S., Agüera, A. and Fernández-Alba, A. (2013) Photo-Fenton and modified photo-Fenton at neutral pH for the treatment of emerging contaminants in wastewater treatment plant effluents: A comparison. Water Res. 47(2), 833-840.

Klymenko, N.A., Kozyatnyk, I.P. and Savchyna, L.A. (2010) Removing of fulvic acids by ozonation and biological active carbon filtration. Water Res. 44(18), 5316-5322.

Kropp, K.G., Andersson, J.T. and Fedorak, P.M. (1997) Biotransformations of three Dimethyldibenzothiophenes by pure and mixed bacterial cultures. Environ. Sci. & Technol. 31(5), 1547-1554.

Lai, J.W.S., Pinto, L.J., Bendell-Young, L.I., Moore, M.M. and Kiehlmann, E. (1996) Factors that affect the degradation of naphthenic acids in oil sands wastewater by indigenous microbial communities. Environ. Toxicology and Chemistry 15(9), 1482-1491.

Lamsal, R., Walsh, M.E. and Gagnon, G.A. (2011) Comparison of advanced oxidation processes for the removal of natural organic matter. Water Res. 45(10), 3263-3269.

Lee, Y., Gerrity, D., Lee, M., Bogeat, A.E., Salhi, E., Gamage, S., Trenholm, R.A., Wert, E.C., Snyder, S.A. and Von Gunten, U. (2013) Prediction of micropollutant elimination during ozonation of municipal wastewater effluents: Use of kinetic and water specific information. Environ. Sci. Technol. 47(11), 5872-5881.

Lee, Y., Kovalova, L., McArdell, C.S. and von Gunten, U. (2014) Prediction of micropollutant elimination during ozonation of a hospital wastewater effluent. Water Res. 64(0), 134-148.

Liao, Y., Shi, Q., Hsu, C.S., Pan, Y. and Zhang, Y. (2012) Distribution of acids and nitrogen-containing compounds in biodegraded oils of the Liaohe Basin by negative ion ESI FT-ICR MS. Organic Geochemistry 47, 51-65.

Lo, C.C., Brownlee, B.G. and Bunce, N.J. (2006) Mass spectrometric and toxicological assays of Athabasca oil sands naphthenic acids. Water Res. 40(4), 655-664.

Mahaffey, A. and Dubé, M. (2016) Review of the composition and toxicity of oil sands process-affected water. Environmental Reviews 25(1), 97-114.

Marentette, J.R., Frank, R.A., Bartlett, A.J., Gillis, P.L., Hewitt, L.M., Peru, K.M., Headley, J.V., Brunswick, P., Shang, D. and Parrott, J.L. (2015) Toxicity of naphthenic acid fraction components extracted from fresh and aged oil

sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (Pimephales promelas) embryos. Aquatic Toxic..y 164, 108-117.

Martin, J.W. (2015) The Challenge: Safe release and reintegration of oil sands process-affected water. Environ. Toxicology and Chemistry 34(12), 2682-2682.

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

Martin, j.w., Han, X.M., Peru, K.M. and Headley, J.V. (2008) Comparison of high- and low-resolution electrospray ionization mass spectrometry for the analysis of naphthenic acid mixtures in oil sands process water. Rapid Commun.in Mass Spec. 22(12), 1919-1924.

McMartin, D.W., Headley, J.V., Friesen, D.A., Peru, K.M. and Gillies, J.A. (2004) Photolysis of naphthenic acids in natural surface water. J. of Environ. Sci. and Health Part A 39(6), 1361-1383.

McGivney W.T. and Kawamura S. (2008) Cost estimating manual for water treatment facilities, John Wiley & Sons, Inc.

McQueen, A.D., Kinley, C.M., Hendrikse, M., Gaspari, D.P., Calomeni, A.J., Iwinski, K.J., Castle, J.W., Haakensen, M.C., Peru, K.M., Headley, J.V. and Rodgers Jr, J.H. (2017a) A risk-based approach for identifying

constituents of concern in oil sands process-affected water from the Athabasca Oil Sands region. Chemosphere 173, 340-350.

McQueen AD, Hendrikse M, Gaspari DP, Kinley CM, Rodgers Jr JH, Castle JW: Performance of a hybrid pilot-scale constructed wetland system for treating oil sands process-affected water from the Athabasca oil sands. Ecological Engineering 2017b;102:152-165.

Misiti, T., Tezel, U. and Pavlostathis, S.G. (2013) Fate and effect of naphthenic acids on oil refinery activated sludge wastewater treatment systems. Water Res. 47(1), 449-460.

Mishra, S., V.M., Dalai, A., McMartin, D., Headley, J. and Peru, K. (2010) Photocatalysis of Naphthenic Acids in Water. Water Resource and Protection 2(7), 644-650 doi: 610.4236/jwarp.2010.27074.

Mitra, S. (2004) Sample Preparation Techniques in Analytical Chemistry, (Vol. 237) pp. 37-138, John Wiley & Sons, Inc (Ed. 2004).

Mohamed, M.H., Wilson, L.D., Shah, J.R., Bailey, J., Peru, K.M. and Headley, J.V. (2015) A novel solid-state fractionation of naphthenic acid fraction components from oil sands process-affected water. Chemosphere 136, 252-258.

Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W. and Giesy, J.P. (2015) Effects-Directed analysis of dissolved organic compounds in oil sands process-affected water. Environ. Sci. & Technol. 49(20), 12395-12404.

Moloney, M.G. (2009) Structure and Reactivity in Organic chemistry.

Morandi, G.D., Wiseman, S.B., Pereira, A., Mankidy, R., Gault, I.G.M., Martin, J.W. and Giesy, J.P. (2015) Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. Environ. Sci. & Technol. 49(20), 12395-12404.

Morandi, G.D., Zhang, K., Wiseman, S.B., Pereira, A.d.S., Martin, J.W. and Giesy, J.P. (2016) Effect of lipid partitioning on predictions of acute toxicity of oil sands process affected Water to Embryos of Fathead Minnow (Pimephales promelas). Environ. Sci. & Technol. 50 (16), pp 8858–8866

Noah, M., Poetz, S., Vieth-Hillebrand, A. and Wilkes, H. (2015) Detection of residual oil-sand-derived organic material in developing soils of reclamation sites by ultra-high-resolution mass spectrometry. Environ. Sci. & Technol. 49(11), 6466-6473.

Noestheden, M.R., Headley, J.V., Peru, K.M., Barrow, M.P., Burton, L.L., Sakuma, T., Winkler, P. and Campbell, J.L. (2014) Rapid characterization of naphthenic acids using differential mobility spectrometry and mass spectrometry. Environ. Sci. & Technol. 48(17), 10264-10272.

Nothe, T., Fahlenkamp, H. and von Sonntag, C. (2009) Ozonation of wastewater: rate of ozone consumption and hydroxyl radical yield. Environ. Sci. & Technol. 43(15), 5990-5995.

Nyakas, A., Han, J., Peru, K.M., Headley, J.V. and Borchers, C.H. (2013) Comprehensive analysis of oil sands processed water by direct-infusion fourier-transform ion cyclotron resonance mass spectrometry with and without offline UHPLC sample prefractionation. Environ. Sci. Technol. 47(9), 4471-4479.

Oh, B.-T., Seo, Y.-S., Sudhakar, D., Choe, J.-H., Lee, S.-M., Park, Y.-J. and Cho, M. (2014) Oxidative degradation of endotoxin by advanced oxidation process (O_3/H_2O_2 & UV/H_2O_2). J. of Hazardous Mat. 279(0), 105-110.

Paillard, H., Brunet, R. and Dore, M. (1988) Conditions optimales d'application du systeme oxydant ozone-peroxyde d'hydrogene. Water Res. 22(1), 91-103.

Pisarenko, A.N., Stanford, B.D., Yan, D., Gerrity, D. and Snyder, S.A. (2012) Effects of ozone and ozone/peroxide on trace organic contaminants and NDMA in drinking water and water reuse applications. Water Res. 46(2), 316-326.

Pocostales, J.P., Sein, M.M., Knolle, W., von Sonntag, C. and Schmidt, T.C. (2010) Degradation of Ozone-Refractory Organic Phosphates in Wastewater by Ozone and Ozone/Hydrogen Peroxide (Peroxone): The Role of Ozone Consumption by Dissolved Organic Matter. Environ. Sci. Technol. 44(21), 8248-8253.

Pereira, A.S., Islam, M.D.S., Gamal El-Din, M. 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 Commun. Mass Spectrom. 27(21), 2317-2326.

Pereira, A.S., Bhattacharjee, S. and Martin, J.W. (2013a) Characterization of oil sands process-affected waters by liquid chromatography orbitrap mass spectrometry. Environ. Sci. & Technol. 47(10), 5504-5513.

Perez-Estrada, L.A., Han, X.M., Drzewicz, P., El-Din, M.G., Fedorak, P.M. and Martin, J.W. (2011) Structure-reactivity of naphthenic acids in the ozonation process. Environ. Sci. Technol. 45(17), 7431-7437.

Peng, H., Sun, J., Alharbi, H.A., Jones, P.D., Giesy, J.P. and Wiseman, S.B. (2016) Peroxisome proliferator-activated receptor γ is a sensitive target for oil sands process-affected water: effects on adipogenesis and identification of ligands. Environ. Sci. & Technol.

Quesnel, D.M., Oldenburg, T.B.P., Larter, S.R., Gieg, L.M. and Chua, G. (2015) Biostimulation of oil sands process-affected water with phosphate yields removal of sulfur-containing organics and detoxification. Environ. Sci. Technol. 49(21), 13012-13020.

Reinardy, H.C., Scarlett, A.G., Henry, T.B., West, C.E., Hewitt, L.M., Frank, R.A. and Rowland, S.J. (2013) Aromatic Naphthenic Acids in Oil Sands Process-Affected Water, Resolved by GCxGC-MS, Only Weakly Induce the Gene for Vitellogenin Production in Zebrafish (Danio rerio) Larvae. Environ. Sci.& Technol. 47(12), 6614-6620.

Ross, M.S., Pereira, A.D., Fennell, J., Davies, M., Johnson, J., Sliva, L. and Martin, J.W. (2012) Quantitative and Qualitative Analysis of Naphthenic Acids in Natural Waters Surrounding the Canadian Oil Sands Industry. Environ. Sci. & Technol. 46(23), 12796-12805.

Rosenfeldt, E.J., Linden, K.G., Canonica, S. and von Gunten, U. (2006) Comparison of the efficiency of OH radical formation during ozonation and the advanced oxidation processes O_3/H_2O_2 and UV/H_2O_2. Water Res. 40(20), 3695-3704.

Rosenfeldt, E.J. and Linden, K.G. (2007) The R-OH,R-UV concept to characterize and the model UV/H_2O_2 process in natural waters. Environ. Sci. Technol. 41(7), 2548-2553.

Rowland, S.J., West, C.E., Scarlett, A.G. and Jones, D. (2011a) Identification of individual acids in a commercial sample of naphthenic acids from petroleum by two-dimensional comprehensive gas chromatography/mass spectrometry. Rapid Commu. in Mass Spec. 25(12), 1741-1751.

Rowland, S.J., West, C.E., Scarlett, A.G., Jones, D. and Frank, R.A. (2011b) Identification of individual tetra- and pentacyclic naphthenic acids in oil sands process water by comprehensive two-dimensional gas chromatography/mass spectrometry. Rapid Commun.in Mass Spec.25(9), 1198-1204.

Rowland, S.J., West, C.E., Jones, D., Scarlett, A.G., Frank, R.A. and Hewitt, L.M. (2011c) Steroidal Aromatic 'Naphthenic Acids' in Oil Sands Process-

Affected Water: Structural Comparisons with Environmental Estrogens. Environ. Sci. & Technol. 45(22), 9806-9815.

Rowland, S.J., Pereira, A.S., Martin, J.W., Scarlett, A.G., West, C.E., Lengger, S.K., Wilde, M.J., Pureveen, J., Tegelaar, E.W., Frank, R.A. and Hewitt, L.M. (2014) Mass spectral characterisation of a polar, esterified fraction of an organic extract of an oil sands process water. Rapid Commun.in Mass Spec. 28(21), 2352-2362.

Rogers, V.V., Liber, K. and MacKinnon, M.D. (2002) Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48(5), 519-527.

Rudzinski, W.E., Oehlers, L. and Zhang, Y. (2002) Tandem mass spectrometric characterization of commercial naphthenic acids and a Maya crude oil. Energy & Fuels 16(5), 1178-1185.

Scarlett, A.G., Clough, R., West, C., Lewis, C.A., Booth, A.M. and Rowland, S.J. (2011) Alkylnaphthalenes: priority pollutants or minor contributors to the poor health of marine mussels? Environ. Sci. & Technol. 45(14), 6160-6166.

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 93(2), 415-420.

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. Environ. Sci. & Technol. 39(21), 8388-8394.

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

Scott, A.C., Young, R.F. and Fedorak, P.M. (2008b) Comparison of GC-MS and FTIR methods for quantifying naphthenic acids in water samples. Chemosphere 73(8), 1258-1264.

Shang, D., Kim, M., Haberl, M. and Legzdins, A. (2013) Development of a rapid liquid chromatography tandem mass spectrometry method for screening of trace naphthenic acids in aqueous environments. J. Chromatogr A 1278, 98-107.

Sharma, J.R.; Najafi M.; and Qasim S.R., Preliminary cost estimation models for construction, operation, and maintenance of water treatment plants. J. of Infrastructure Systems, 2013, 19(4), 451-464.

Shell Canada Limited, (2016) Oil Sands Performance Report 2015 (Dated April 22, 2016). Accessed online April 2017: http://www.shell.ca/en_ca/energy-and-innovation/oil-sands/oil-sands-performance-report/_jcr_content/par/textimage_78af.stream/1463441702776/00cfc57a8625b41538ec24a2fc9d2e7160147b85367300b8dd8b9cb735aa1736/she-2055-oil-sands-performance-report-2015-final1.pdf.

Shrestha, N., Chilkoor, G., Wilder, J., Gadhamshetty, V. and Stone, J.J. (2017) Potential water resource impacts of hydraulic fracturing from unconventional oil production in the Bakken shale. Water Research.

Shu, Z., Li, C., Belosevic, M., Bolton, J.R. and Gamal El-Din, M. (2014) Application of a solar UV/chlorine advanced oxidation process to oil sands process-affected water remediation. Environ. Sci. & Technol. 48(16), 9692-9701.

Smith, B.E., Lewis, C.A., Belt, S.T., Whitby, C. and Rowland, S.J. (2008) Effects of alkyl chain branching on the biotransformation of naphthenic acids. Environ. Sci. & Technol. 42(24), 9323-9328.

Sohrabi, V., Ross, M.S., Martin, J.W. and Barker, J.F. (2013) Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. Chemosphere 93(11), 2698-2703.

Suh, J.H. and Mohseni, M. (2004) A study on the relationship between biodegradability enhancement and oxidation of 1,4-dioxane using ozone and hydrogen peroxide. Water Res. 38(10), 2596-2604.

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

Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M. and Teiser, B. (2003) Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. 37(8), 1976-1982.

Tembhekar, P., Padoley, K., Chandra, T., Malik, S., Sharma, A., Gupta, S., Pandey, R. and Mudliar, S. (2015) Environmental Waste Management, pp. 299-339, CRC Press.

Thomas, K.V., Langford, K., Petersen, K., Smith, A.J. and Tollefsen, K.E. (2009) Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in north sea offshore produced water discharges. Environ. Sci. & Technol. 43(21), 8066-8071.

Tollefsen, K.E., Petersen, K. and Rowland, S.J. (2012) Toxicity of synthetic naphthenic acids and mixtures of these to fish liver cells. Environ. Sci. & Technol. 46(9), 5143-5150.

Tuan Vo, D. (1978) Multicomponent analysis by synchronous luminescence spectrometry. Anal. Chem. 50(3), 396-401.

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 Heuvel, M.R. (2015) In Response: An academic perspective on the release of oil sands process–affected water. Environ. Toxicology and Chemistry 34(12), 2682-2684.

Verbeek, A.G., Mackay, W.C. and Mackinnon, M.D. (1994) Proceedings of the Twentieth Annual Aquatic Toxicity Workshop.

Von Gunten, U. and von Sonntag, C. (2012) The Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications, IWA Publishing, 2012.

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

Wang, B., Wan, Y., Gao, Y., Zheng, G., Yang, M., Wu, S. and Hu, J. (2015) Occurrences and behaviors of naphthenic acids in a petroleum refinery wastewater treatment plant. Environ. Sci. &Technol. 49(9), 5796-5804.

Wang, C., Alpatova, A., McPhedran, K.N. and Gamal El-Din, M. (2015) Coagulation/flocculation process with polyaluminum chloride for the remediation of oil sands process-affected water: Performance and mechanism study. J. of Environ. Management (160), 254-262.

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. Environ. Sci. & Technol. 47(12), 6518-6526.

Wang, C., Huang, R., Klamerth, N., Chelme-Ayala, P. and Gamal El-Din, M. (2016) Positive and negative electrospray ionization analyses of the organic fractions in raw and oxidized oil sands process-affected water. Chemosphere 165, 239-247.

Wang, C., Klamerth, N., Messele, S.A., Singh, A., Belosevic, M. and Gamal El-Din, M. (2016) Comparison of UV/hydrogen peroxide, potassium ferrate(VI), and ozone in oxidizing the organic fraction of oil sands process-affected water (OSPW). Water Res. 100, 476-485.

West, C.E., Jones, D., Scarlett, A.G. and Rowland, S.J. (2011) Compositional heterogeneity may limit the usefulness of some commercial naphthenic acids for toxicity assays. Sci. Total Environ. 09(19), 4125-4131.

Weltens, R., Deprez, K. and Michiels, L. (2014) Validation of microtox as a first screening tool for waste classification. Waste Manag. 34(12), 2427-2433.

West, C.E., Scarlett, A.G., Tonkin, A., O'Carroll-Fitzpatrick, D., Pureveen, J., Tegelaar, E., Gieleciak, R., Hager, D., Petersen, K., Tollefsen, K.-E. and Rowland, S.J. (2014) Diaromatic sulphur-containing 'naphthenic' acids in process waters. Water Res. 51, 206-215.

Whitby, C. (2010) Microbial naphthenic acid degradation. Advances in Applied Microbiology, Vol. 70 70, 93-125.

Wiseman, S.B., He, Y., Gamal-El Din, M., Martin, J.W., Jones, P.D., Hecker, M. and Giesy, J.P. (2013) Transcriptional responses of male fathead minnows

exposed to oil sands process-affected water. Comp Biochem Physiol C Toxicol Pharmacol 157(2), 227-235.

Woudneh, M.B., Coreen Hamilton, M., Benskin, J.P., Wang, G., McEachern, P. and Cosgrove, J.R. (2013) A novel derivatization-based liquid chromatography tandem mass spectrometry method for quantitative characterization of naphthenic acid isomer profiles in environmental waters. J Chromatogr A 1293, 36-43.

Wu, D., Yang, Z., Wang, W., Tian, G., Xu, S. and Sims, A. (2012) Ozonation as an advanced oxidant in treatment of bamboo industry wastewater. Chemosphere 88(9), 1108-1113.

Wu, T. and Englehardt, J.D. (2015) Peroxone mineralization of chemical oxygen demand for direct potable water reuse: Kinetics and process control. Water Res. 73, 362-372.

Xue, J., Zhang, Y., Liu, Y. and Gamal El-Din, M. (2016) Treatment of raw and ozonated oil sands process-affected water under decoupled denitrifying anoxic and nitrifying aerobic conditions: a comparative study. Biodegradation 27(4), 247-264.

Yi, Y., Gibson, J., Birks, J., Han, J. and Borchers, C.H. (2014) Comment on "Profiling oil sands mixtures from industrial developments and natural groundwaters for source identification". Environ. Sci. & Technol. 48(18), 11013-11014.

Young, R.F., Orr, E.A., Goss, G.G. and Fedorak, P.M. (2007) Detection of naphthenic acids in fish exposed to commercial naphthenic acids and oil sands process-affected water. Chemosphere 68(3), 518-527.

Young, R.F., Wismer, W.V. and Fedorak, P.M. (2008) Estimating naphthenic acids concentrations in laboratory-exposed fish and in fish from the wild. Chemosphere 73(4), 498-505.

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J. (2015a) Toxicity and composition profiles of solid phase extracts of oil sands process-affected water. Sci. Total Environ. 538, 573-582.

Yue, S., Ramsay, B.A., Brown, R.S., Wang, J. and Ramsay, J.A. (2015b) Identification of estrogenic compounds in oil sands process waters by effect directed analysis. Environ. Sci. & Technol. 49(1), 570-577.

Yue, S., Ramsay, B.A., Wang, J. and Ramsay, J.A. (2016) Biodegradation and detoxification of naphthenic acids in oil sands process affected waters. Sci. Total Environ. 572, 273-279.

Zhang, K., Pereira, A.S. and Martin, J.W. (2015) Estimates of Octanol–Water partitioning for thousands of dissolved organic species in oil sands process-affected water. Environ. Sci. &Technol. 49(14), 8907-8913.

Zhao, B., R. Currie and H. Mian (2012) Catalogue of analytical methods for naphthenic acids related to oil sands operations. Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, Edmonton, Alberta. OSRIN Report No. TR-21.65 pp.

Zubot, W., MacKinnon, M.D., Chelme-Ayala, P., Smith, D.W. and El-Din, M.G. (2012) Petroleum coke adsorption as a water management option for oil sands process-affected water. Sci. Total Environ. 427, 364-372.

APPENDIX A. STANDARD CURVE FOR FLUKA AND OSPW EXTRACT; OZONATION EXPERIMENTS, CHANGE OF CONCENTRATIONS WITH TIME

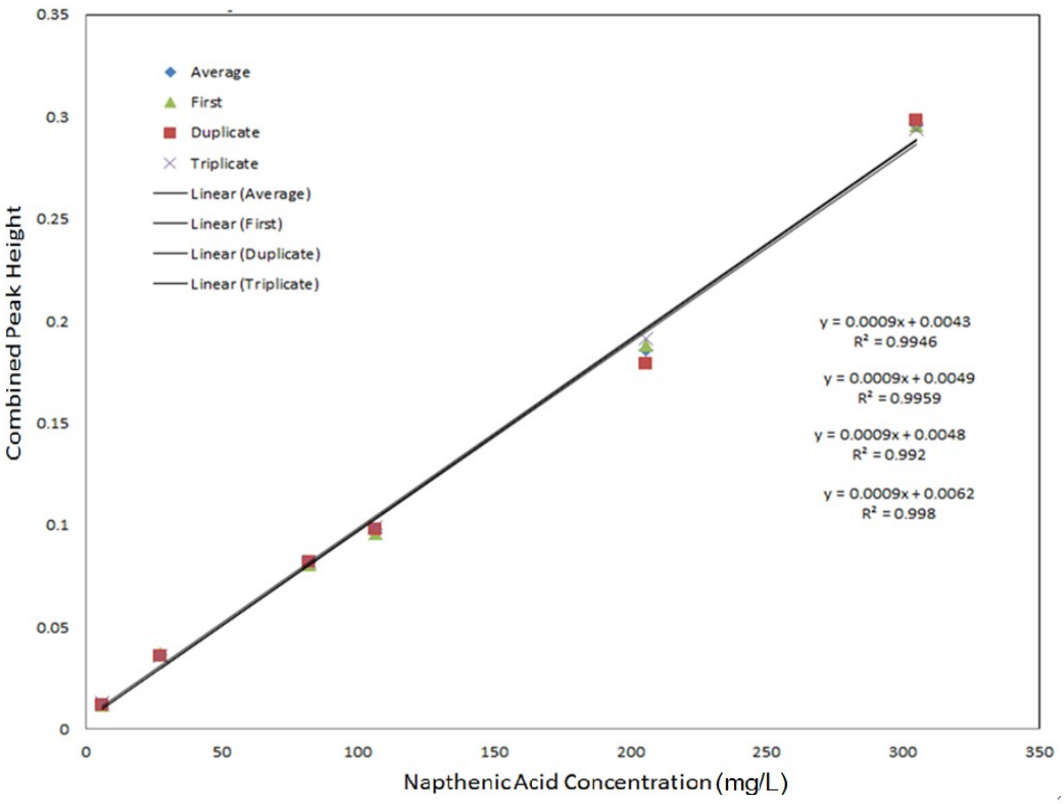

Figure A1. Naphthenic acid determination calibration curve using Fluka standard.

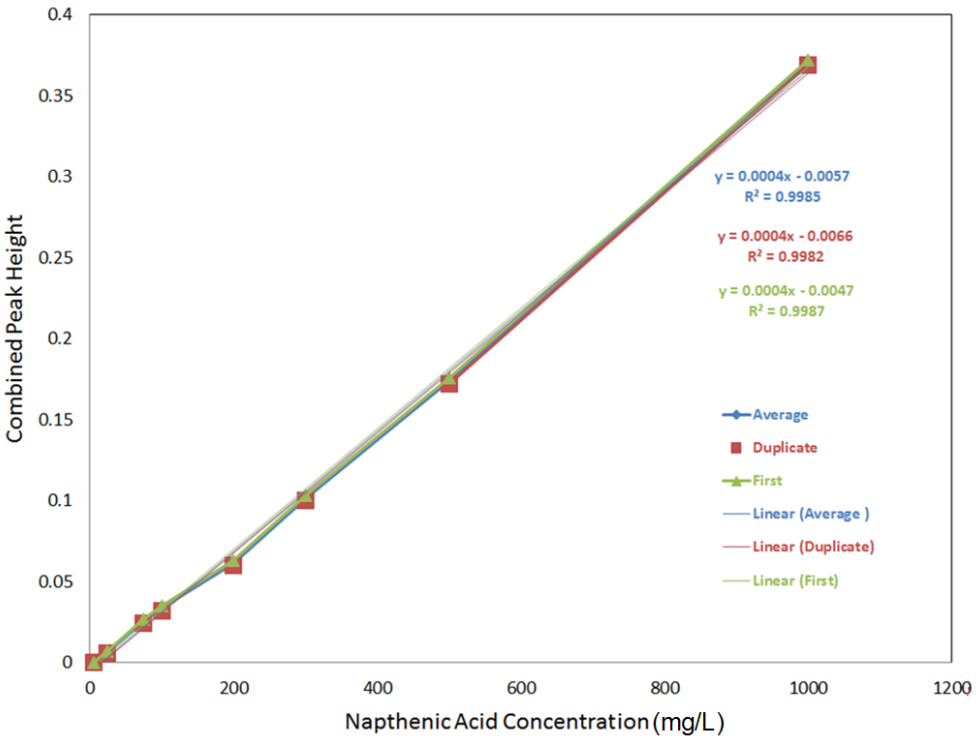

Figure A2. Naphthenic acid determination calibration curve using OSPW extract.

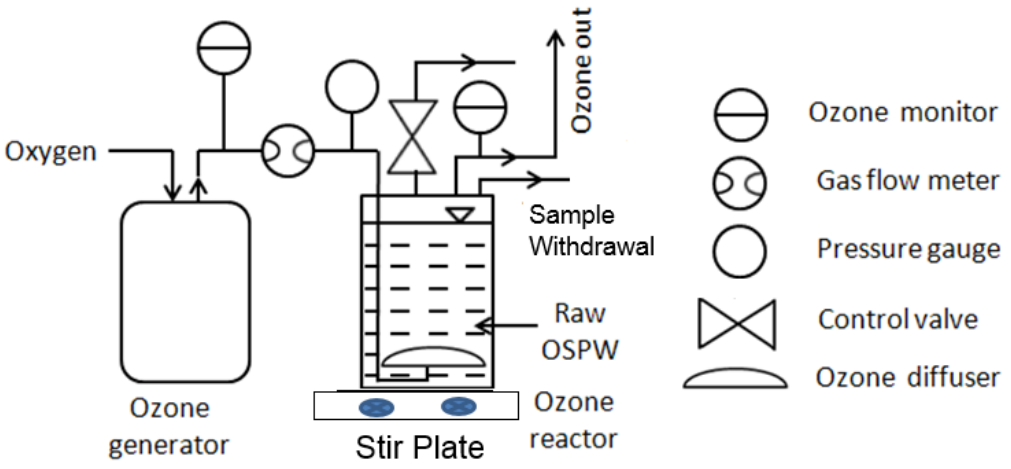

Figure A3. Schematic of ozonation system using semi-batch reactor.

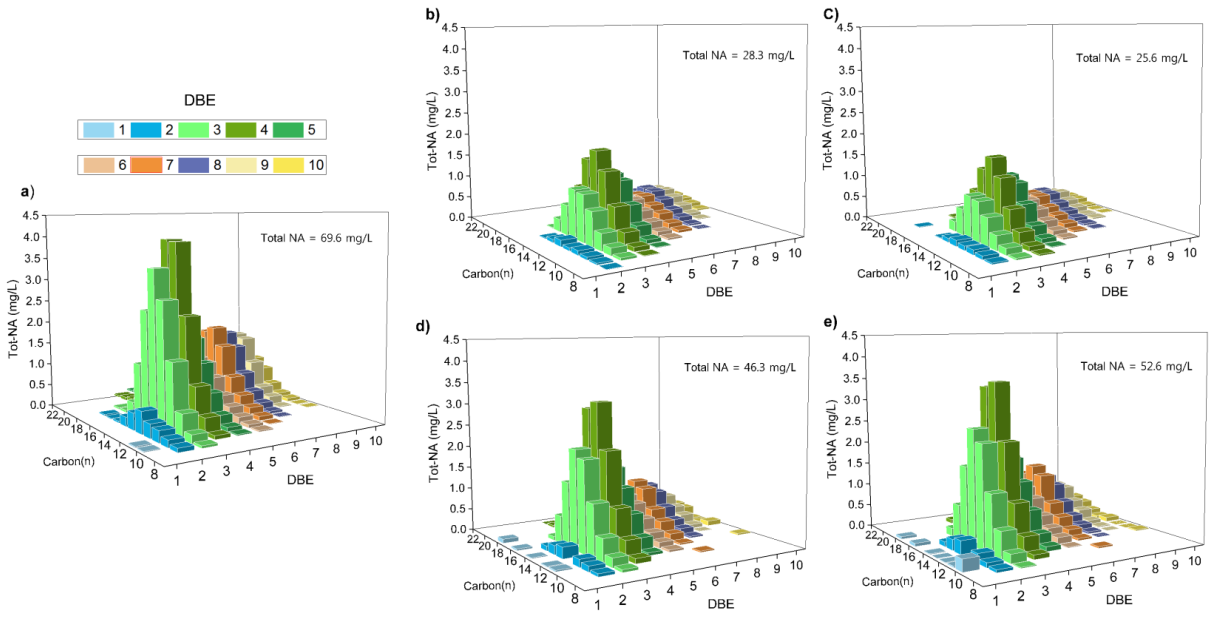

Figure A4. Concentration profiles for O_x-NA or Total-NAs in a) raw OSPW; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; d) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and e) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

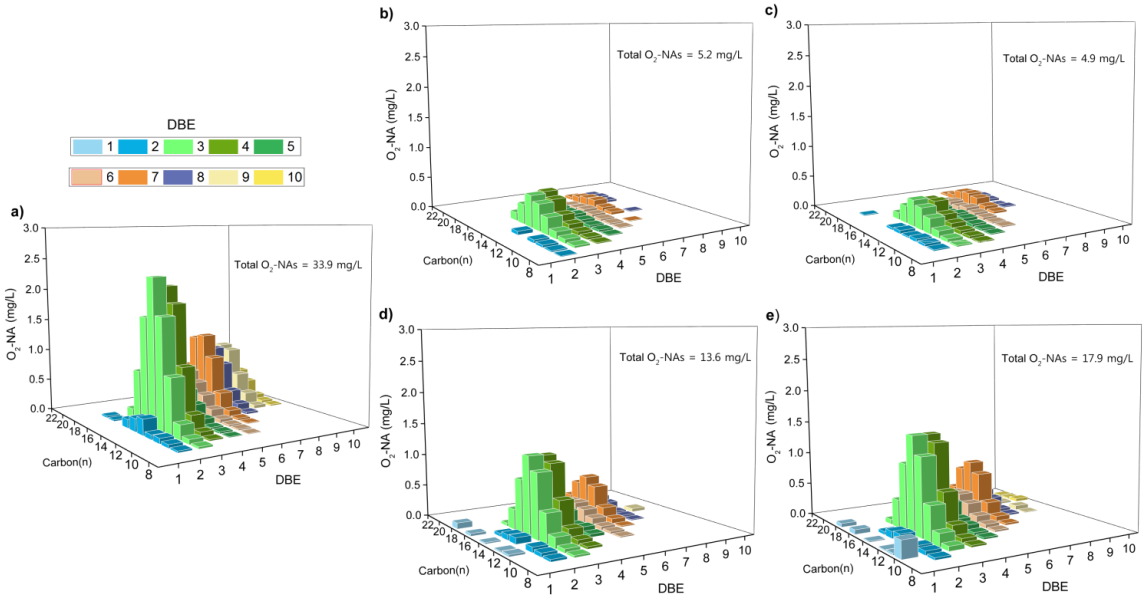

Figure A5. Concentration profiles for O_2-NAs in a) raw OSPW; b) 50 mg/L utilized O_3 dose; c) Peroxone (1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; d) Peroxone (1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 dose] ; and e) Peroxone (1:2) [11 mg/L H_2O_2 and 30 mg/L utilized O_3 dose].

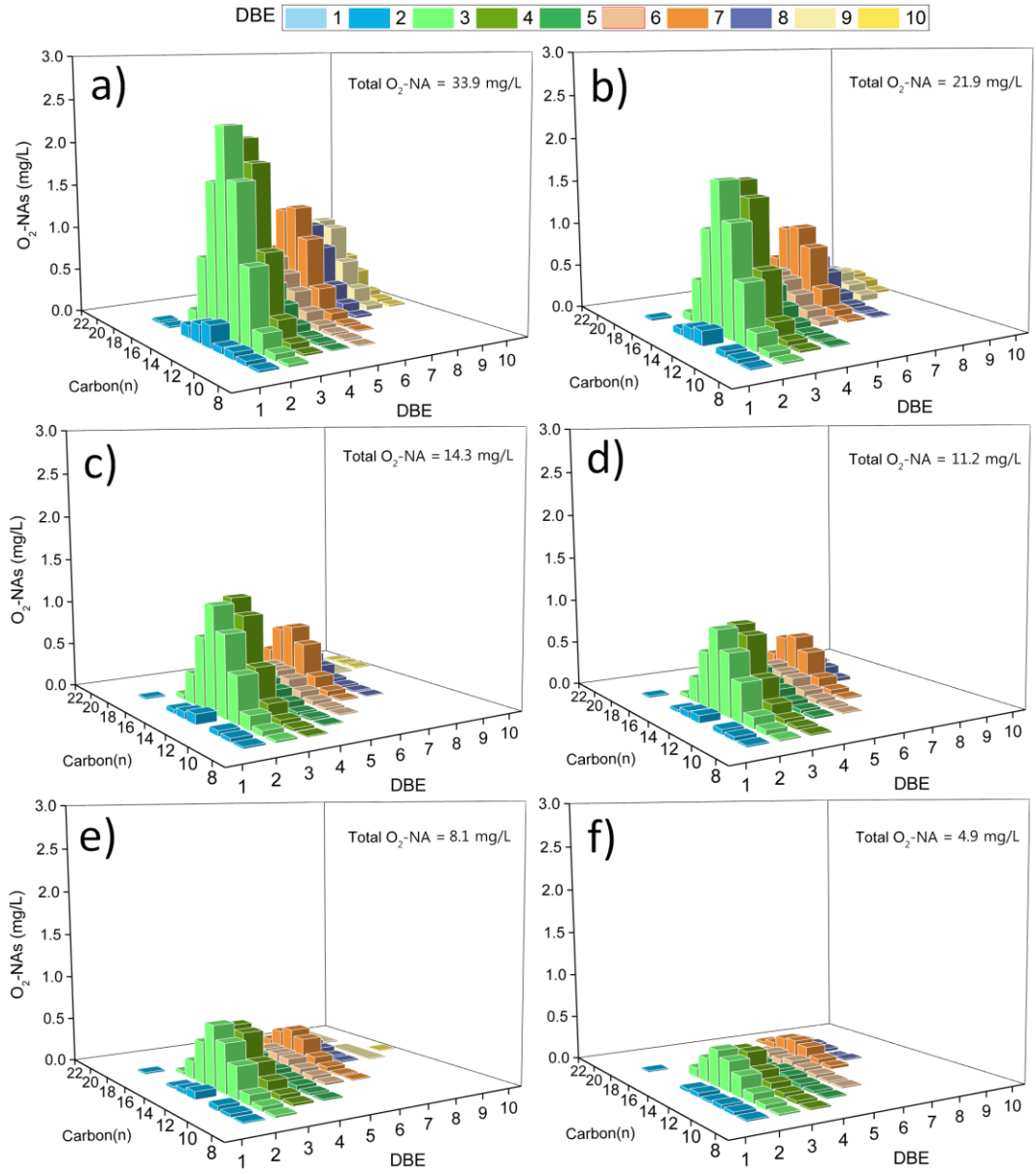

Figure A6. Oxidative degradation O_2-NAs in a) raw OSPW; b) 1 minute; b) 3 minutes; d) 5 minutes; e) 7 minutes; and f) 9 minutes after applying P(1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 doses].

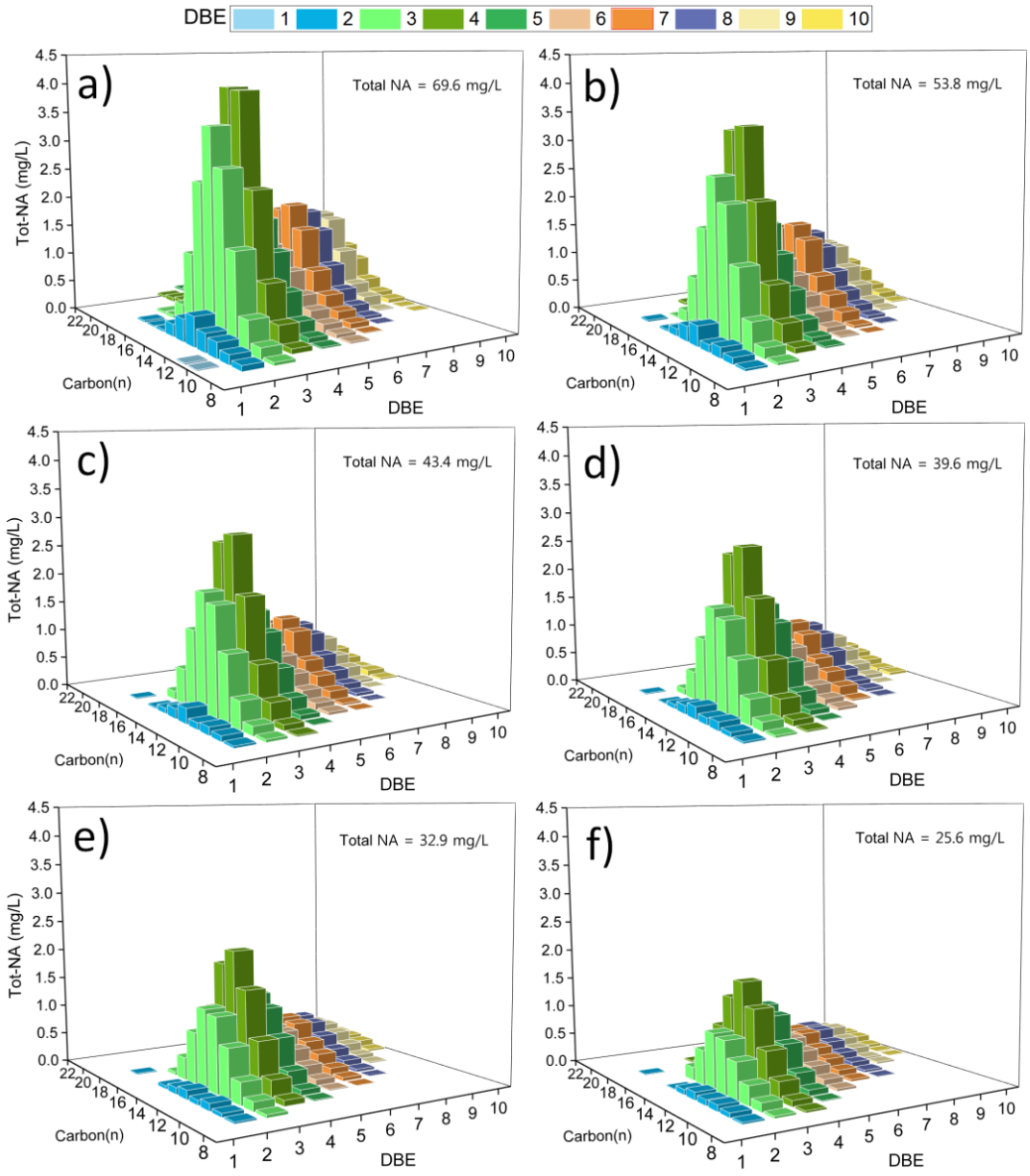

Figure A7. Oxidative degradation O_x-NA or Total-NAs in a) raw OSPW; b) 1 minute; b) 3 minutes; d) 5 minutes; e) 7 minutes; and f) 9 minutes after applying P(1:2) [20 mg/L H_2O_2 and 50 mg/L utilized O_3 doses].

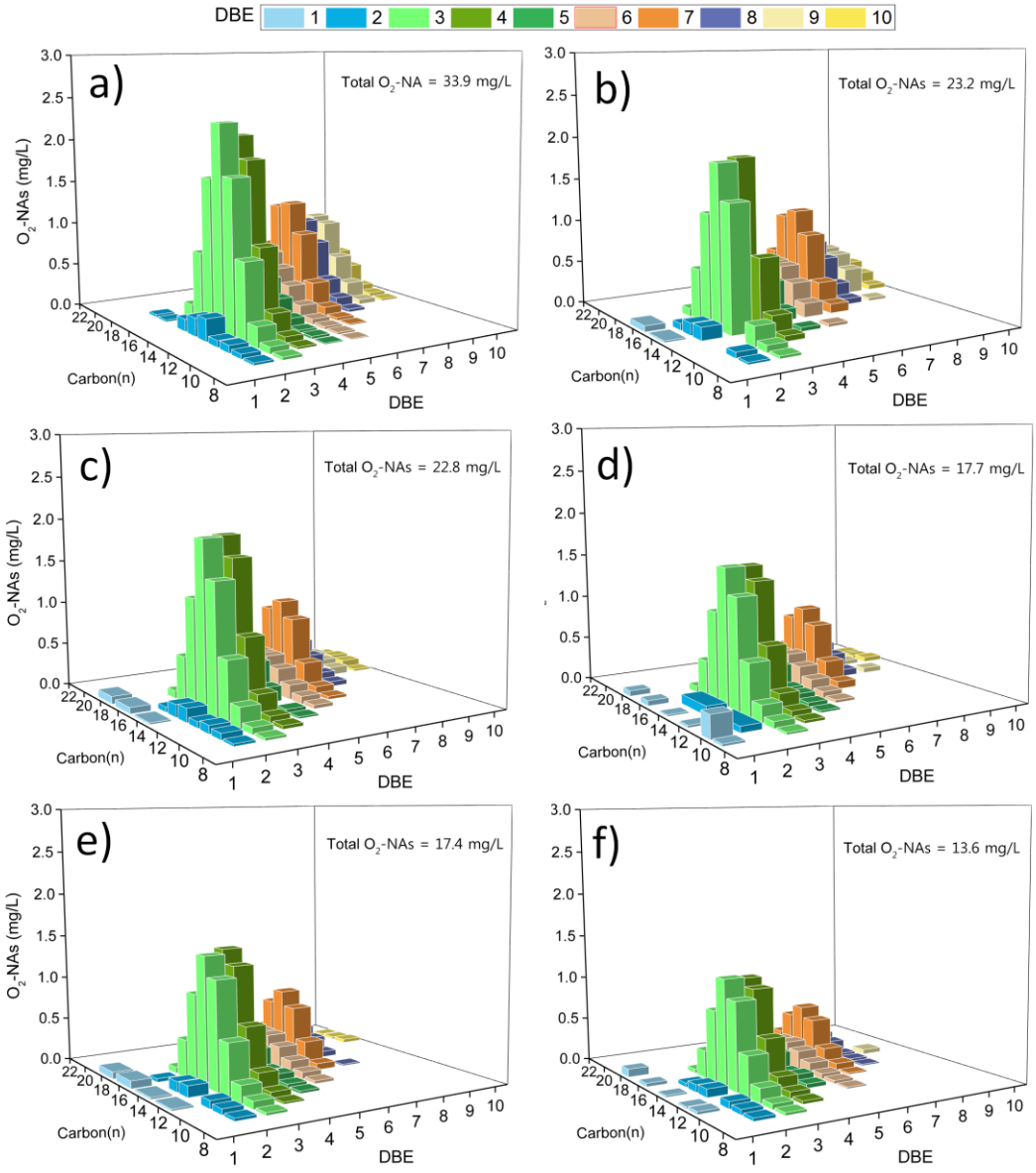

Figure A8. Oxidative degradation O_2-NAs in a) raw OSPW; b) 1 minute; b) 3 minutes; d) 5 minutes; e) 7 minutes; and f) 9 minutes after applying P(1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 doses].

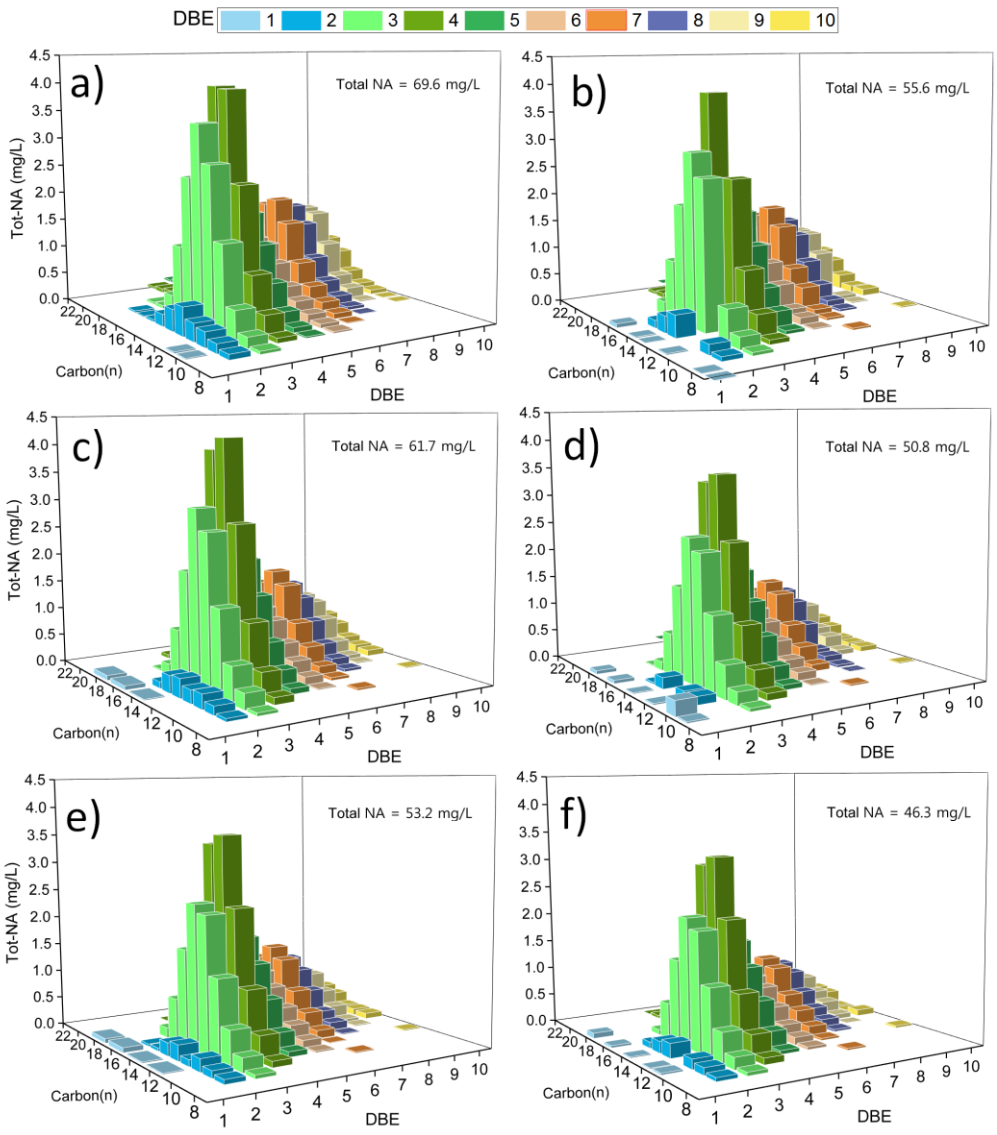

Figure A9. Oxidative degradation O_x-NA or Total-NAs in a) raw OSPW; b) 1 minute ;b) 3 minutes; d) 5 minutes; e) 7 minutes; and f) 9 minutes after applying P(1:3) [10 mg/L H_2O_2 and 50 mg/L utilized O_3 doses].

APPENDIX B. SUMMARY OF THE STANDARDS AND STATISTICAL TEST

Table B1. Fluka standards prepared for the calibration curve

Calibration Curve		**First**			**Duplicate**			**Triplicate**			**Average (cm^{-1})**
Working STD (mg/L)	Actual (mg/L)	Height 1743 cm^{-1}	Height 1706 cm^{-1}	**Total Height**	Height 1743 cm^{-1}	Height 1706 cm^{-1}	**Total Height**	Height 1743 cm^{-1}	Height 1706 cm^{-1}	**Total Height**	
300	305.14	0.202	0.094	0.296	0.204	0.094	0.298	0.201	0.093	0.294	0.296
200	205.68	0.138	0.05	0.188	0.134	0.045	0.179	0.141	0.05	0.191	0.186
100	106.35	0.074	0.022	0.096	0.076	0.022	0.098	0.077	0.022	0.099	0.097
75	81.90	0.061	0.02	0.081	0.062	0.02	0.082	0.062	0.02	0.082	0.082
25	27.33	0.023	0.014	0.037	0.022	0.014	0.036	0.022	0.014	0.036	0.036
5	5.87	0.001	0.011	0.012	0.001	0.011	0.012	0.002	0.011	0.013	0.012

Table B2. Summary of Kruskal-Wallis test results for the sub group of OSPW samples, sub group of groundwater samples and the entire group of samples.

Test	Sample locations	Kruskal-Wallis result		Comment
		Chi-squared	P-Value	
TOF-SPE	All	28.0	<0.05	Not similar
	Groundwater	18.6	<0.05	Not similar
	OSPW	5.42	0.07	Similar
TOF-LLE	All	27.8	<0.05	Not similar
	Groundwater	18.6	<0.05	Not similar
	OSPW	2.76	0.0608	Similar
FTIR-Fluka-SPE	All	27.9	<0.05	Not similar
	Groundwater	18.4	<0.05	Not similar
	OSPW	5.4	0.07	Similar
FTIR-Fluka-LLE	All	28.2	<0.05	Not similar
	Groundwater	18.9	<0.05	Not similar
	OSPW	5.6	0.07	Similar
FTIR-OSPW Ext-SPE	All	27.5	<0.05	Not similar
	Groundwater	18.2	<0.05	Not similar
	OSPW	3.3	0.1931	Similar
FTIR-OSPW Ext-LLE	All	28.0	<0.05	Not similar
	Groundwater	18.6	<0.05	Not similar
	OSPW	5.42	0.07	Similar

Table B3. Calculated SPE/LLE ratio for all samples using UPLC-TOFMS and FTIR analyses. OSPW samples: 1, 2 and 3; Groundwater (GW) samples: 4, 5, 6, 7, 8, 9 and 10. Note: solid-phase extraction is denoted as (SPE); and liquid-liquid extraction is denoted as (LLE).

Batch	Sample #	UPLC-TOFMS	FTIR-Fluka
		SPE/LLE Ratio	
Batch 1	1	1.04	0.62
	2	0.41	0.21
	3	1.12	0.65
	4	0.93	0.48
	5	1.10	1.09
	6	0.84	0.85
	7	1.08	1.13
	8	1.02	1.50
	9	1.03	1.46
	10	1.14	1.15
Batch 2	1	1.35	0.98
	2	2.93	1.37
	3	1.09	1.52
	4	1.45	1.41
	5	1.07	1.14
	6	1.08	0.97
	7	1.7	0.98
	8	1.14	1.53
	9	1.18	1.70
	10	1.12	0.96
Batch 3	1	1.34	1.08
	2	1.02	0.29
	3	1.08	1.42
	4	1.37	0.87
	5	1.81	1.44
	6	0.99	0.87
	7	1.65	2.65
	8	1.05	1.42
	9	0.92	1.26
	10	1.08	0.81