University of Alberta

Degradation of Naphthenic Acids from Oil Sands Process Water and Groundwater Using Chemical Oxidation

By

Weibing Lu

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ABSTRACT

Naphthenic acids attribute to the toxicity of oil sands process water. Naphthenic acids quantification by Fourier Transform Infrared Spectroscopy depends significantly on which standard is used and Merichem naphthenic acids are a good standard.Naphthenic acids can be removed from oil sands process water using sodiumpersulfate as the oxidant in the presence of iron sulfate or zero-valent iron as the activator. Increasing temperature increased reaction rate between naphthenic acids and sodium persulfate. The chemical oxidation process has a significant effect on oil sands process water chemistry, especially, the pH dropped from 8.3 to about 2.4 after chemical oxidation.Naphthenic acids can be degraded from groundwater in the presence of soil using sodium persulfate with 95 % efficiency within 40 days at 22 ^{0}C. The chemical oxidation did not significantly decrease the number of total bacteria in the DNA extracted from the soil.

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Chapter 1 Introduction

1.1. Introduction

1.1.1. The oil sands industry and naphthenic acids

The oil sands regions located in Northern Alberta, Canada, contain an estimated 1.7 trillion barrels of oil in the form of bitumen (Kannel and Gan, 2012). Compared with conventional fossil fuel, bitumen is more costly to recover and process, however, increasing global demand for oil coupled with technological advances in bitumen production have stimulated rapid growth in the Alberta oil sands industry (Allen, 2008). The primary method of extracting bitumen from oil sands has been the Clark hot water extraction. In this process, sodium hydroxide is added to increase mixture pH to around 8, resulting in solubility increase of oil sands asphaltic acids, which can promote the release of surfactants and improve bitumen separation from sand and clay (Allen, 2008). The hot water extraction process consumes large volumes of fresh water, approximately 2 to 3 m^3 per m^3 of oil produced, and produces approximately up to 4 m^3 of wastewater of which 80 % is recycled. This process affected water, commonly known as oil sands process water (OSPW) is a complex mixture which contains sands, silts, clays, water, dissolved ions (primarily Na^+, HCO_3^-, Cl^- and SO_4^{2-}), heavy metals, unrecoverable bitumen, and inorganic and organic compounds (Allen, 2008; Kannel and Gan, 2012).

OSPW is toxic as it contains a wide variety of contaminants including heavy metals, polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, phenol and naphthenic acids (NAs). NAs are a complex mixture of naturally occurring alkyl-substituted acyclic and

cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$; where *n* is the carbon number and Z is zero or a negative even integer defining the hydrogen deficiency resulting from ring formation (Headley and McMartin, 2004; Clemente and Fedorak, 2005; Whitby, 2010; Kannel and Gan, 2012). NAs are the major contaminant in OSPW which can cause acute and chronic aquatic toxicity. Depending on their composition and age, NAs can have toxic effects at relatively low concentrations. And concentrations of NAs in OSPW range from 20 to 70 mg/L, but can be as high as 130 mg/L in fresh process water (Holowenko et al., 2002).

1.1.2. Degradation of naphthenic acids

Rapid expansion of the Alberta's oil sands industry presents huge challenges with respect to the protection of surrounding environment. Remediation of OSPW will allow for the potential future discharge into the local aquatic environment. This requirement has led to substantial research on OSPW remediation, primarily focusing on the degradation of NAs found in OSPW (Quagraine et al., 2005; Whitby, 2010). Among the reported approaches, two have been extensively studied: biodegradation and ozonation of OSPW. Microbial biodegradation has been expected to be the most cost-effective method for removing NAs, but it is slow,andlimited success has been achieved in decreasing the concentration of NAs to below 19 mg/L due to a persistent fraction of NAs remaining after decades of storage (Martin et al., 2010; Pérez-Estrada et al., 2011; Kannel and Gan, 2012); Ozonation has been shown to be able to significantly reduce concentrations of NAs in OSPW, while increasing their biodegradability, and consequently reduce the toxicity (Scott et al., 2008; Martin et al., 2010; Pérez-Estrada et al., 2011). However, ozonation

has its limitations: it is expensive to generate ozone, and the low water solubility (Watts and Teel, 2006) and the short half life of ozone in water ($t_{1/2}$≈ 20 min at 20 ^{0}C) limit the distances overwhich it can be delivered into water (Seol et al., 2003). Taking into account the large existing stores of OSPW and ongoing expansion of the Alberta oil sands industry, a more cost-effective and rapid approach for removing NAs from OSPW is highly desirable.

1. 2. Research objectives

In the last decade, in situ chemical oxidation (ISCO), which is based on the delivery of oxidants to a contaminated source zone and the subsequent oxidation of contaminants into harmless end products within the natural environment, has been increasingly studied to develop novel remediation strategies. With the aim in mind of developing chemical oxidation to an in situ technology for OSPW and groundwater remediation, the goal of this research is to establish the technical feasibility of using chemical oxidation with inexpensive oxidants as an innovative and efficient strategy for the removal of naphthenic acids from OSPW and groundwater. In detail, the following objectives are being evaluated:

1. For NAs removal from OSPW, (1). To optimize the experimental condition of degradation of NAs in OSPW including oxidant dose, activator dose, and reaction period;(2). To study the reaction kinetics of NAs degradation; and (3). To study the impact of chemical oxidation on OSPW water chemistry.

2. For NAs removal from groundwater, (1). To optimize the experimental condition of degradation of NAs in groundwater including oxidant selection, oxidant dosage, and

reaction period; (2) To study the persistence of oxidants in groundwater; and (3) To demonstrate the effect of chemical oxidation on the microbial community.

For OSPW, these objectives will be explored through the use of batch tests on OSPW after being oxidized by sodium persulfate in the presence of iron sulfate or zero valent iron as the activator. NAs degradation efficiency will be determined through the use of Fourier Transform Infrared Spectroscopy (FT-IR), the amount of oxidants will be determined by Ultra Violet Visible Spectrophotometry (UV-vis). Influence of chemical oxidation on water chemistry will be examined through the following tests and procedures: pH, ion chromatography (IC), total alkalinity, inductively coupled plasma mass spectroscopy (ICP-MS), total organic carbon (TOC), total inorganic carbon (TIC), chemical oxygen demand (COD), biochemical oxygen demand (BOD). Microtox^{TM} tests will provide a bigger picture understanding how the chemical oxidation process affects water quality of OSPW.

For groundwater, these objectives will be explored through the use of batch tests on groundwater after being oxidized by different oxidant: sodium persulfate, and potassium permanganate. NAs degradation efficiency will be determined through the use of FT-IR, the persistence of the oxidants in groundwater will be determined by UV-Vis.Real-time quantitative polymerase chain reaction (RT–qPCR) will be performed to determine the number of total bacteria and sulfate reducing bacteria in the extracted DNA.

Mitigation of the impact of oil sands operation on surrounding environment has become a critical issue for Canada's oil sands industry. NAs are the major contaminant in OSPW and have the potential to migrate from the tailings ponds into surrounding environment.

This research establishes the technical feasibility of using chemical oxidation for NAs removal from OSPW and groundwater. With the goal of developing in-situ remediation strategy for OSPW and groundwater, the findings in this research will provide guidance for field tests in the future. This research is significant to the oil sands industry because it provides a promising remediation strategy both for OSPW and groundwater. Overall, this treatment technology will assist sustainable oil sands development within the Athabasca region.

1.3. Thesis outline

This thesis consists of five chapters. Chapter 2 presents an overall review of the research conducted in this area completed so far. Chapter 3 focuses on the chemical oxidation of naphthenic acids in oil sands process water. The removal of naphthenic acids from groundwater is presented in chapter 4. Chapter 5 will provide the final conclusion as well as the engineering significance of the research conducted in this thesis to the oil sands industry.

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Chapter 2 Literature Review

Naphthenic acids (NAs) are a complex mixture of predominantly alkyl-substituted acyclic and cyclo-aliphatic saturated carboxylic acids. The general chemical formula $C_nH_{2n+Z}O_2$ has been used to describe the structure of NAs, where n is the carbon number and Z is zero or a negative even integer defining the hydrogen deficiency resulting from ring formation, and the absolute value of Z divided by 2 gives the number of rings which may be fused or bridged (Headley and McMartin, 2004; Clemente and Fedorak, 2005; Whitby, 2010; Kannel and Gan, 2012). For a given Z homolog, more than one isomer will exist and the carboxylic acid group is usually attached to a side chain, rather than directly to the cycloaliphatic ring (Dzidic et al., 1988; Fan, 1991). The molecular weights differ by 14 between n-series and by 2 between Z-series. Figure 2-1 shows examples of NAs structures with Z = 0 (acyclic), -2 (monocyclic), -4 (bicyclic), -6 (tricyclic), -8 (tetracyclic), -10 (pentacyclic) and -12 (hexacyclic). In recent years, the definition of NAs has broadened to describe a wider range of organic acids. This is because NAs solutions also contain other organic acids which do not adhere to the classical formula $C_nH_{2n+Z}O_2$, as well as impurities with various levels of unsaturation and aromaticity, It has been suggested that NAs should instead be called “acid-extractable organics” (Headley et al., 2009; Grewer et al., 2010), a term which would more accurately describe the diversity of organics present.

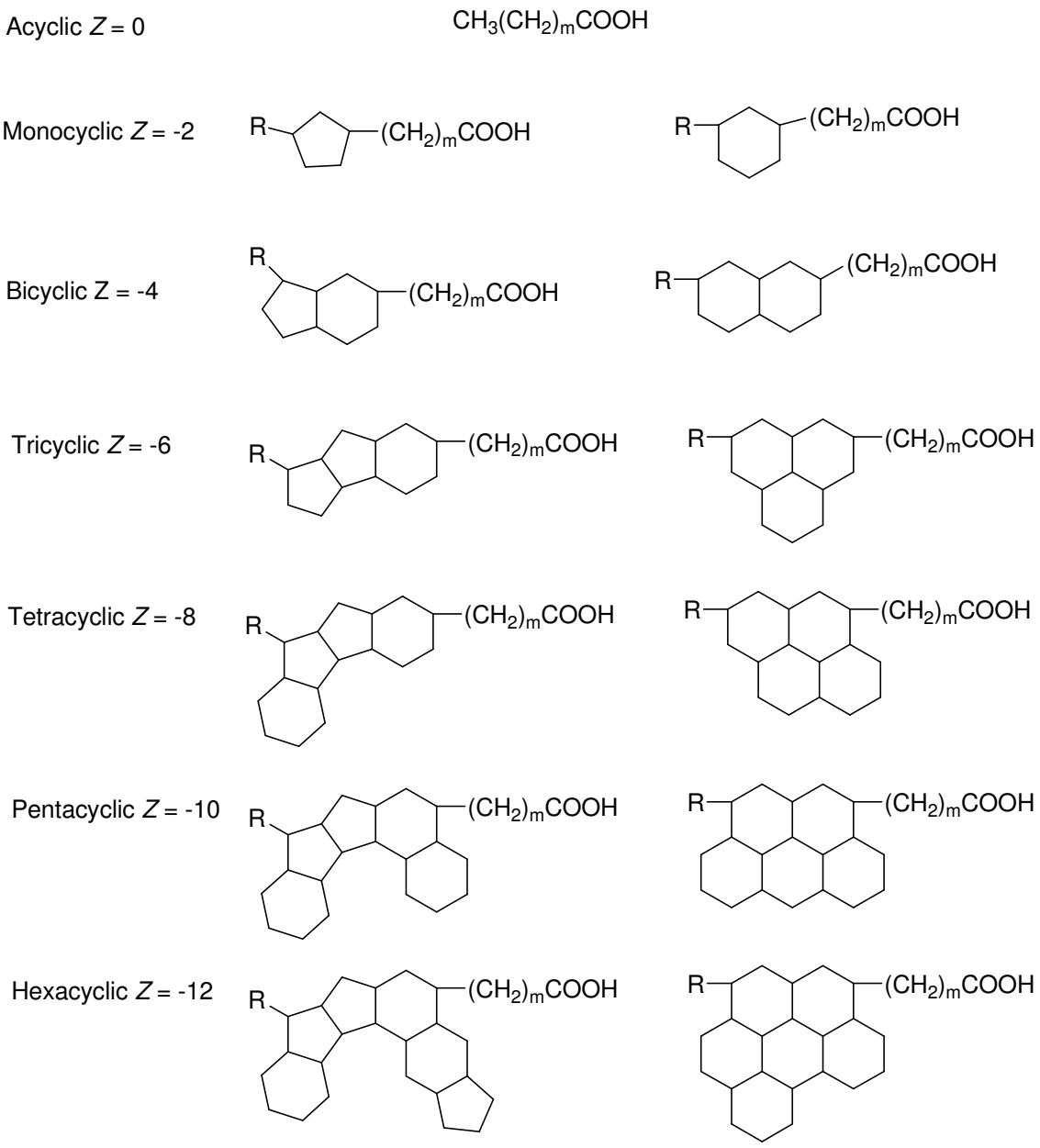

Figure 2- 1 Example NAs where R represents an alkyl chain, *Z* describes the hydrogen deficiency, and *m* is the number of CH_2 units

2.1. Properties of NAs

NAs are a complex mixture, and thus their physical and chemical properties vary depending on chemical composition and molecular structures (Kannel and Gan, 2012). However, broadly speaking, the overall mixture of NAs can be characterized by several physical and chemical properties as summarized in Table 2-1. In general, molecular weights of NAs range from 150 to 450 g/mol (Mohammed and Sorbie, 2009), with an average molecular weight of 300 g/mol. NAs are chemically stable (Rogers et al., 2002) and non-volatile (vapor pressure = 2.4×10^{-6} atm and Henry's law constant = 8.6×10^{-6} atm m^3/mole). Their polarity and non-volatility increase with increasing molecular weights. The boiling points of NAs are high, ranging between 250 ^{0}C and 350 ^{0}C (Brient et al., 1995). Their dissociation constants (pK_a) range between 5 and 6 (Kanicky et al., 2000), values which are typical of most carboxylic acids (acetic acid = 4.74, propionic acid = 4.87, and palmitic acid = 4.78). NAs can act as surfactants and concentrate at the aqueous/non-aqueous interfaces as they have both a hydrophilic (carboxylic group) head and a hydrophobic (non-polar aliphatic) end in the structure. NAs are soluble in organic solvents, like dichloromethane, ethyl acetate and methanol. In water, their solubility is pH dependent and increases with increasing pH (Quagraine et al., 2005). For example, at 25 ^{0}C, in water, NAs have solubility values of 0.07 g/L at pH 0.91, and 5.04 g/L at pH 9.16. Also, the solubility of NAs depends on the structure of NAs, for example, higher molecular weight NAs (i.e., multi-cyclic NAs) are less soluble compared to lower molecular weight NAs (with fewer cyclic rings). NAs show moderately strong sorption to

soils (K_d = 1.3-17.8 mL/g; logK_{ow} = 2.08-4.3), which is the key factor that limits the bioavailability of oil sands NAs in aqueous environments (Providenti et al., 1993).

Table 2- **1**Physical and chemical properties of NAs

Parameters	**Properties**
Color	Pale yellow, dark amber
Odor	Musty hydrocarbon odor
State	Viscous liquid
Molecular weight	Generally between 150 and 450 atomic mass units
Solubility	Completely soluble in organic solvents, soluble in water at neutral or alkaline pH (at NAs < 50 mg/L)
Density	0.97~0.99 g/cm^3
Boiling point	250~350 ^{0}C
Dissociation constant (pK_a)	5~6
Henry's law constant	8.6×10^{-6} atm m^3/mole
Vapor pressure	2.4×10^{-6} atm
Log (K_{ow})	4.3 at pH 1.07, 2.4 at pH 7.1 and 2.1 at pH 10
Partition coefficient (mL/g)	0.2 for coarse sands and 2.5 for oil sands fines; 1.3 for water and 17.8 for synthetic groundwater

2.2. Sources of NAs

NAs can be obtained from either commercial sources(Merichem, Kodak, Acros and Sigma) which produce synthetic NAs, or can be extracted from OSPW. Both NAs from commercial preparations and from OSPW are complex mixtures. However, commercial NAs mixtures are generally further refined, making them purer than NAs extracted from OSPW. This purification process includes caustic extraction of petroleum distillates carried out between 200 and 370°C, followed by an ethanol extraction to remove unsaponifiable material, and this purified extract is then acidified to return to their protonated form of NAs (Brient et al., 1995).

The compositions of these NAs mixtures can also vary significantly(Headley and McMartin2004).Commercial NAs are composed mainly of low molecular mass compounds, with *n* of about 10-14(Clemente et al., 2003; Clemente et al., 2004),with an averageof around 12-14(Martin et al., 2008). However, the NAs in OSPW are typically composed of a broader range of molecular mass, with *n*ranging from 5-28 (Holowenko et al., 2002)or even, in rare instances, 40(Lo et al.,2003). This difference in composition between commercial NAs and OSPW extracted NAs is illustrated in Figure 2-2. Furthermore, even among OSPW-extracted NAs derived from different sources, compositions can vary significantly (Headley and McMartin, 2004).

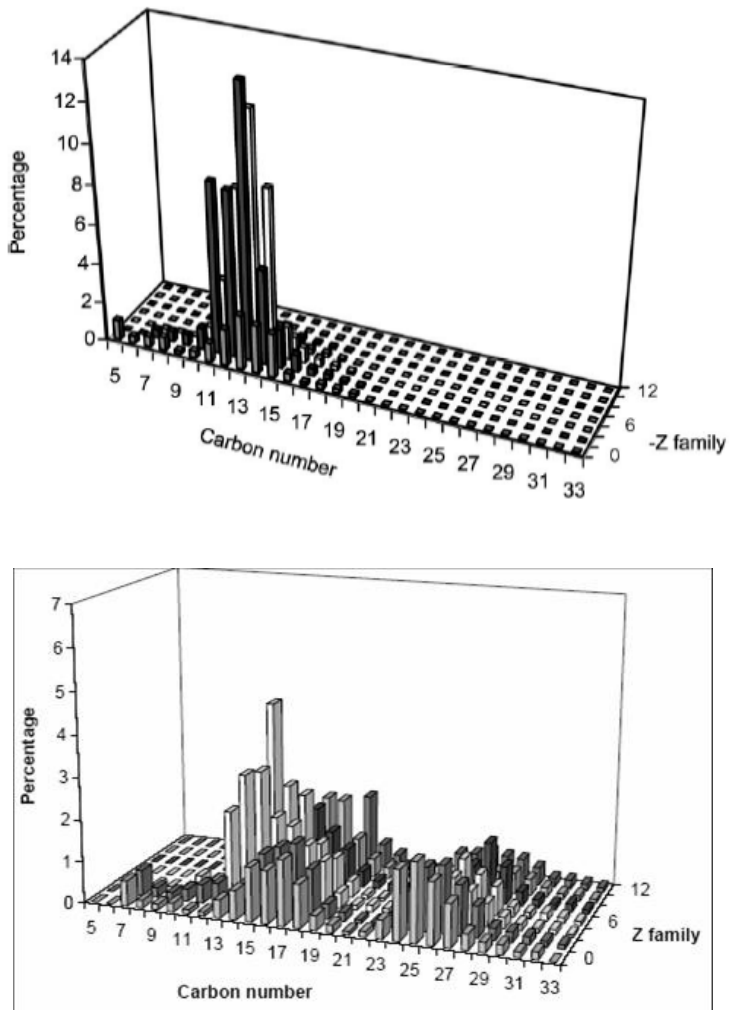

Figure 2- 2NAs extracted from OSPW (bottom) shows a broader distribution in composition than commercial NAs (top) (*adapted from Clemente et al., 2003*)

2.3. Corrosivity of NAs

One concern associated with NAs in an industry setting is that NAs are corrosive to processing and refining infrastructure. Even steel alloys which are generally resistant to corrosion caused by sulfide-containing compounds can be susceptible to corrosion by NAs (Kane and Cayard, 1999). It is not entirely clear why NAs cause corrosion, but it is

known to involve the chelation of the metal ion of the corroding surface by the carboxylic group of the NAs with the production of hydrogen gas. Thus, corrosivity by NAs also depends on the availability of the carboxylic group of NAs to form metal complexes and the molecular composition of the present metal compounds (Slavcheva et al., 2009).

2.4. Toxicity of NAs

NAs are acutely and chronically toxic to a variety of organisms including: plants, fish, mammals, zooplanktons, phytoplanktons and bacteria (Allen, 2008; Whitby, 2010). More specifically, NAs have been shown to be toxic to fish at concentrations greater than 2.5 to 5 mg/L (Clemente and Fedorak, 2005; Whitby, 2010). Due to the presence of NAs, fresh tailings water is acutely toxic to aquatic organisms (LC_{50}< 10 % v/v for rainbow trout) (MacKinnon and Boerger, 1986) and mammals (oral LC_{50} = 3.0 g/kg body weight) (Headley and McMartin, 2004). Because of the inherent complexity of NAs mixtures, it is not feasible at this time to determine which individual compounds contribute to the toxicity of NAs (Clemente and Fedorak, 2005). An important observation about NAs toxicity is that toxic effects of NAs are not directly concentration-dependent, but are more a function of content and complexity of NAs mixtures (Brient et al., 1995; Lai et al., 1996). For example, Holowenko et al. (2002) demonstrated that the toxicity of NAs is primarily caused by lower molecular weight NAs, a finding that was later confirmed by NMR studies and Microtox toxicity testing by Frank et al. (2009). In addition to molecular weight, other factors including pH, salinity, molecular size and hydrophobicity have also been shown to affect the toxicity of NAs. The toxicity of NAs is often associated with their surfactant characteristics: as surfactants, NAs can easily penetrate the cell wall of a biological membrane (Rogers et al., 2002a; Rogers et al., 2002b). Due

to the surfactant characteristics of NAs and their relative small molecular size (molecular weight ranging between 150 and 450), the primary proposed action mechanism to aquatic organisms for NAs is believed to be narcosis leading to membrane disruption(Roberts, 1991; Frank et al., 2008; Frank et al., 2009).

2.5. Quantification of NAs

NAs are a complex mixture, so analysis and quantification of NAs in environmental samples has been a significant challenge (Grewer et al., 2010). In the last decade, due to the prevalence and toxicity of NAs, a lot of analytical methods have been developed to characterize and quantify NAs.

2.5.1. Common issues associated with quantification of NAs

To date, no absolute quantification method for NAs in environmental samples has been reported, primarily because NAs are present in the environment as a complex mixture and currently there is no procedure that can separate and identify individual NAs in a mixture. All of the reported quantification methods for NAs treat NAs as a group and use standards for calibration. The accuracy of these measurements is based on two assumptions.First, the standard used for calibration is representative of the NAs in the samples, and secondly, samples and the calibration standard have the same response factor under the same experimental conditions.

Unfortunately, this is rarely the case for environmental NAs as they are always present as complex mixtures.As a result, no perfect standard is available for calibration. Currently, the reported calibration standards available include commercial NAs, NAs extracted from OSPW and single carboxylic acids.

A common standard used for calibration is commercial mixtures of NAs (such as Merichem, Kodak, Acros and Sigma NAs). However, the commercial NAs may not be ideal calibration standards for quantifying NAs in OSPW due to the factors discussed above and because: the composition of NAs differs among the suppliers of the commercial NA mixtures, which can affect the quantitative results obtained (Scott et al. 2005). Additionally, commercial NAs and NAs extracted from OSPW have different composition: in terms of carbon number (*n*), commercial NAs are composed of mainly low molecular mass, with *n* of about 10-14(Clemente et al., 2003; Clemente et al., 2004), but the NAs in OSPW are typically composed of a broader range of molecular mass, with *n* of 5-28 (Holowenko et al., 2002)or even 40(Lo et al., 2003); in terms of *Z*, commercial and OSPW extracted NAs also show different distributions. For example, Merichem NAs have an even distribution of Z = 0 to -4; Kodak NAs have Z = -2 and -4 as the most abundant acids; and Acros NAs have acids with Z = 0. However, NAs extracted from Syncrude and Suncor OSPW show a relatively low content of Z = 0 acids, with more acids of Z = -2 to -6. Therefore, using commercial NAs to represent NAs from OSPW and fresh surface water is oversimplified since the isomer groups in these reference materials are different from those in OSPW (Grewer et al., 2010). Taken together, these findings suggest that commercial NAs may not represent the complexity of real NAs extracted from OSPW samples.

An alternative to using commercial NAs as standard is to use OSPW extracted NAs (Martin et al., 2008). This is also problematic as compositions of NAs from OSPW greatly depend on what sources they are extracted from. For example, a comparative study (Clemente et al., 2003) revealed that the complexity of NAs from tailings pond

water vary significantly. For this reason, it is imperative that research should always include the source of NAs being studied so that results can be compared across studies and interpreted correctly without confusion.

Sometimes, single commercial available carboxylic acids (i.e., 1-pyrenebutyric acid) can be used as calibration standards for NAs quantification. This approach simplifies quantification, reporting and data interpretation compared to the calibration methods using commercial or OSPW extracted NAs as standards. However, this method is also oversimplified because NAs present in OSPW are complex mixtures, not a single carboxylic acid.

The following section provides a summary for the widely used methods for NAs quantification. For each method, its working principle and detection limit will be discussed, and advantages and disadvantages will be reviewed. This information is summarized in Table 2-1.

2.5.2. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy is the oil sands industry standard method for quantifying NAs, and was developed by Syncrude Canada Ltd. (Jivraj et al., 1995). In the FTIR method, OSPW samples, usually pH 8-9(conditions under which, NAs are deprotonated to their conjugate bases and are soluble in water) are acidified to pH $\leq$ 2.5 (at this pH, NAs are protonated and are soluble in organic solvents), and then the NAs are extracted into dichloromethane. The extract is then dried and is analyzed by FTIR. The absorbance of the dimeric and monomeric forms of the carboxylic groups (at 1706 cm^{-1} and 1743 cm^{-1}, respectively) is measured. The sum of the absorbance at these two wavenumbers is compared to those in

a calibration curve prepared with commercial NAs. The detection limit of FTIR method for NAs quantification is typically 1 mg/L, but this depends on the volume of OSPW sample extracted, and the extent to which the dichloromethane extract is concentrated prior to FTIR analysis (Jivraj et al., 1995).

2.5.3. High performance liquid chromatography (HPLC)

Based on Miwa's HPLC method for measuring carboxylic acids (Miwa 2000), Clemente et al. (2003) developed a HPLC method for NAs quantification. In this method, NAs are reacted with 2-nitrophenylhydrazine (NPH) in the presence of the coupling agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in aqueous solution (Figure 2-3). Then the derivatized products are injected onto a HPLC reversed phase column and can be detected by Ultraviolet-Visible spectrophotometry (UV-Vis). The derivatized NAs are eluted as an unresolved hump, which is integrated to get the area under the HPLC chromatogram. The same procedure is used for a series of commercial NAs with known concentrations to prepare a calibration curve. Historically, the detection limit of this method is about 15 mg/L. This method was then optimized by Yen et al. (2004) by modifying the reaction conditions to improve the detection limit to about 5 mg/L.

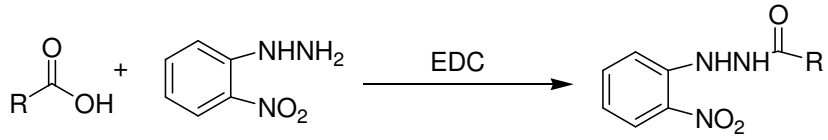

Figure 2- 3 Derivatization of NAs to their hydrazine derivatives, which can be separated by HPLC and detected by UV-Vis

2.5.4. Gas chromatography (GC)

NAs have boiling points ranging between 250 to 350 ^{0}C and are non-volatile, so they have to be derivatized to volatile derivatives which can be separated by GC. In GC-Flame ionization detection (FID) method, NAs are extracted from aqueous solutions and derivatized to their methyl esters by reacting with either BF_3 in MeOH or diazomethane, which are then separated by GC and detected by FID. In GC-MS method, NAs are extracted from aqueous solutions and derivatized to their *tert*-butyldimethylsilyl esters by reacting with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) (Figure 2-4), which are then separated by GC and detected by MS. The esters are eluted from the GC column as an unresolved hump in the chromatogram and the total area of the hump is integrated and then compared to the area of an internal standard to calculate the total NAs concentration in an unknown sample. Both GC-FID and GC-MS methods have detection limit of 0.01 mg/L.

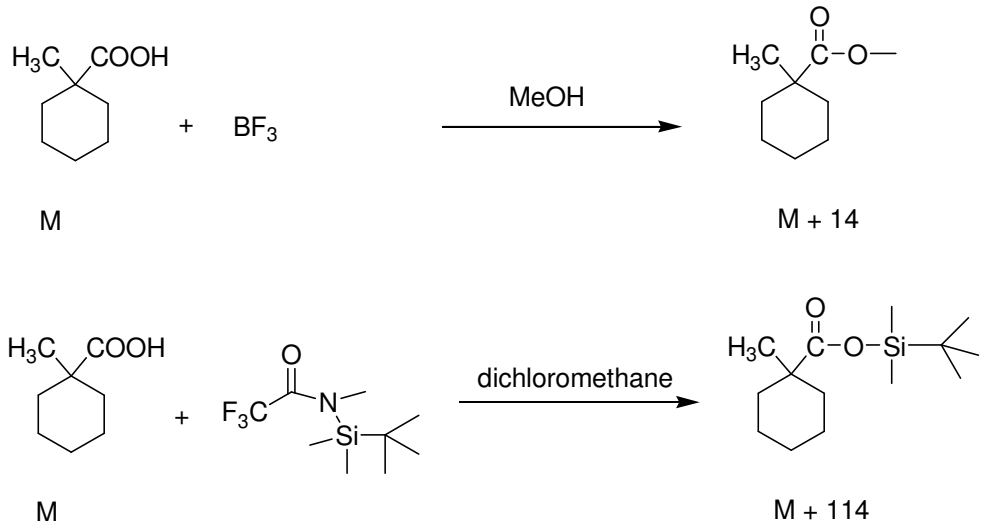

Figure 2- 4 Derivatization of example NAs to the volatile methyl ester or *tert*-butyldimethyl ester for GC separation

2.5.5. Fluorescence spectrophotometry

The classical formula used to describe NAs, $C_nH_{2n+Z}O_2$,indicates that NAs are not fluorescent. However, various levels of unsaturation and aromaticity in NAs enable the absorption of ultraviolet-visible light and subsequent fluorescence emission (Mohamed et al., 2008; Kavanagh et al., 2009). This characteristic of NAs provides an opportunity to use fluorescence as a detection method for NAs quantification by taking advantage of its high sensitivity. Either the peak intensity of the fluorescence emission spectrum or the area under the fluorescence emission spectrum of the unknown samples can be compared to that of commercial NAs and the concentrations can be calculated (Mohamed et al., 2008; Lu et al., 2013). This method has a detection limit of around 1 mg/L.

2.5.6. Electrospray ionization mass spectroscopy (ESI-MS)

ESI is one of the soft ionization techniques in mass spectroscopy resulting in less fragmentation and has been used to determine the concentration and molecular composition of NAs in environmental samples. ESI-MS with or without HPLC separation has been used to quantify NAs in environmental samples (Headley et al., 2009). Headley et al. (2002) concentrated NAs from aqueous samples and then purified NAs by solid phase extraction using a divinyl benzene support sorbent. The pretreated NAs were then eluted from the solid phase sorbent with acetonitrile and the solution was analyzed by ESI-MS with commercial Fluka NAs as the standard. The detection limit based on a 500 mL sample was reported to be 0.01 mg/L.

The methodology developed by Lo et al. (2003) involved negative ion ESI-MS without chromatographic preseparation. Briefly, NAs were extracted from tailings pond water into dichloromethane after adjusting the pH to ~2. Then the extract was dried and dissolved in a 1:1 (*v*/*v*) solution of 2-propanol and 1% isopropylamine in water. "Semi-quantitative" analysis of NAs was achieved using a standard addition method, spiking with cyclohexanebutyric acid as the internal standard.

Bataineh et al. (2006) combined HPLC and ESI-MS for quantification and characterization of NAs. In this method, congeners (*n* and *Z* combinations) in NAs were separated on a capillary reversed-phase HPLC column and detected by negative mode ESI-MS with tetradecanoic acid-1-^{13}C as the internal standard. The concentration of each congener was calculated based on its relative response to the internal standard and the total NAs concentration extracted from OSPW or commercial NAs are the sum of all analytes which adhere to the formula $C_nH_{2n+Z}O_2$ for values of n = 9 to 20 and Z = 0 to -12.

This method was further optimized by Pérez-Estrada et al. (2011) to improve the separation efficiency by using ultra-pressure liquid chromatography (UPLC) to separate congeners of NAs before ESI-MS analysis.

Wang and Kasperski (2011) developed a HPLC-MS-MS method for quantification of NAs. In this method, separation was performed on a reverse-phase column and detection was performed in the negative ion mode by either selective ion monitoring san or enhanced MS scan mode. Commercial Fluka NAs were used as the standard for quantification.

The utilization of HPLC in MS can significantly reduce matrix effects in MS analysis, also, chromatographic separation before MS analysis can help minimize possible false positive errors (Bataineh et al., 2006; Headley et al., 2009) as HPLC can separate NAs from other impurities.

Each of these NAs quantification methods has its own advantages and disadvantages in terms of rapidity, applicability, accuracy and cost.Table 2-1 compares the above mentioned NAs quantification techniques. FTIR measurements usually overestimate the concentrations of NAs (Grewer et al., 2009), this overestimation may be caused by the carboxylic groups of naturally occurring non-NAs in the aqueous sample and which also have a carboxylic group and therefore contribute to the measured IR absorbance at 1706 and 1743 cm^{-1}. However,FTIR is a fast and affordable analytical technique. The HPLC/MS (UPLC/MS) technique doesn't require sample pretreatment (NAs aqueous sample can be directly injected into HPLC/UPLC reversed phase column), but this technique is sophisticated because it requires specialized and expensive instrumentation as well as expertise in operation and data analysis, and relatively fewlaboratories have

this specialized mass spectrometry equipment. GC/FID and GC/MS involve derivatization of NAs, and the derivatization step can be time-consuming, and more importantly, sometimes incomplete conversion of NAs to their derivative takes place, which means that it is difficult to obtain quantitative data using GC/FID or GC/MS. FTIR and fluorescence spectrophotometry treat NAs as a group, which are not capable ofproviding information about molecular structures and compositions of NAs, furthermore, the quantification results by fluorescence emission spectrophotometry are much higher than those obtained by UPLC/MS (Lu et al., 2013). Compared with other methods, one advantage of MS methods is that they are able to provide information about molecular structures and compositions of NAs by categorizing NAs according to their *n* and *Z* number, which helps identify individual NAs. An important observation is that MS instrument settings affect quantification results by affecting signal intensity in a structure-dependent manner and it was suggested that different investigators should calibrate their instruments to obtain uniform results using reference NAs samples (Lo et al., 2003). And this suggestion raised a question about what reference NAs samples should be used as the calibration standard.

Table 2- 2Comparison of different NAs quantification methods

Method	**Detection limit**	**Advantage**	**Disadvantage**
FT-IR	1 mg/L	cost-effective, simple, no derivatization needed	not capable of separating congeners

HPLC-UV	5 mg/L	cost-effective	derivatization needed, not capable of separating congeners
GC-MS	0.01 mg/L	capable of separating congeners	derivatization needed, relatively expensive
UPLC-HRMS	0.1 mg/L	capable of separating congeners even isomers, no extraction needed	expensive, data analysis time consuming
Fluorescence	1.0 mg/L	cost-effective, simple, no derivatization needed	not capable of separating congeners, quantification results much higher than other methods

2.6. Degradation of NAs

Regulations do not allow for the discharge of OSPW into local aquatic environment and must be kept in tailings ponds until it can be remediated (Allen, 2008). These regulations have led to substantial research on OSPW remediation, primarily focusing on the degradation of NAs found in OSPW (Quagraine et al., 2005; Whitby, 2010). Among the reported approaches, two have been studied extensively: biodegradation and ozonation of OSPW.

2.6.1. Biodegradation of NAs

Laboratory investigations have been carried out to study the biodegradation of NAs. NAs used in these investigations fall into three categories: model NAs (individual carboxylic acids that fit with the formula $C_nH_{2n+Z}O_2$), commercially available NAs, and NAs extracted from OSPW (Clemente and Fedorak, 2005). When model NAs were used, usually, the goal of the investigations was to elucidate the biodegradation pathways of the individual carboxylic acids. On the other hand, investigations that utilized commercial NAs and OSPW-extracted NAs focused more on the biodegradation of these compounds as a group, because current analytical methods do not allow for the study of individual compounds in the complex mixture. Some of these investigations monitored how the toxicity of NAs was affected by biodegradation.

In the last decade, both aerobic and anaerobic cultures of indigenous microbial communities from oil sands tailings water have been investigated for their ability to degrade NAs, and both cultures have been shown promise if the appropriate nutrient conditions are provided (Herman et al., 1993; Hsu et al., 2000).

Herman et al. (1993) reported that both commercially available Kodak NAs and NAs extracted from OSPW could be biodegraded by aerobic consortia obtained from tailings pond water. This microbial community showed higher biodegradation efficiency for commercial NAs, releasing 48 % of the NAs carbon as CO_2, while the same community was only able to release 20 % of carbon from OSPW-extracted NAs as CO_2. Biodegradation of both commercial and OSPW extracted NAs corresponded to a decrease in toxicity, as determined by Microtox test (Herman et al., 1993).

The aerobic biodegradation of two commercial preparation of NAs (from Kodak and Merichem) and NAs extracted from OSPW were studied in detail (Clemente et al., 2004). It was found that commercial NAs are more biodegradable than OSPW-extracted NAs, additionally, their results indicate that the NAs with lower molecular weights and fewer rings are most susceptible to biodegradation. Further investigations indicate that concentrations of NAs and their toxicity can be reduced with the biodegradation of lower molecular mass NAs, preferentially $n \leq 21$, this is consistent with early findings that NAs toxicity is mainly caused by lower molecular mass NAs (Holowenko et al., 2002; Frank et al., 2009). In most recent findings, it has been suggested that biodegradation rates of NAs are affected primarily by their chemical structure. The more recalcitrant NAs are those with higher degree of alkyl substituted aliphatic chains (Han et al., 2008; Smith et al., 2008), tertiary substitution at positions other than the β-position to the carboxylic of the main carbon chain, methyl substitution on the cycloalkane rings (Herman et al., 1993), increased cyclicity (Han et al., 2008), evenness of carbon side chain, and *cis*-isomerism in alicyclic acids (Headley et al., 2002; Holowenko et al., 2002).

2.6.2. Ozonation of NAs

Scott et al. (2008) presented the first experimental evidence that ozone can react with OSPW NAs. It was shown that after 130 min of ozonation, more than 95 % of the initial NAs were degraded.

Similarly, investigations regarding ozonation of NAs used three kinds of NAs: model compounds; commercial NAs and NAs extracted from OSPW. NAs model compounds were mainly used to study ozonation reaction pathways.

How ozonation of NAs changed distribution of NAs isomers was also studied (Scott et al., 2008). Figure 2-5 shows the three-dimensional plots of the NAs present before and after 50 and 130 min of ozonation. After 130 min of ozonation, the residual ions were clustered between n = 5 to 15 with Z = 0, -2, and –4, this distribution is significantly different than that of the untreated NAs (Figure 2-5a). The data in Figure 2-5c and Table 2-2 clearly illustrates that extended ozonation decreases the average molecular weight of NAs, probably because higher molecular weight isomers of OSPW NAs had higher reactivity during ozonation.

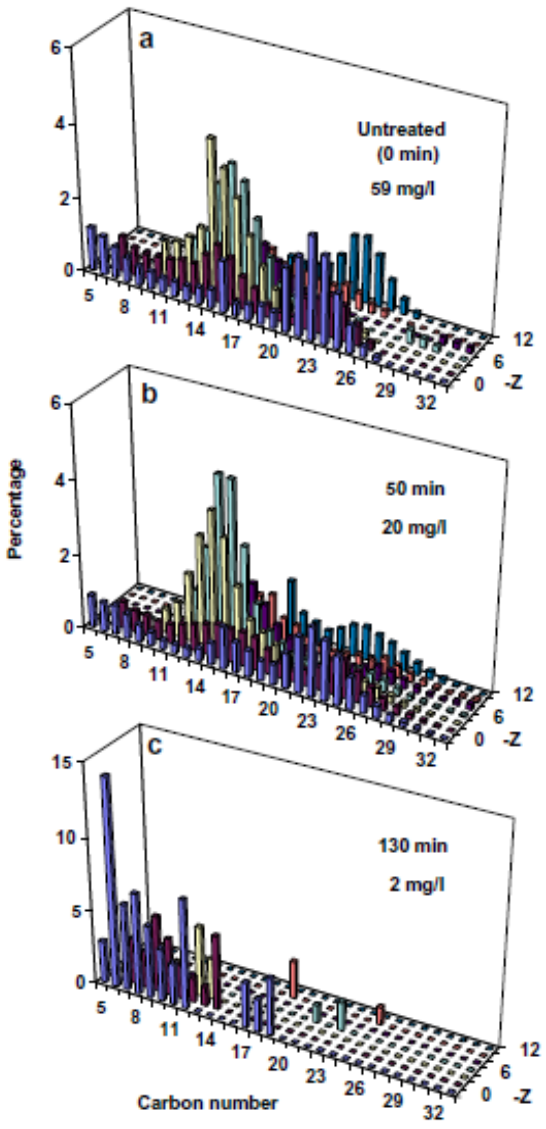

Figure 2- 5 Three-dimensional plots showing changes in the distribution of NAs isomers in OSPW treated with ozone (*adapted from Scott et al., 2008*)

Table 2- 3Proportion of ions in each carbon-number group before ozonation and after 50 and 130 min of ozonation of OSPW (*Modified from Scott et al., 2008*)

Carbon-number (*n*) group	Proportion of ions (%)		
	Time 0	Ozonated (50 min)	Ozonated (130 min)
5-13	19	19	79
14-21	53	54	18
22-33	28	27	3

Pérez-Estradaet al. (2011) studied ozonation of both the commercial Merichem NAs and OSPW NAs. For commercial Merichem NAs, it was found that NAs oxidation efficiency was pH dependent, showing highest efficiency at pH 8. The authors contributed the higher efficiency at higher pH to the deprotonation of NAs at alkaline pH because the pK_a of NAs ranges between 5 and 6 (Whitby, 2010) and studies have shown that for some weak acids, such as phenol, the reactivity of zone is greater for the deprotonated pheoxide anion (Joshi and Shambaugh, 1982).

The reaction kinetics of ozonation of commercial Merichem NAs was also studied (Pérez-Estradaet al.,2011). In each isomer class of the studied commercial Merichem NAs, degradation during the ozonation process at pH 8 and 10 followed second order kinetics. The overall structure-reactivity relationship based on the carbon number (*n*) and

the number of rings (Z/2) of NAs was summarized: reactivity generally increased with increasing *n* and with increasing number of rings. However, for OSPW NAs, reaction followed different kinetics from commercial Merichem NAs. Instead of following second order kinetics, the ozonation followed pseudo first order kinetics and, in general, reactivity increased with increasing carbon number (*n*) and number of rings (Z/2).

The reaction mechanism of ozonation of commercial NAs and OSPW extracted NAs was also studied ((Pérez-Estradaet al.,2011). The ozonation of commercial NAs primarily involved reactive hydroxyl radicals for NAs degradation, these hydroxyl radicals were produced by ozone under the natural pH of OSPW (ranging between 8-9). The mechanism of ozonation of OSPW NAs was difficult to study because of the complexity of the dissolved organics of OSPW (Allen, 2008).

A number of studies have been carried out to investigate the relationship between biodegradation and ozonation of NAs. Martin et al. (2010) reported that ozonation of OSPW accelerated microbial bioremediation, allowing residual NAs to be biodegraded significantly faster compared to non-ozonated OSPW. Similarly, Gamel El-Din et al. (2011) reported that ozonation of OSPW NAs increased the biodegradability of NAs by showing that the ratio between biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of NAs increased after ozonation. Given these results, it has been proposed that ozonation can be used in combination with other technologies (namely biodegradation) to remove NAs from OSPW. This combination is based on the capability of ozone to preferentially degrade the most recalcitrant NAs fraction from OSPW, creating an environment that will maximize the biodegradation capacity of microbial populations in OSPW (Martin et al., 2010).

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Chapter 3 Removal of naphthenic acids from oil sands process water

3. 1. Introduction

The oil sands regions located in Northern Alberta, Canada, contain an estimated 1.7 trillion barrels of oil in the form of bitumen (Kannel and Gan, 2012). The surface mining oil sands industry uses a caustic hot water extraction method to extract bitumen from the oil sands. The resulting oil sands process water (OSPW) is recycled into the extraction process to minimize the draw of fresh water resources and is retained on site in tailings ponds. Through the recycling process, compounds accumulate in the OSPW. One such compound, known as naphthenic acids (NAs), has been attributed to the acute and chronic aquatic toxicity of OSPW (Whitby, 2010). NAs are a complex mixture of naturally occurring alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$; where n is the carbon number and Z is zero or a negative even integer defining the hydrogen deficiency resulting from ring formation (Headley and McMartin, 2004; Clemente and Fedorak, 2005; Whitby, 2010; Kannel and Gan, 2012).

The concentration range of NAs in OSPW is approximately 20-120 mg/L (Clemente et al., 2004). Hence, remediation of OSPW will allow for the potential future discharge into the local aquatic environment. This requirement has led to substantial research on OSPW remediation, primarily focusing on the degradation of NAs found in OSPW (Quagraine et al., 2005; Whitby, 2010). Among the reported approaches, two have been extensively studied: biodegradation and ozonation of OSPW. Microbial biodegradation was expected to be the most cost-effective method for removing NAs, but it is slow and limited success has been achieved in decreasing the concentration of NAs to below 19 mg/L due to a

persistent fraction of NAs remaining after even decades of storage (Martin et al., 2010;Pérez-Estrada et al., 2011; Kannel and Gan, 2012); Ozonation has been shown to be able to significantly reduce concentrations of NAs in OSPW, while increasing their biodegradability, and consequently reduce the toxicity (Scott et al., 2008; Martin et al., 2010; Gamal El-Din et al., 2011; Garcia-Garcia et al., 2011; He et al., 2011; Pérez-Estrada et al., 2011). However, ozonation has its limitations: it is expensive to generate ozone, and the low water solubility (Watts and Teel, 2006) and the short half-life of ozone in water ($t_{1/2}$≈ 20 min at 20 ^{0}C) limit the distances over which it can be delivered into water (Seol et al., 2003). Taking into account the large existing stores of OSPW and ongoing expansion of the Alberta oil sands industry, a more cost-effective and rapid approach for removing NAs from OSPW is highly desirable.

In the last decade, in situ chemical oxidation, which is based on the delivery of oxidants to a contaminated source zone and the subsequent oxidation of contaminants into harmless end products within the natural environment, has been increasingly studied to develop novel remediation strategies. Four oxidation systems are commonly used in chemical oxidation of contaminants: catalyzed hydrogen peroxide (Fenton's reagent), permanganate, ozone and persulfate (Watts and Teel, 2006). Table 3-1 lists the commonly used oxidizing agents with their chemical formula and redox potential (E^0).

Table 3- 1Commonoxidizing agents for contaminant removal

Oxidant	**Chemical formula**	**Redox potential** (V)

Hydroxyl radical[1]	$OH^{\cdot}$	2.7
Sulfate radical[2]	$SO_4^{-\cdot}$	2.6
Ozone	O_3	2.2
Persulfate anion	$S_2O_8^{2-}$	2.1
Hydrogen peroxide	H_2O_2	1.8
Permanganate ion	MnO_4^-	1.7

[1]Usually generated by the combination of oxidizing agents with catalysts or UV irradiation

[2]Generated from the activation of the persulfate anion $S_2O_8^{2-}$

Each of the oxidants has different characteristics such as (1) their stability in the environment, (2) selective reactivity to different types of contaminants, and (3) their oxidative power, which is reflected by the different redox potentials as given in Table 3-1. Compared with the other oxidants, persulfate has the advantage of both high stability and wide spread reactivity, also, it can oxidize contaminants under a wide range of pH conditions, which has led to increasing research on its use (Watts and Teel, 2006). Persulfate itself is a strong oxidant (E^0 = 2.1 V), however, the reaction between the persulfate anion itself with contaminants is usually kinetically slow (House, 1962). The persulfate anion can be activated by heat, transitionmetal ions, ultraviolet light or ultrasound to generate a sulfate anion radical ($SO_4^{-\cdot}$), which reacts with contaminants at a

kinetically faster rate and is an even stronger oxidant (E^0 = 2.6 V) than the persulfate ion (Equation 1):

$$S_2O_8^{2-} + \text{activator} \rightarrow 2\ SO_4^{-\cdot} \qquad (1)$$

Transition metal ions are often used in this activation reaction. Ferrous ion (Fe^{2+}) is most commonly used (Equation 2) because it is inexpensive, readily available and environmentally benign (Tsitonaki et al., 2010).Zero-valent iron (Fe^0) can also be used to activate persulfate to produce the sulfate anion radical as Fe^0 can serve as a slow releasing source of dissolved Fe^{2+} as described in the following equations (Equations 3 and 2):

$$S_2O_8^{2-} + Fe^{2+} = SO_4^{-\cdot} + SO_4^{2-} + Fe^{3+} \qquad (2)$$

$$Fe^0 + S_2O_8^{2-} = Fe^{2+} + 2\ SO_4^{2-} (3)$$

We hypothesized that NAs can be removed by chemical oxidation from OSPW and chose to focus on persulfate because of its above-mentioned advantages. The objective of this research was to establish the technical feasibility of using activated persulfate as an innovative and efficient strategy for the removal of NAs from OSPW. To elucidate this goal, the present study explored the oxidation of NAs by persulfate activated with Fe^{2+} and Fe^0inbatch experiments to determine: (1) the optimal ratio of persulfate-to Fe^{2+} or Fe^0 in terms of NAs degradation efficiency, (2) the reactivity of the oxidation process at temperatures (12-32 ^{0}C), which mimick field conditions and to determine the influence of temperature on the rate of reaction, and (3) the impact of chemical oxidation on OSPW water chemistry.

3.2. Materials and methods

3.2.1. Chemicals and tailings water collection

Chemicals used to prepare buffer solutions and eluents were of reagent grade and were used without purification. Sodium persulfate and ferrous sulfate heptahydrate as well as zero valent iron were purchased from Sigma Aldrich Co. (St. Louis, MO, USA).

Suncor OSPW was collected from the South Tailings Pond (June 2011) by Suncor Energy Inc. Syncrude OSPW was collected from the clarified zone of its "West-In-Pit" (May, 2011). Albian Sands OSPW was collected from the External Tailings Facility Pond (June, 2009). All of the OSPW samples were stored in the dark at $4^{\circ}C$ in sealed polyethylene pails prior to use.

3.2.2. Chemical oxidation experiments

Oxidation experiments were conducted in 1.0 L Erlenmeyer flasks containing 500 mL of Suncor OSPW at fixed temperatures (12, 22 and 32 ^{0}C). Sodium persulfate was added into the solution and shaken on a shaker at 200 rpm, followed by addition of $FeSO_4$ or Fe^0. The concentrations of sodium persulfate and $FeSO_4$ or Fe^0 were varied between experiments and are reported in the Results section. All the experiments in this study were carried out in the dark. At selected time intervals, 10 mL samples were collected from each flask and were immediately added to a 50 mL vial containing 2 mL of methanol (CH_3OH) as a radical scavenger to quench the oxidation reaction. In each vial, the pH was adjusted to around 2.5 with 1 M HCl and the remaining NAs were extracted with dichloromethane. All experiments were conducted in duplicate and data presented in all figures are mean values.

3.2.3. Chemical analysis

NAs were extracted from OSPW by liquid-liquid extraction and the quantification of NAs was performed using a PerkinElmer 100FT-IR spectrometer. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were measured using Standard Methods (APHA et al., 2005). Total organic carbon (TOC) and the total inorganic carbon (TIC) were measured using a TOC analyzer (Shimadzu, model TOC-L CPH, Japan). Aqueous lithium, sodium, ammonium, potassium, magnesium and calcium concentrations were quantified using a Dionex ICS-2000 Ion Chromatography system. Aqueous fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate concentrations were determined using a Dionex ICS-2500 Ion Chromatography system. The concentrations of metal ions iron and aluminum were quantified using a PerkinElmer ELAN 9000 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The concentration of total carbonate (including carbonate and bicarbonate) was determined by titration with hydrochloric acid using methyl orange as the indicator.

3.2.4. Toxicity measurements

The acute toxicity of OSPW samples was measured using a Microtox® 500 analyzer (Azur Environmental, Carlsbad, CA, USA), with an incubation time of 5 min and the 81.9 % Basic Test Protocol. All materials were purchased from Azur Environmental (Carlsbad, CA, USA). The percentage of inhibition after incubation and the toxicological parameters EC_{20} and EC_{50} (effective volume concentration percentage required to decrease bacterial luminescence by 20 % and 50 %, respectively) was calculated from the change in luminescence intensity. Phenol toxicity was used as quality control to verify

the sensitivity of the luminescent bacteria prior to the analysis of OSPW samples (phenol EC_{20} between 5 and 8 mg/L and phenol EC_{50} between 13 and 26 mg/L, respectively).

3.3. Results and discussion

3.3.1. Equation describing oxidation of NAs by sulfate anion radical

The complete oxidation of NAs by sulfate anion radicals to carbon dioxide and water is represented stoichiometrically as

$$C_{20}H_{28}O_2 + 104\ SO_4^{-\cdot} + 38\ H_2O = 104\ SO_4^{2-} + 20\ CO_2 + 104\ H^+ \quad (4)$$

in which NAs are represented by $C_{20}H_{28}O_2$ with a molecular weight of 300 g/mol, as NAs are a complex mixture containing different molecules with molecular weights ranging between 150 and 450 g/mol, averaged at 300 g/mol (Holowenko et al., 2002). To determine the optimal ratio of persulfate-to Fe^{2+} or Fe^0 needed to create $SO_4^{-\cdot}$(which are subsequently used to oxidize NAs), batch experiments were conducted at 22 ^{0}C and the results are outlined and discussed in Sections 3.2, 3.3 and 3.4.

3.3.2. NAs quantification

Quantification methods of NAs were reviewed in Chapter 2. FTIR is the industrial standard method that has been most commonly used in the measurement of NAs. It was reported that FTIR measurements usually overestimate the concentrations of NAs (Grewer et al., 2009). However, FTIR is a fast and affordable analytical technique. FTIR uses certain standard for calibration curve so it is anticipated that the measured NAs concentrations depend on which standard is used. In order to choose an appropriate standard, the FTIR spectra of NAs extracted from OSPWs were compared with those of

the commercial NAs. As shown in Figure 3-1, FTIR spectra of commercial NAs solutions of the same concentration (1 mg/g in CH_2Cl_2) display different characteristics in terms of peak wavenumber and intensity. The maximum absorbances of Merichem and Kodak NAs are located at 1706 and 1743 cm^{-1}, with Merichem NAs showing higher absorbance at both wavenumbers. In contrast, Acros and Sigma-Aldrich NAs absorb at relatively lower wavenumbers: 1697 and 1737 cm^{-1} for Acros NAs and 1700 and 1738 cm^{-1} for Sigma-Aldrich NAs. The maximum absorbances of the NAs extracted from three different OSPWs are all at 1706 and 1743 cm^{-1}, the same as those of Merichem and Kodak NAs. In this sense, the Merichem and Kodak NAs more closely resemble the NAs originating from all three OSPW sources (Syncrude, Suncor, and Albian Sands). Table 3-2 lists the FTIR results of NAs concentrations with different commercial NAs as the standards, it can be seen that the quantification results significantly depend on which standard is used.

Table 3-3 compares the concentrations measured by FTIR with Merichem NAs as the standards to the concentrations measured by fluorescence emission spectrophotometry and UPLC-TOF/MS. The fluorescence emission spectrophotometry results were presented here is because our group has spent long time on developing it as a quantification method for NAs (Ewanchuk, 2010). The concentrations determined by fluorescence emission spectrophotometry are much higher than those measured by the FTIR and UPLC-TOF/MS methods and the concentrations determined by FTIR are comparable to those measured by UPLC-TOF/MS methods. All of the quantification of NAs in this thesis was done by FTIR with Merichem NAs as the standards.

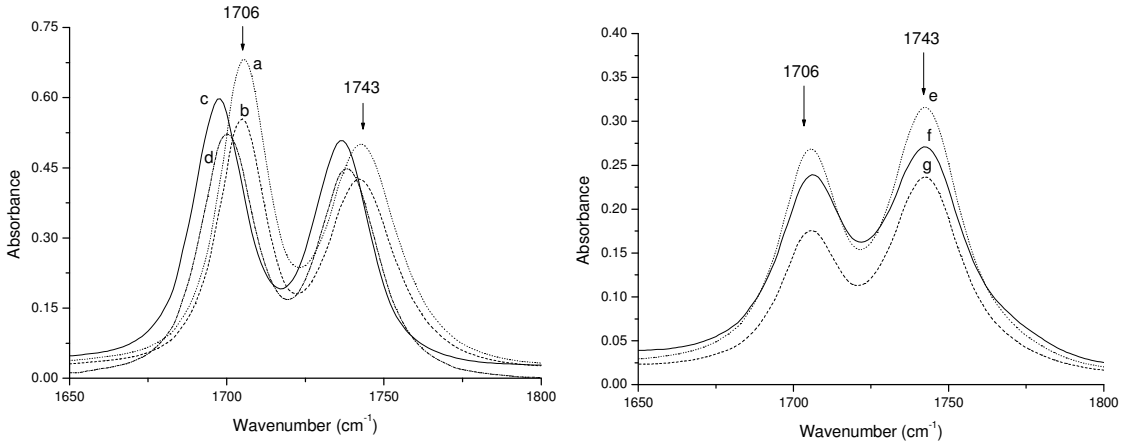

Figure 3- 1FTIR spectra of NAs from four commercial resources and from oil sands tailings ponds: (a) Merichem NAs; (b) Kodak NAs; (c) Acros NAs; (d) Sigma-Aldrich NAs; (e) NAs extracted from Syncrude OSPW; (f) NAs extracted from Albian Sands OSPW; and (g) NAs extracted from Suncor OSPW

Table 3- **2**NAs concentration measured by FTIR with different commercial NAs as standards

Standard	**NAs concentration (mg/L)**		
	Suncor OSPW	Syncrude OSPW	Albian-Sands OSPW
Merichem	11.0 ± 0.4	25.5 ± 0.1	8.3 ± 0.2
Kodak	13.5 ± 0.4	30.9 ± 0.7	10.1 ± 0.5

Sigma-Aldrich	16.1 ± 0.4	37.2 ± 1.1	12.1 ± 0.4
Acros	17.1 ± 0.3	39.6 ± 0.8	12.9 ± 0.9

Table 3- **3**Comparison of FTIR and fluorescence, UPLC-TOF/MS analyses of NAs extracted from OSPWs

NAs Source	Fluorescence[a] (Peak area) (mg/L)	Fluorescence[a] (Intensity) (mg/L)	FTIR (mg/L)[a]	UPLC-TOF/MS (mg/L)
Syncrude	594	576	25.5	20.7
Suncor	541	535	11.0	12.9
Albian-Sands	407	398	8.3	6.8

[a] Merichem NAs as standard.

3.3.3. Oxidation of NAs by Fe^{2+}-activated persulfate

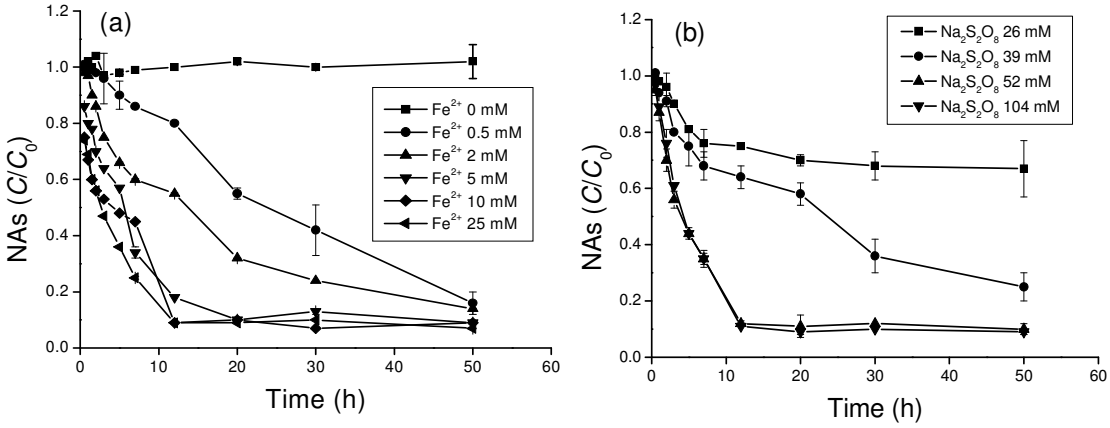

Figure 3- 2(**a**) NAs concentration change as a function of Fe^{2+} concentration (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; pH = 8.3); (**b**) NAs concentration change as a function of persulfate concentration (Initial conditions: NAs = 0.05 mM; Fe^{2+} = 10 mM; pH = 8.3)

Figure 3-2a represents the NAs concentration over time in the presence of persulfate and varying doses of Fe^{2+}. The batch reactors contained 0.05 mM (15 mg/L) NAs and 52 mM persulfate(the ratio of persulfate over NAs is 1040, or persulfate is 10 times in excess of the amount required by Equation 4). It can be seen from Figure 3-2a, in the absence of ferrous ion, little change in the NAs concentration was observed within 50 h. On the other hand, a 91 % decrease of NAs was recorded with 5 mM Fe^{2+} within 20 h. When 10 mM Fe^{2+} was added, the reaction was further accelerated and 91 % removal of NAs was achieved within 12 h. Further increases in Fe^{2+} did not benefit NAs removal.

Figure 3-2b shows the effect of persulfate concentration on the oxidation of NAs in OSPW. The initial NAs concentration was 0.05 mM and initial Fe^{2+} concentration was 10

mM. It can be observed that the rate of NAs transformation increased with increasing persulfate concentration until 52 mM, after which, further increase in persulfate concentration did not increase NAs removal efficiency. As seen from Equation 2, Fe^{2+} is needed to activate $S_2O_8^{2-}$ togenerate $SO_4^{-\cdot}$ and increasing Fe^{2+} would lead to fast production of $SO_4^{-\cdot}$, however according to Equation 5, Fe^{2+} in excess may lead to consumption of produced $SO_4^{-\cdot}$ (Tsitonaki et al., 2010).

$$S_2O_8^{2-} + Fe^{2+} = SO_4^{-\cdot} + SO_4^{2-} + Fe^{3+} \qquad (2)$$

$$SO_4^{-\cdot} + Fe^{2+} = SO_4^{2-} + Fe^{3+} \qquad (5)$$

The optimal ratio of Fe^{2+} over $S_2O_8^{2-}$ (when the highest NAs oxidation efficiency was achieved) from Figure 3-2a, was 0.19, which is the same as that obtained from Figure 3-2b.

3.3.4. Oxidation of NAs by Fe^0-activated persulfate

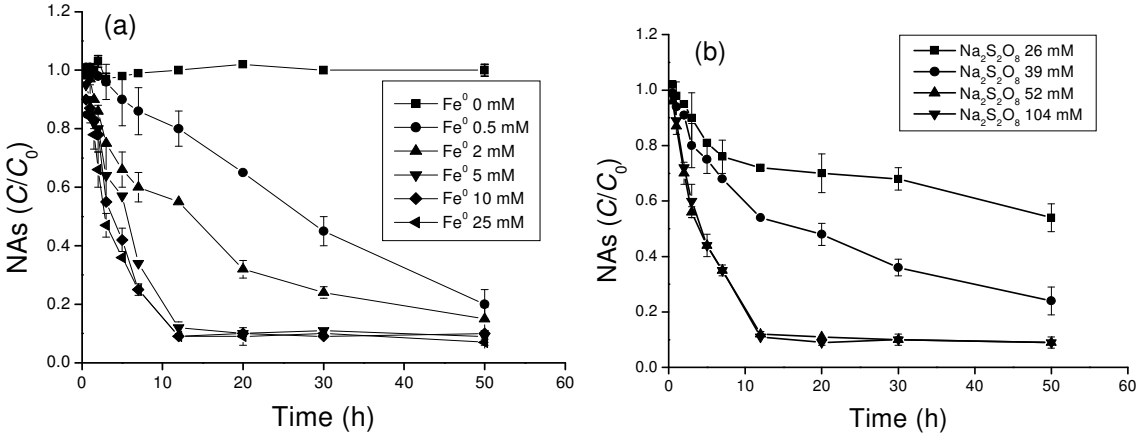

Figure 3- 3 (**a**) NAs concentration change as a function of Fe^0 concentration (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; pH = 8.3); (**b**) NAs concentration

change as a function of persulfate concentration (Initial conditions: NAs = 0.05 mM; Fe^0 = 5 mM; pH = 8.3)

Figure 3-3a represents the NAs concentration over time in the presence of persulfate and varying doses of Fe^0. Similar to the addition of Fe^{2+}, the addition of Fe^0 could enhance the oxidation of NAs in the persulfate system. In the absence of Fe^0, little change in the NAs concentration was observed within 50 h. In the presence of 5 mM of Fe^0 (persulfate to Fe^0 molar ratio of 10.4:1), approximately 90 % of the NAs were oxidized in 12 h. Increasing Fe^0 to 10 mM slightly increased the oxidation efficiency within the same time period. After that, further increase in Fe^0 did not increase NAs oxidation efficiency.

Figure 3-3b shows the effect of persulfate concentration on NAs oxidation in OSPW. The initial NAs concentration was 0.05 mM and initial Fe^0 concentration was 5 mM. It can be observed that the rate of NAs oxidation increased with increasing persulfate concentration and after 52 mM, further increase in persulfate concentration did not increase NAs removal rate or efficiency. The optimal ratio of Fe^0 to persulfate (when the highest NAs oxidation efficiency was achieved), from either Figure 3-3a or Figure 3-3b was 0.10.

3.3.5. Comparison between Fe^{2+} and Fe^0-activation

Theoretically, both Fe^{2+} and Fe^0 can be used to activate persulfate to generate sulfate anion radicals according to the equations below (Oh et al., 2009):

$$S_2O_8^{2-} + Fe^{2+} = SO_4^{\cdot-} + SO_4^{2-} + Fe^{3+} \qquad (2)$$

$$Fe^0 + S_2O_8^{2-} = Fe^{2+} + 2\ SO_4^{2-} (3)$$

However, activation of persulfate with Fe^{2+} is preferable from an environmental protection perspective because its natural abundance in porous media and benign nature (Tsitonaki et al., 2010). From Figures 3-2 and 3-3, oxidation of NAs by persulfate in the presence of Fe^{2+} or in the presence of Fe^0 gave very similar effects in terms of oxidation efficiency and reaction time period. In the present study, oxidation of NAs by persulfate in the presence of Fe^{2+} did not involve pH adjustment, which meant the reaction mixture has a pH of 8.3 at the beginning of the reaction. This raised a concern that the low concentration of available Fe^{2+} for activating persulfate will lead to low efficiency of NAs oxidation because under this pH, Fe^{2+} may form insoluble $Fe(OH)_2$ (Drzewicz et al., 2012). In fact, it was observed that as the oxidation of NAs proceeded, the solution pH dropped gradually (see Section 3.6.1), which increased the concentration of available Fe^{2+}. The reaction mixture of OSPW with persulfate in the presence of Fe^{2+} was somewhat turbid in the beginning, but after 2 h, the mixture was completely clear. This observation served as evidence that a pH drop led to increasing the availability of Fe^{2+}, which was used for activating persulfate. It should be noted that compared with Fe^{2+}, Fe^0 gave higher activating efficiency where a molar ratio of Fe^0 over persulfate (0.10) was half that of Fe^{2+} over persulfate (0.19) as shown in Figures 3-2 and 3-3.

The mechanism of OSPW NAs oxidation by persulfate in the presence of Fe^{2+} or Fe^0 is difficult to study due to the complexity of the OSPW. In the present study, persulfate was in large excess compared to NAs, and it can be concluded that oxidation of NAs by the sulfate radical is the dominant process, but it can not be excluded that the hydroxyl

radical also participates in NAs oxidation. Hydroxyl radicals can be generated by the reaction of the sulfate anion radical with the hydroxide anion or water (Peyton, 1993):

$$SO_4^{-\cdot} + OH^- = SO_4^{2-} + OH^\cdot \qquad (6)$$

$$SO_4^{-\cdot} + H_2O = SO_4^{2-} + OH^\cdot + H^+ \qquad (7)$$

The sulfate radical is an equally powerful oxidant as the hydroxyl radical (Table 3-1), but the sulfate radical is relatively more stable in water than the hydroxyl radical, and thus may be able to disperse a greater distance in water. The sulfate radical is also capable of decomposing carboxylic acids which are not very reactive with the hydroxyl radical, for example, perfluorocarboxylic acids (Hori et al., 2005), cyanuric acid (Manoj et al., 2002) and cyclohexanoic acid (Drzewicz et al., 2012). The sulfate radicals react with aliphatic carboxylic acids by decarboxylation but hydroxyl radicals react preferentially with carboxylic acids by abstracting H at the α-position (Madhavan et al., 1978). NAs are a complex mixture of aliphatic or alicyclic carboxylic acids and the mechanism should be different between the reaction of sulfate anion radicals and hydroxyl radicals with NAs.

3.3.6. Oxidation of NAs byFe^{2+} or Fe^0-activated persulfate under different temperatures

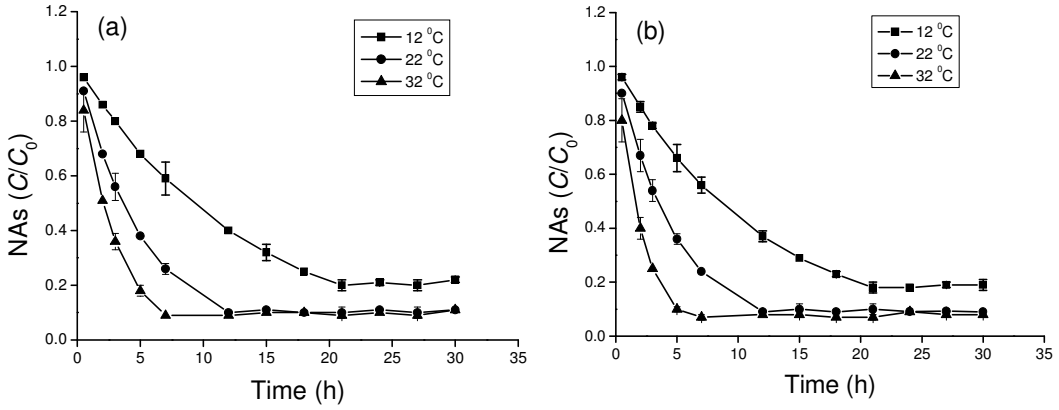

Figure 3- 4 (**a**) Influence of temperature on persulfate oxidation of NAs in the presence of Fe^{2+} (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; Fe^{2+} = 10 mM; pH = 8.3); (b) Influence of temperature on persulfate oxidation of NAs in the presence of Fe^0 (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; Fe^0 = 5 mM; pH = 8.3)

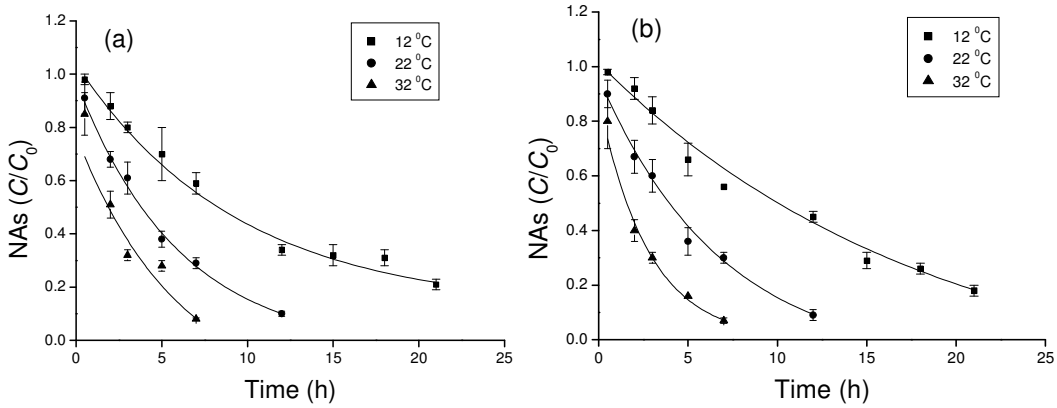

Figure 3- 5(**a**) Pseudo-first-order model for the influence of temperature on persulfate oxidation of NAs in the presence of Fe^{2+} (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; Fe^{2+} = 10 mM; pH = 8.3); (**b**) Pseudo-first-order model for the influence of

temperature on persulfate oxidation of NAs in the presence of Fe^0 (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; Fe^0 = 5 mM; pH = 8.3)

Underoptimal conditions (Fe^{2+} to persulfate molar ratio = 0.19 and Fe^0 to persulfate molar ratio = 0.10, respectively), the oxidation of NAs by persulfate was further enhanced by temperature elevation. As shown in Figure 3-4a and 3-4b, the oxidation of NAs was significantly enhanced in the Fe^{2+} or Fe^0-persulfate system as temperature increased from 12 to 32 ^{0}C. Under both cases, the normalized remaining concentration $[NAs]/[NAs]_0$ vs. reaction time (t) (before the time point when the highest maximum NAs oxidation efficiency was obtained) exhibits a good fit of the experimental data in all experiments to a pseudo-first-order model (Figure 3-5a and 3-5b). It was found that the ozonation of OSPW NAs followed pseudo-first-order kinetics as ozone and hydroxyl radicals were likely present in great excess compared with NAs through the ozonation experiment (Pérez-Estrada et al.,2011).Similarly, in the present study, sulfate radicals were in large excess compared with NAs, which could help explain the pseudo-first-order kinetics. The kinetic data including reaction rate constant and half-life under different temperatures were presented in Table 3-4.

Table 3- 4Comparison of pseudo-first-order model parameters for NAs degradation via persulfate oxidation as a function of temperature

Activator	Temperature	k_{obs}	$t_{1/2}$

	(^{0}C)	(h^{-1})	(h)
	12	0.076	9.12
[a]Fe^{2+}	22	0.19	3.65
	32	0.34	2.04
	12	0.081	8.56
[b]Fe^0	22	0.20	3.47
	32	0.45	1.54

[a]NAs = 0.05 mM, persulfate = 52 mM, Fe^{2+} = 10 mM; and [b]NAs = 0.05 mM, persulfate = 52 mM, Fe^0 = 5 mM.

The pseudo-first-order rate constant (k_{obs}) of NAs degradation was found to increase with increasing temperature. Increasing reaction temperature has two effects: (1) it can increase the production rate of $SO_4^{-\cdot}$ by thermally activating persulfate anion (Tsitonaki et al., 2010), and (2) it can increase the reaction rate between NAs and $SO_4^{-\cdot}$. At a relatively higher temperature (32 ^{0}C), a higher rate of $SO_4^{-\cdot}$ production would occur or the reaction rate between NAs and $SO_4^{-\cdot}$ would be kinetically faster, leading to a higher rate of NAs degradation. The observation that heat can effectively accelerate NAs oxidation should be a combination of the two above-mentioned effects.

Both heat and Fe^{2+} or Fe^{0} can be used to activate persulfate for NAs oxidation (Oh et al., 2009; Tsitonaki et al., 2010). Thermal activation of persulfate has been experimently shown for temperatures ranging from 35 to 130 ^{0}C (House, 1962; Peyton, 1993; Tsitonaki et al., 2010). The studied temperature range (12-32 ^{0}C) covers natural in situ and ex situ temperature encountered in the field. However, it is worth noting that increasing the system temperature requires additional cost, and in view of an in situ remediation technology, increasing temperature to activate persulfate might not be a promising method because it would be very difficult to heat up soil or an aquifer in a cost-effective way (Liang et al., 2007). It is likely that activation of persulfate by Fe^{2+} or Fe^{0} is a better method in subsurface environments as long as transportation of the activator (Fe^{2+} or Fe^{0}) to the oxidizing agent (persulfate) is effectively controlled.

3.3.7. Effect of oxidation process on water chemistry

3.3.7.1. pH change during oxidation process

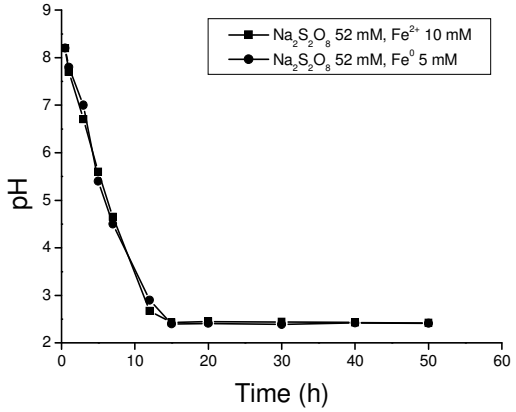

Figure 3- 6pH change during oxidation process (Initial conditions: NAs = 0.05 mM; persulfate = 52 mM; Fe^{2+} = 10 mM or Fe^{0} = 5 mM; pH = 8.3)

The pH of the reaction mixture may influence NAs oxidation efficiency and water chemistry such as solubility and speciation of metal ions in OSPW. The pH of the reaction mixture was monitored during the oxidation process and Figure 3-6 shows the reaction mixture pH change over time at 22 ^{0}C when highest NAs degradation efficiency was obtained in the presence of Fe^{2+} or Fe^0 (persulfate = 52 mM, Fe^{2+} = 10 mM and persulfate = 52 mM, Fe^0 = 5 mM, respectively). The whole oxidation process did not involve pH adjustment. The pH of the solutions at the beginning of the chemical oxidation experiment was 8.3, which was the pH of the original OSPW. Under this pH, NAs (pK_a = 5~6) were present as their conjugate bases and were soluble in the reaction mixture.It can be seen that during the oxidation process, the pH decreased gradually over time, then reached a stable value of about 2.4 after 15 h. From Figures 3-2a and 3-3a, the oxidation of NAs took place within 12 h (after this time point, NAs concentration did not change over time). The decrease in pH can be explained by Equation (4), which shows the release of H^+ during the NAs oxidation process. Moreover, from Figure 3-6, a faster pH decrease rate was observed during the first 12 h of reaction reflecting the higher oxidation rate of NAs during this period, which is in agreement with the faster removal rate of NAs during the first 12 h as shown in Figures 3-2a and 3-3a.

$$C_{20}H_{28}O_2 + 104\ SO_4^{\cdot-} + 38\ H_2O = 104\ SO_4^{2-} + 20\ CO_2 + 104\ H^+ \quad (4)$$

Also the production of hydroxy radicals expressed by Equations 6 and 7 (Peyton, 1993) also contributes to pH drop.

$$SO_4^{\cdot-} + OH^- = SO_4^{2-} + OH^{\cdot} \quad (6)$$

$$SO_4^{\cdot-} + H_2O = SO_4^{2-} + OH^{\cdot} + H^+ \quad (7)$$

3.3.7.2. Major ions

Table 3-5 shows how the water chemistry of OSPW changed before and after chemical oxidation.

Table 3- **5**Water chemistry data of raw OSPW and oxidized OSPW

Parameters	**Raw OSPW**	**Oxidized OSPW in the presence of Fe^{2+}**	**Oxidized OSPW in the presence of Fe^{0}**
Lithium (mg/L)	0.30	0.29	0.29
Ammonium (mg/L)	2.05	n.a.	n.a.
Sodium (mg/L)	640.70	2456.60	2358.37
Potassium (mg/L)	10.71	10.13	10.41
Calcium (mg/L)	7.40	7.10	7.32
Magnesium (mg/L)	6.30	6.27	6.22
Iron (mg/L)	3.21	2655.67	2550.57
Aluminum (mg/L)	0.25	0.27	0.24
Fluoride (mg/L)	2.23	2.10	2.17

Chloride (mg/L)	641.94	643.92	642.00
Nitrite (mg/L)	0.087	n.a.	n.a.
Nitrate (mg/L)	4.65	4.73	4.77
Bromide (mg/L)	0.94	n.a.	n.a.
Sulfate (mg/L)	224.51	8523.00	9310.26
Carbonate + bicarbonate (mg/L)	875.20	n.a.	n.a.
Alkalinity (as $CaCO_3$ mg/L)	494.20	n.a.	n.a.
pH	8.30	2.41	2.40

As shown in Table 3-5, compared with the original OSPW, sodium, iron ion and sulfate anion concentrations in the treated OSPW were increased significantly because of the introduction of the oxidant and the activator into OSPW. The concentrations of the following ions did not change: lithium, calcium, magnesium, potassium, fluoride, aluminium and chloride. The concentrations of magnesium and calcium did not change, which means the hardness of the OSPW was not changed by the oxidation process. Ammonium, nitrite and bromide completely disappeared after oxidation because they were easily oxidized by the strong oxidant: sulfate anion radicals. Total carbonate (including carbonate and bicarbonate) decreased from 875.20 mg/L to zero because the

carbonate and bicarbonate ions were consumed by the H^+ produced by the NAs oxidation. Similarly, the alkalinity decreased significantly because of the H^+ produced by the oxidation process. Carbonate and bicarbonate ions in OSPW can act as $SO_4^{-\cdot}$ scavengers through Equations 8 and 9 (Liang et al, 2006), which can decrease the availability of $SO_4^{-\cdot}$ to oxidize NAs. The complete total inorganic carbon removal observed in this study benefits NAs oxidation by $SO_4^{-\cdot}$.

$$SO_4^{-\cdot} + HCO_3^- = SO_4^{2-} + HCO_3^{\cdot} \quad (8)$$

$$SO_4^{-\cdot} + CO_3^{2-} = SO_4^{2-} + CO_3^{-\cdot} \quad (9)$$

3.3.7.3. COD, BOD, TOC, TIC analyses and toxicity

Table 3- 6TOC, TIC, COD, BOD and toxicity measurements for NAs oxidation

Samples	**TOC** (mg/L)	**TIC** (mg/L)	**COD** (mg/L)	**BOD** (mg/L)	**EC_{20}** (total) **%**	**EC_{20}** (organic) **%**
Control	64	167	240	12	22	22
Oxidation with Fe^{2+} (50 h)[a]	35	0.2	180	20	51	>100
Oxidation	30	0.2	165	24	56	>100

with Fe^0	
(50 h)[b]	

[a]NAs = 0.05 mM, persulfate = 52 mM, Fe^{2+} = 10 mM; and [b]NAs = 0.05 mM, persulfate = 52 mM, Fe^0 = 5 mM.

As shown in Table 3-6, for oxidation of NAs by Fe^{2+}-activated persulfate, 45 % of the initial TOC, 25 % of the initial COD and 91 % of the total NAs were removed. For oxidation of NAs by Fe^0-activated persulfate, 53 % of the initial TOC, 31 % of the initial COD and 91 % of the total NAs were removed. The low COD and TOC removal may be due to the degradation of NAs into simple organic compounds, which still contribute to the COD and TOC of the OSPW samples. The finding of low COD removal in this study is similar to those reported in previous research using ozone as the oxidizing agent. The ozonation of wastewater with complex matrices results in the partial oxidation of contaminants rather than complete mineralization, showing limited COD removal due to the partial oxidation of contaminants by ozone (Sevimli and Sarikaya, 2002; Scott et al., 2008; Fanchiang et al., 2009; Gamel El-Din et al., 2011). The initial TIC of the OSPW sample is 167 mg/L, which was reduced to almost 0 after both oxidation treatments. This is consistent with the result of the total carbonate measurement (carbonate + bicarbonate) shown in Table 3-5 and can be explained by the above mentioned pH change after NAs oxidation, in which excess H^+ was produced, leading to consumption of carbonate and bicarbonate ions (Equations 10 and 11).

$$2\ H^+ + CO_3^{2-} = CO_2 + H_2O \qquad (10)$$

$$H^+ + HCO_3^{2-} = CO_2 + H_2O \qquad (11)$$

The results in Table 3-6 also show that the BOD increased from 12 to 20 and 24 mg/L after Fe^{2+}-activated and Fe^0-activated oxidation process, respectively. The higher BOD value indicated that the oxidation-treated OSPW becomes more biodegradable. The BOD/COD ratio increased from 0.05 to 0.11 and 0.15 after Fe^{2+}-activated and Fe^0-activated oxidation process, respectively. Similar observation of BOD/COD ratio increase was reported after oxidation of OSPW using ozone as the oxidizing agent. For example, the BOD/COD ratio increased from 0.01 to 0.15 after 130 min of ozonation of OSPW with an ozone dose of 35 mg/L (Scott et al., 2008) and Gamel El-Din et al. (2011) observed an increase in BOD/COD ratio from 0.04 to 0.13 with a utilized ozone dose of 148 mg/L. Generally, a wastewater sample is considered as readily biodegradable with the BOD/COD value of higher than 0.4 (Tchobanoglous et al., 2003). Although the BOD/COD ratio obtained in the present study was too low to consider the oxidized OSPW as readily biodegradable, the results indicate that the persulfate treated OSPW was more amenable to biological treatment. It was reported that ozonation was complementary to microbial biodegradation of NAs in OSPW (Martin et al., 2010), and the finding of this study that persulfate treated OSPW is more biodegradable provides a possibility of using biodegradation as a polishing step after persulfate oxidation for OSPW treatment.

NAs are thought to contribute to the toxicity of OSPW (Kannel and Gan, 2012; Whitby, 2010). Microtox® bioassay has been successfully used as a fast and simple toxicity assay to determine the toxicity of OSPW. The EC_{50} values of the untreated and oxidized OSPW

could not be determined due to their low toxicity to the Microtox® assay. Therefore, the EC_{20} values were used here as a reliable relative measurement of the toxicity of OSPW during chemical oxidation. As shown in Table 3-6, the EC_{20} of untreated OSPW was 22 %. The toxicity decreased after chemical oxidation in both cases (either Fe^{2+} or Fe^{0} used as the activator). In each case, two different samples were assessed for their toxicity, one was the aqueous sample after chemical oxidation (total), the second one was the organic fraction extracted from the aqueous mixture with dichloromethane (organic). After chemical oxidation, the toxicity of the aqueous mixture decreased but certain toxicity remained (EC_{20} value was 51 % in the case of Fe^{2+} and EC_{20} value was 56 % in the case of Fe^{0}), for the organic fraction, the toxicity was removed completely in both cases (EC_{20}> 100 %). The toxicity of the aqueous mixture may be caused either by the remaining oxidant (sodium persulfate in water) or by the strong acidic condition after chemical oxidation (pH around2.4 in both cases, Table 3-5). It should be noted that although Microtox® bioassay is a simple and fast test, more comprehensive tests like fish toxicity assays should be used to assess the OSPW toxicity (Kerr et al., 2008; Stalter et al., 2010; Gamal El-Din et al., 2011).

3.4. Conclusion

It was demonstrated that within the studied temperature range (12-32 ^{0}C) which covers natural in situ and ex situ temperature encountered in the field, in the presence of Fe^{2+} or Fe^{0}, persulfate can remove NAs from OSPW. From an environmental protection perspective, this treated water was expected to contain NAs in very low concentration (less than 1 mg/L), which would meet water quality criteria for safe discharge into the local aquatic environment. This process has the potential of being developed to an

attractive in situ remediation technology to remove NAs from OSPW, as this process is simple (which does not involve any pretreatment like pH adjustment), operates at low temperature, uses readily available and cheap chemicals as well as removes NAs with an efficiency of more than 90 %.

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Chapter 4 Removal of naphthenic acids from groundwater

4. 1. Introduction

The Athabasca oil sands, in northern Alberta, Canada, contain approximately 1.7 trillion barrels of crude oil in the form of bitumen (Kannel and Gan, 2012). For shallow deposits, the Alberta surface mining industry uses an alkaline hot water extraction technology to facilitate the separation of bitumen from the oil sands ore. The resulting oil sands process water (OSPW) is retained onsite in large tailings ponds. It is estimated that 720 million m^3 of OSPW are currently stored (Ross et al., 2012), and the volume of OSPW is expected to increase due to the further expansion of the Alberta oil sands surface-mining industry.

OSPW is acutely toxic to various aquatic organisms (Whitby, 2010), and can cause endocrine disruption and immunological impairments (He et al., 2010; Garcia-Garcia et al., 2011; He et al., 2011). The acute and chronic toxicity of OSPW has been attributed to naphthenic acids (NAs). NAs are a complex mixture of aliphatic and cycloaliphatic monocarboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$, where n is the carbon number and Z is zero or a negative even integer defining the hydrogen deficiency resulting from ring formation or presence of double bonds (Headley and McMartin, 2004; Clemente and Fedorak, 2005; Whitby, 2010; Kannel and Gan, 2012).

NAs are of particular concern in oil sands tailings as they become concentrated in OSPW during bitumen extraction (Schramm et al., 2000). Also, NAs pose a serious threat to shallow groundwater and surface water (McMartin et al., 2004; Oiffer et al., 2009; Ahad et al., 2012) because of the potential of seepage or runoff of OSPW into surface and

groundwater potentially to take place (Oiffer et al., 2009; Ross et al., 2012). Also, it was demonstrated that coal is a source of NAs, and that NAs can leach into groundwater (Scott et al., 2009).

To date, most of the research regarding the environmental fate of NAs has been focused on surface water, especially OSPW. Biodegradation and ozonation have been the most intensively studied strategies for the removal of NAs from OSPW (Quagraine et al., 2005; Scott et al., 2008; Kannel and Gan, 2012). Only a couple of studies regarding NAs in groundwater have been reported. MacKinnon et al. (2004) demonstrated that the natural attenuation of NAs in process-affected groundwater occurred because of aerobic biotransformation of NAs (Clemente et al., 2004). Gervais and Barker (2004) reported that in three NAs plumes, NAs concentrations significantly decreased due to dispersive dilution at all three sites. Oiffer et al. (2009) investigated fate and transport of NAs in subsurface by performing quantitative and qualitative analyses on groundwater samples and they found that significant attenuation of NAs by biodegradation was not observed even after more than 20 years of subsurface residence time.

NAs are toxic and NAs can enter groundwater through seepage of OSPW or leaching from coal sources (Oiffer et al., 2009; Scott et al., 2009; Ross et al., 2012), which means that groundwater can be contaminated by NAs causing concerns in water quality, public health and environmental risks. Groundwater contamination can persist for decades, requiring development of efficient and cost-effective strategies to be used in NAs-contaminated groundwater remediation.

In the past two decades, in situ chemical oxidation (ISCO) has proven to be a useful remediation technology for the most prevalent organic contaminants in groundwater (Seol et al., 2003; Tsitonaki et al., 2010). Its advantages include its applicability to a wide variety of contaminants, its adaptability to a wide range of subsurface conditions, its potential to treat a site rapidly, and its ability to be combined with other in situ remediation technologies (Huling and Pivetz, 2006).

ISCO involves the subsurface delivery of a chemical oxidant to a contaminated source zone and the subsequent oxidative degradation of contaminants into harmless end products within the natural environment. While research and development still continue, ISCO is a relatively mature technology for the remediation of contaminated groundwater (Siegrist et al., 2011). The chemical oxidants commonly used for ISCO are: hydrogen peroxide, potassium and sodium permanganate, ozone and sodium persulfate. Their characteristics are summarized in Table 4-1.

Table 4- **1** Characteristics of chemical oxidants used for destruction of organic contaminants (*adapted from Huling and Pivetz, 2006*)

Oxidant	**Oxidant chemical**	**Commercial form**	**Activator**	**Reactive species**
Permanganate	$KMnO_4$ or $NaMnO_4$	powder, liquid	None	MnO_4^-
Hydrogen	H_2O_2	liquid	None, Fe^0, Fe^{2+},	$OH^{\cdot}$, $O_2^{-\cdot}$,

peroxide			Fe^{3+}	$HO_2^{\cdot}$, HO_2^{-}
Ozone	O_3	gas	None	O_3, $OH^{\cdot}$
Persulfate	$Na_2S_2O_8$	powder	None, Fe^0, Fe^{2+}, Fe^{3+}, heat, H_2O_2, high pH	$S_2O_8^{2-}$, $SO_4^{-\cdot}$

It was shown in Chapter 3 that NAs can be removed from OSPW using chemical oxidation processes with sodium persulfate as the oxidant in the presence of an activator. To go one step further, our goal was to develop ISCO as a remediation technology for NAs-contaminated groundwater. An ISCO project begins with a laboratory study aimed at selecting the most appropriate oxidant. In the present system, H_2O_2 and O_3 were discarded because of the following considerations: H_2O_2 is too reactive to effectively handle the oxidants in subsurface environments and would be rapidly consumed during transportation through the soil and aquifer before reaching the contaminants due to its high reactivity (Watts and Teel, 2005; Watts and Teel, 2006). Also, pH has a strong effect on H_2O_2 chemistry and effectiveness. The optimum pH for H_2O_2 activity is 2-4, however, the pH of OSPW ranges between 8-9. ISCO using O_3 also has its limitations. It is expensive to generate ozone, has low solubility in water (Watts and Teel, 2006), and the short half life of ozone in water ($t_{1/2}\approx$ 20 min at 20 ^{0}C) limits the distances over which it can be delivered into water (Seol et al., 2003). For this reason, it is difficult to supply enough O_3 to effectively remediate contaminants in subsurface environments.

Instead, we focused on using potassium permanganate ($KMnO_4$) and sodium persulfate ($Na_2S_2O_8$) as oxidants to remove NAs from groundwater. $KMnO_4$ and $Na_2S_2O_8$ were chosen because of the following considerations: $KMnO_4$ is relatively easy to use and it is persistent in the environment (Waldemer and Tratnyek, 2006). $Na_2S_2O_8$ is a strong oxidizing reagent and has several advantages. Compared with H_2O_2 and O_3, $Na_2S_2O_8$ has the advantage of both high stability and wide spread reactivity, and can also degrade contaminants under a wide range of pH conditions (Watts and Teel, 2006).

The present bench scale study was conducted to identify a chemical oxidant suitable for the use of removing NAs from groundwater, with the ultimate goal of developing ISCO as an innovative and efficient strategy for remediation of NAs-contaminated groundwater. To elucidate this goal, the present study explored the oxidation of NAs by potassium permanganate or sodium persulfate inbatch experiments to determine: (1) the optimal oxidant dosage in terms of NAs degradation efficiency, (2) the persistence of chemical oxidants in groundwater, and (3) the impact of chemical oxidation on the microbial community.

4.2. Materials and methods

4.2.1. Chemicals, tailings water collection and soil collection

Chemicals used to prepare buffer solutions and eluents were of reagent grade and were used without purification. Potassium permanganate and sodium persulfate were purchased from Sigma Aldrich Co. (St. Louis, MO, USA).

The OSPW was collected from the South Tailings Pond (June 2011) by Suncor Energy Inc. and stored in the dark at 4°C in sealed polyethylene pails prior to use. The soil cores

were collected (September 2012) by Suncor Energy Inc. and were then shipped to the University of Alberta Applied Geoenvironmental Research Facility and stored at -20 ^{0}C. Core sections for laboratory use were cut using a radial arm saw, then wrapped in cling wrap and aluminum foil, sealed in air-tight plastic bags, and placed in cold storage at 4°C. Two soil cores were used, the first one (4B) is from the lower glacial till and the second one (4D) is from the lower sand channel. The respective depth is: 19.6-21.1 m below ground surface (mbgs) and 36.6-38.1 mbgs.

The groundwater used in the oxidation experiments was not real groundwater, instead, it was a mixture between OSPW and soil with an weight ratio of 10:1.

4.2.2. Soil characterization and NOD measurement

Soil samples were analyzed by *Exova* for specific gravity, grain size distribution, moisture content, fraction of organic content (FOC).

NOD was measured in two soil samples. 10 g of each soil sample was mixed with ultra pure water (100 g) in a 250 mL Erlenmeyer flask. Potassium permanganate or sodium persulfate (10 g) was added. Flasks were capped and kept at 22 ^{0}C in the dark. They were shaken at the beginning of the experiment and then twice a day. After 60 days, the supernatant was sampled and the residual oxidant concentration was measured, enabling us to calculate NOD. NOD was calculated according to the following equation:

$NOD = \{[Ox]_{control}V_{control} - [Ox]_{sup}V_{sup}\}/m$, where NOD is the natural oxidant demand (g oxidant/kg soil), $[Ox]_{sup}$ is the oxidant concentration in the supernatant (g/L), V_{sup} is the supernatant volume (L), $[Ox]_{control}$ is the oxidant concentration in the control (g/L),

$V_{control}$ is the control solution volume (L), m is the mass of wet soil (kg).

4.2.3. Chemical oxidation experiments

Oxidation experiments were conducted in 500 mL Erlenmeyer flasks containing 250 mL of Suncor OSPW and 25 g of soil at 22 ^{0}C. Different amount of oxidant was added into the solution and shaken on a shaker at 200 rpm. The concentrations of oxidant were varied between experiments and are reported in the Results section. All the experiments in this study were carried out in the dark. At selected time intervals, 10 mL samples were collected from each flask and were immediately added to a 50 mL vial containing 2 mL of methanol (CH_3OH) as a radical scavenger to quench the oxidation reaction. In each vial, the pH was adjusted to around 2.5 with 1 M HCl and the remaining NAs were extracted with dichloromethane. All experiments were conducted in duplicate and data presented in all figures are mean values.

4.2.4. Chemical analysis

NAs were extracted from OSPW by liquid-liquid extraction and the quantification of NAs was performed using a PerkinElmer 100FT-IR spectrometer (Jivraj et al., 1995). The persulfate concentration was determined using a UV-vis spectrophotometer according to the reported method (Liang et al., 2008). The permanganate concentration was determined by titration with hydrogen peroxide (Vonderbrink, 2006).

4.2.5. DNA extraction

DNA was extracted from soil following the protocol provided in the PowerSoil® DNA isolation Kit (MO BIO Laboratories, Inc.) and stored at -20 ^{0}C prior to use.

4.2.6. Total bacteria and sulfate reducing bacteria measurement

The *rpoB* primers (Table 4-2) targeting the RNA polymerase β subunit (*rpoB*) gene were used to enumerate total bacteria [Dahllof et al., 2000]. The *dsrB* primers (Table 4-2) targeting the *dsrB* gene were used to quantify the sulfate-reducing bacteria (SRB) (Wagner et al., 1998; Geets et al., 2006). Real-time quantitative polymerase chain reaction (RT–qPCR) was performed on a C1000 Thermal cycler (Bio-Rad Laboratories Inc.). Each reaction contained 10 µL of SsoFast EvaGreen Supermix (Bio-Rad Laboratories Inc.), 5 pmol each of the forward and reverse *rpoB* primers or 10 pmol each oftheforward and reverse *dsrB* primers, 1 µL of extracted DNA, and sterile ultra pure water to a final volume of 20 µL with sterile ultra pure water. The thermocycling program for *rpoB* primers was as follows: initial denaturation for 3 min at 95 °C and then 35 cycles (95 °C for 30 s, 47 °C annealing for 90 s). The thermocycling program for *dsrB* primers was as follows: initial denaturation for 3 min at 95 °C and then 35 cycles (95 °C for 45 s, 58 °C annealing for 45 s). Calibration was performed with serial dilutions of a known quantity of DNA amplified from either the *rpoB* or *dsrB* gene using the specified primers. Triplicate reactions were performed for each standard dilution and for each sample. The specificity and identity of the PCR products were verified by a final melting curve analysis from 65 to 95 °C, measuring fluorescence every 0.5 °C.

Table 4- **2**Quantitative PCR primers used in this study

	Primer name	Sequence
Total bacteria	*rpoB*1698F	5'-AACATCGGTTTGATCAAC-3'

	*rpoB*2041R	5'-CGTTGCATGTTGGTACCCAT-3'
SRB	DSRp2060F	5'-CAACATCGTYCAYACCCAGGG-3'
	DSR4R	5'-GTGTAGCAGTTACCGCA-3'

4.3. Results and discussion

During the selection, design and implementation of any ISCO project, a number of key issues need to be addressed including measurement of natural oxidant demand (NOD), degradation efficiency of the target contaminants, optimal oxidant loading, byproduct characterization, and capability of combining ISCO with other remediation technologies (Huling and Pivetz, 2006).

4.3.1. NOD

NOD is a key design parameter for ISCO projects, and is a measurement of the oxidant consumed by non-target reduced species (natural organic matter, reduced inorganic species, and some mineral phases) in the subsurface. It is suggested that ISCO is not technically and/or economically feasible at very high NOD (Lemaire et al., 2011).

Table 4-3 lists the characterization data and NOD for two different soils. The NOD of soil 4B was 0.84 g /kg wet soil and 0.67 g /kg wet soil for $KMnO_4$ and $Na_2S_2O_4$, respectively. The NOD of soil 4D was 0.65 g /kg wet soil and 0.54 g /kg wet soil for $KMnO_4$ and $Na_2S_2O_4$, respectively. Soil 4B had higher NOD than soil 4D, which is correlated to the finding that its fraction of organic content (FOC %) is higher than that of soil 4D (1.66 % for 4B vs. 0.28 % for 4D). The NOD values for $Na_2S_2O_4$ are within in the range of reported NOD values measured with other soils (Liang et al., 2008). For both

soils and both oxidants, NOD < 1 % of the initial oxidant content was observed, which suggests ISCO is potentially technically and economically feasible for both soils with either oxidant.

Table 4- 3 Characterization data and NOD for the soils

Sample ID	**Soil core sampling location**	**Grain size summary**	**Moisture**	**FOC by weight**	**Texture**	**NOD**
04B	19.6-21.1 m	50 µm-2 mm, 57.4%; 2 µm-50 µm, 25.8%; <2 µm, 16.8%	11.2%	1.66%	Sandy loam	0.84 g /kg soil for permanganate; 0.67 g /kg soil for persulfate
04D	36.6-38.1 m	50 µm-2 mm, 93.4%; 2 µm-50	10.8%	0.28%	Sand	0.65 g/kg soil for permanganate; 0.54 g/kg soil

		µm, 2.4%; <2 µm, 4.2%				for persulfate

4.3.2. NAs degradation in groundwater

4.3.2.1. NAs degradation with $KMnO_4$

Figure 4-1 shows the change in NAs concentrations following exposure to different concentrations of $KMnO_4$ in OSPW mixed with soil 4B. When the concentration of the oxidant increased from 1.5 g/L to 15 g/L, the NAs degradation efficiency increased over the same reaction time period. It can be seen that the highest degradation efficiency was around 77 % with 15 g/L of $KMnO_4$ (the initial concentration of NAs in the mixture is 15 mg/L) within a reaction period of 40 days, after which, the concentration of NAs remained stable.

Similarly, Figure 4-2 shows the change in NAs concentrations following exposure to different concentrations of $KMnO_4$ in OSPW mixed with soil 4D, and similar results were obtained. When the concentration of $KMnO_4$ was increased from 1.5 g/L to 15 g/L, the NAs degradation efficiency also increased, and the highest degradation efficiency was found to be around 80 % with 15 g/L of $KMnO_4$ within a reaction period of 40 days.

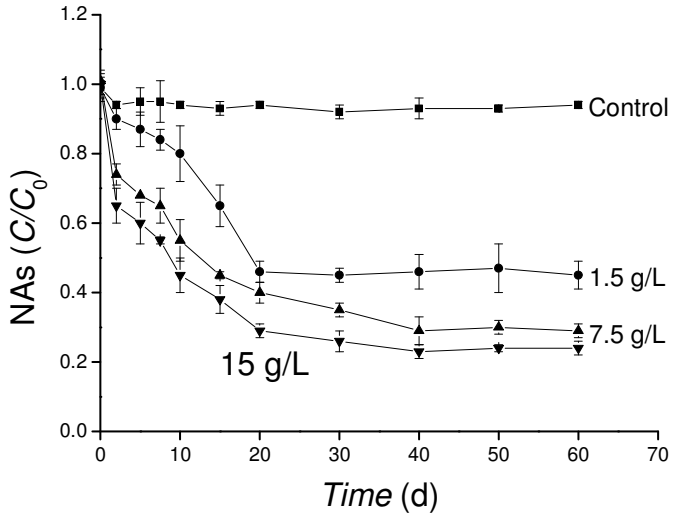

Figure 4- 1NAs concentration change in the presence of potassium permanganate in OSPW mixed with soil 4B (Initial conditions: NAs = 15 mg/L; pH = 8.3)

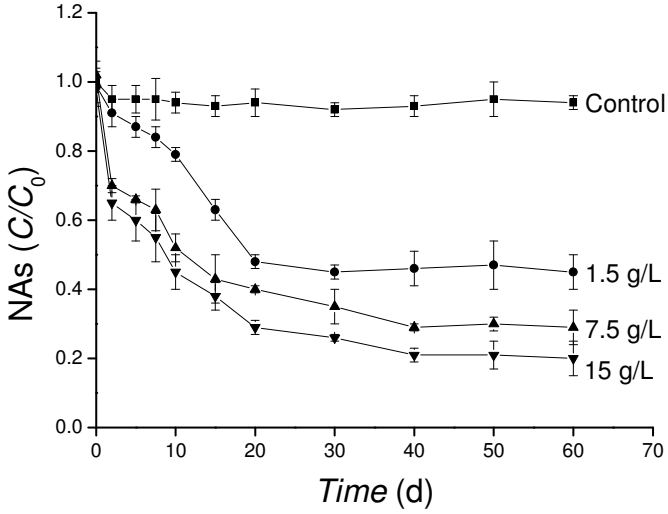

Figure 4- 2NAs concentration change in the presence of potassium permanganate in OSPW mixed with soil 4D (Initial conditions: NAs = 15 mg/L; pH = 8.3)

4.3.2.3. NAs degradation with $Na_2S_2O_4$

The change of NAs concentration in the presence of different concentrations of sodium persulfate in OSPW mixed with soil 4B or 4D was shown in Figure 4-3 and 4-4 respectively. It can be seen that in both cases, the highest degradation efficiency was up to 95 % with 12 g/L of $Na_2S_2O_4$ (the initial concentration of NAs in the mixture is 15 mg/L) within a reaction period of 40 days.

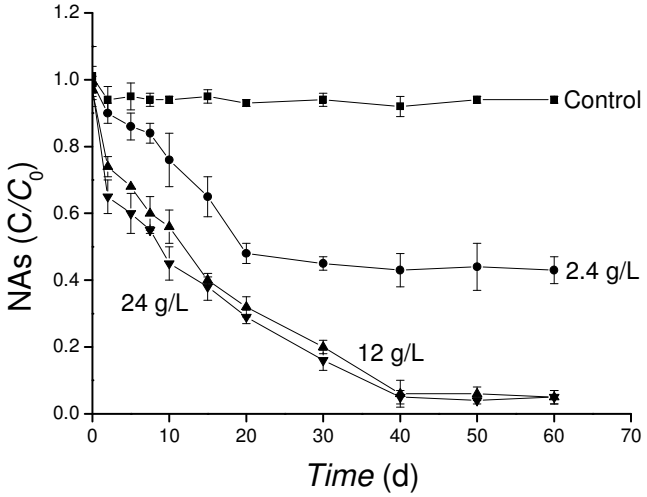

Figure 4- 3NAs concentration change in the presence of sodium persulfate for soil 4B (Initial conditions: NAs = 15 mg/L; pH = 8.3)

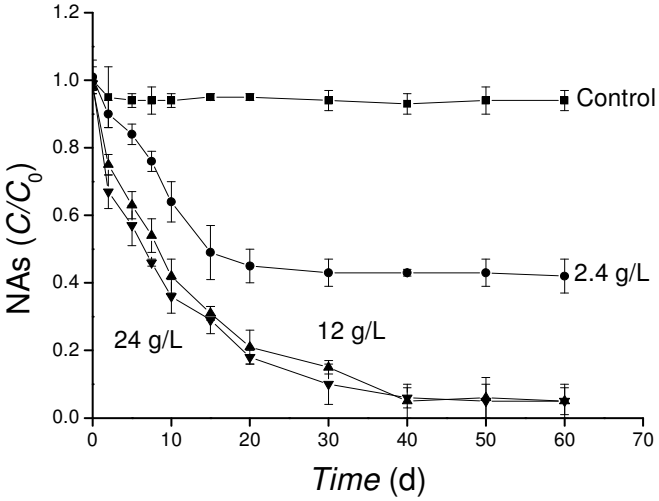

Figure 4- 4NAs concentration change in the presence of sodium persulfate for soil 4D (Initial conditions: NAs = 15 mg/L; pH = 8.3)

4.3.3. Oxidant persistence

The above results indicated that $Na_2S_2O_4$ is thebest of thetested oxidants as it degrades NAs with higher efficiency (95 %) than $KMnO_4$ does (77 %). Before the technology can be moved into the field, however, the persistence of $Na_2S_2O_4$ inthe environment must be elucidated. The persistence of $Na_2S_2O_4$ in the system was monitored continuously for three months, and the change of remaining oxidant concentration over time was shown in Figure 4-5. The oxidative degradation of NAs in both systems went to completion within 40 days (Figures 4-3 and 4-4) and the oxidant can be persistent in the system for 90 days (Figure 4-5), after which, the concentration of oxidant in both systems was no longer monitored. The fact that the oxidant persistence time (90 days) is much longer that the time period which the NAs are completely degraded can prevent rebound of NAs from happening. Rebound, where post-ISCO concentrations of target contaminant of concern

in groundwater return to levels near or even above those present prior to ISCO, is a relatively common occurrence (Tsitonaki, et al., 2010).

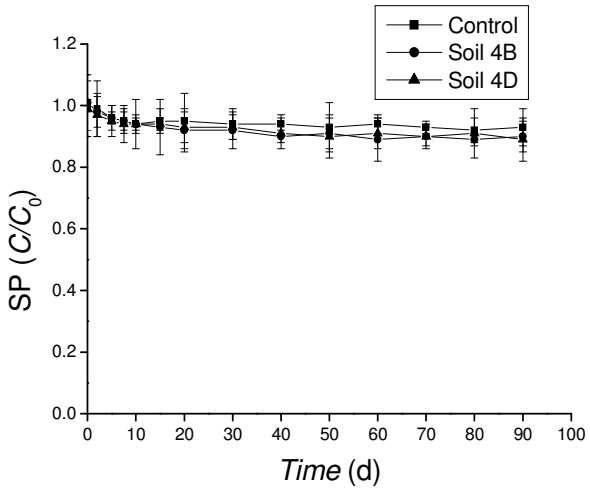

Figure 4- 5 Sodium persulfate (SP) concentration change during the NAs oxidation process (Initial conditions: NAs = 15 mg/L; pH = 8.3)

4.3.4. Effect on microbial community

To achieve the more stringent remediation objectives, ISCO is almost always combined with another technology (e.g., bioremediation) or approach (e.g., monitored natural attenuation). ISCO can enhance or interfere with the use of bioremediation or monitored natural attenuation. To investigate if this chemical oxidation process can be combined with biological process, the impact of NAs oxidation by $Na_2S_2O_4$ on sulfate reducing bacteria (SRB) and total bacteria was studied. Table 4-4 lists the number of SRB and total bacteria represented in the DNA extracted from both soil samples.

Table 4- **4**Impact of chemical oxidation on SRB and total bacteria counts

Samples	**SRB** (copies/g soil)	**Total Bacteria** (copies/g soil)
4B	1.89E+02	9.82E+04
4D	1.14E+02	5.68E+04
4B after oxidation with SP	N/A	5.28E+04
4D after oxidation with SP	N/A	2.75E+04
4B (control)	N/A	6.45E+04
4D (control)	N/A	3.37E+04

Soil 4D has lesser numbers of SRB and total bacteria, which is expected as the sampling location of soil 4D (around 37 m below ground surface) is much deeper than that of soil 4B (around 20 m below ground surface). After chemical oxidation, the SRB were killed completely in both type of soil, but it is not certain whether this occurred due to the specified chemical oxidation process, or by other processes (like exposure to air), as the SRB in both control samples were also killed (Table 4-4). For both soils, the number of

the total bacteria decreased but did not change significantly, which means that it is theoretically possible to use chemical oxidation in conjunction with biodegradation processes. Further studies, including the effect of chemical oxidation on the makeup of the natural microbial community of the soil and groundwater are underway in our laboratory.

4.4. Conclusion

With the goal of developing ISCO treatments for NAs-contaminated groundwater remediation, we present a bench scale study that was conducted to select the most appropriate oxidant and optimal dosage. Permanganate and persulfate were both effective for NAs degradation, and it was found that NAs can be oxidatively degraded with 95 % efficiency by sodium persulfate at room temperature within 40 days. Sodium persulfate can also be persistent for more than 90 days, allowing the time needed to complete NAs degradation. The finding that the number of total bacteria is not significantly affected by long-time exposure to sodium persulfate suggests it may be possible to combine sodium persulfate chemical oxidation with biodegradation for more efficient remediation of NAs-contaminated groundwater.

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Chapter 5 Summary, Conclusions and Recommendations

This research has added to the understanding of how chemical oxidation can be used as a remediation tool for the oil sands industry. In this study, tools were developed to better measure and characterize NAs. It was also demonstrated that chemical oxidation has the ability to remove naphthenic acids from oil sands process water and groundwater. This is significant because it potentially provides an innovative and cost-effective strategy for the oil sands industry to remediate oil sands process water and contaminated groundwater, promoting sustainable oil sands development in the Athabasca region.

5.1. Naphthenic acids quantification with FTIR

The FTIR spectra of naphthenic acids from different sources were obtained and compared. The concentrations of naphthenic acids in oil sands process water were measured by FTIR and compared with other methods. The subsequent conclusions summarize the results of the FTIR quantification:

1. The FTIR spectra of naphthenic acids extracted from three oil sands process water sources (Suncor, Syncrude and Albian Sands) show a maximum absorbance at 1706 and 1743 cm^{-1}. Among the four commercial naphthenic acids tested, Merichem and Kodak naphthenic acids show a similar maximum absorbance pattern, while Acros and Sigma-Aldrich naphthenic acids have FTIR spectra distinct from those of the OSPW sources. Thus, this research suggests that Merichem naphthenic acids should be used as standard for the quantification of environmental naphthenic acids with FTIR.

2. The results of naphthenic acids quantification by FTIR depend significantly on which standard is used. With Acros naphthenic acids as the standard, the quantification results are more than 50 % higher than those using Merichem naphthenic acids as the standard.

3. Using Merichem naphthenic acids as the standard, the quantification results of naphthenic acids by FTIR are comparable to those obtained by ultra pressure liquid chromatography-mass spectroscopy.

5.2. Naphthenic acids removal from oil sands process water

Naphthenic acids can be removed from oil sands process water by chemical oxidation using sodium persulfate as the oxidant. The subsequent conclusions summarize the results of naphthenic acids removal from oil sands process water:

1. Naphthenic acids can be removed from oil sands process water using sodium persulfate as the oxidant in the presence of iron sulfate as the activator. Under these conditions, naphthenic acids can be degraded with 91 % efficiency within 12 h at 22 ^{0}C. The optimal ratio of iron sulfate:sodium persulfate was 0.19.

2. Naphthenic acids can be removed from oil sands process water using sodium persulfate as the oxidant in the presence of zero valent iron as the activator. Under these conditions, naphthenic acids can be degraded with 90 % efficiency within 10 h at 22 ^{0}C. The optimal ratio of zero valent iron:sodium persulfate was 0.10.

3. The chemical oxidation of naphthenic acids at temperatures between 12-32 ^{0}C followed pseudo first order kinetics. Increasing temperature increased reaction rate

between naphthenic acids and sodium persulfate in the presence of iron sulfate or zero valent iron.

4. The addition of a chemical oxidant has a significant effect on oil sands process water chemistry. After chemical oxidation, the pH dropped from 8.3 to about 2.4 and the concentrations of sodium, iron, sulfate significantly increased. Additionally, bicarbonate and carbonate were completely removed from the solution and the alkalinity decreased significantly.

5. Following Fe^{2+}-activated sodium persulfate oxidation of NAs, 45 % of the initial TOC, and 25 % of the initial COD were removed. For oxidation of NAs by Fe^{0}-activated sodium persulfate, 53 % of the initial TOC, and 31 % of the initial COD were removed. The low COD and TOC removal may be due to the degradation of NAs into simple organic compounds, which still contribute to the COD and TOC of the OSPW samples. The initial TIC of the OSPW was reduced from 167 mg/L to almost 0 after both oxidation treatments.

6. The BOD/COD ratio increased from 0.05 to 0.11 and 0.15 after Fe^{2+}-activated and Fe^{0}-activated oxidation process, respectively, which means that the oxidation-treated OSPW becomes more biodegradable. This indicates that using biodegradation as a polishing step after sodium persulfate oxidation is a possibility for OSPW treatment.

7. After chemical oxidation, the toxicity (as measured by the Microtox assay) of the OSPW decreased to some extent, but the OSPW still remained toxic.This may be the

result of the low pH (about 2.4) of the OSPW after chemical oxidation, or due to the presence of residual chemical oxidant. The organic fraction of the OSPW remaining after chemical oxidation was not found to be toxic.

5.3. Naphthenic acids removal from groundwater

Naphthenic acids can be removed from groundwater (in the presence of soil)by chemical oxidation using sodium persulfate or potassium permanganate as the oxidant. The subsequent conclusions summarize the results of naphthenic acids removal from groundwater:

1. For both soils (sand and clay), the natural organic demand was less than 1 % for either potassium permanganate or sodium persulfate, which suggests ISCO is potentially technically and economically feasible for both soils with either oxidant.

2. Naphthenic acids can be removed from groundwater using potassium permanganate as the oxidant. Naphthenic acids can be degraded with 77 % efficiency within 40 days at 22 ^{0}C.

3. Naphthenic acids can be removed from groundwater using sodium persulfate as the oxidant. Naphthenic acids can be degraded with 95 % efficiency within 40 days at 22 ^{0}C.

4. Sodium persulfate can be persistent in the mixture for more than 90 days, a time period much longer than the time it took naphthenic acids to be degraded (40 days).

5. After chemical oxidation, for both soils, the number of the total bacteria decreased but did not change significantly, which means that it is theoretically possible to use chemical oxidation in conjunction with biodegradation processes.

5.4. Recommendations

Future work may include the following:

1. Future experiments should examine how the activated chemical oxidation process affects the mobility and speciation of metal ions in the oil sands process water, particularly in regards to the effects of lowered pH.

2. Additionally, the breakdown products of NAs due to the chemical oxidation process should be examined. Does this process produce toxic by-products (i.e. bromate) in oil sands process water?

3. More studies should also be carried out to determine how the chemical oxidation process affects the makeup of the natural microbial community of the soil and groundwater.

4. Lastly, can the findings of chemical oxidation laboratory studies be applied to the field?This possibility should be investigated using column studies where the oxidant will be fed and degradation of NAs will be monitored downstream,then the findings of column studies will be used in field studies.

This future work will help to build a better understanding how the chemical oxidation process affects oil sands process water, groundwater and the surrounding community. Further research could ultimately turn these laboratory findings into a remediation

technology for oil sands process water and groundwater. Additional benefits to the implementation of this technology may include the ability to increase recycling of oil sands process water, thereby limiting the amount of imported fresh water taken from the AthabascaRiver. The removal of toxicity from the tailings ponds will also aid in the reclamation of these ponds, potentially allowing for the stocking of aquatic species. This research is a preliminary step towards creating sustainable development within the Athabasca oil sands region.

Chapter 6 Appendices

Appendix A: Procedure of DNA extraction from soil samples

Appendix B: Schematic of monitoring wells

Appendix A: Procedure of DNA extraction from soil samples

DNA Extraction from soil Samples

Obtain **PowerSoil® DNA Isolation Kit** (MO BIO Laboratories, Inc.)- stored at room temperature

- *Utilizes Inhibitor Removal Technology® (IRT) on environmental samples containing a high humic acid content, including difficult soil types allowing for more successful PCR amplification of organisms from the sample. Samples are added to a bead beating tube for rapid and thorough homogenization and cell lysis occurs by mechanical and chemical methods. The total genomic DNA is captured on a silica membrane in a spin column format and the DNA is then washed and eluted from the membrane. Once this is complete, the DNA is then ready for PCR analysis.*

Need:

1. Clean surface of work area with 'DNA Away' and isopropyl alcohol
2. To the **PowerBead Tubes** provided, add 0.25 g of soil sample
    a. *The PowerBead Tube contains a buffer that will help disperse the soil particles, begin to dissolve the humic acids, and protect the nucleic acids from degradation.*
3. Gently vortex to mix
    a. This begins to disperse the sample in the PowerBead solution
4. Check **Solution C1**. If **Solution C1** is precipitated, heat solution to 60^{o}C until the precipitate has dissolved before use.
    a. *This solution contains SDS and other disruption agents required for complete cell lysis. The SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Solution C1 can be used when it is still warm*.
5. Add 60 µL of **Solution C1** and invert several times or vortex briefly
6. Secure **PowerBead Tubes** horizontally using the MO BIO Vortex adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at a maximum speed for 10 minutes.
    a. *This allows for complete homogenization and cell lysis. The collision of the beads with microbial cells will cause the cells to break open.*
7. Make sure the **PowerBead Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature.
    a. *If you exceed this speed, the tubes may break*
8. Transfer the supernatant to a clean **2 mL collection tube** (provided)
    a. *Expect between 400-500 µL; however, the exact volume recovered depends on the absorbancy of your starting material and is not critical for the procedure to be effective. The supernatant may be dark or still contain some soil particles.*
9. Add 250 µL of **Solution C2** and vortex for 5 seconds. Incubate at 4^{o}C for 5 minutes.
    a. *This solution contains a reagent to precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins.*

10. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g
11. Avoiding the pellet, transfer up to 600 µL of supernatant to a clean **2 mL collection tube** (provided)
    a. *This pellet contains non-DNA organic and inorganic material*
12. Add 200 µL of **Solution C3** and vortex briefly. Incubate at 4°C for 5 minutes
    a. *This solution is a second reagent to precipitate additional non-DNA organic and inorganic material.*
13. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g
14. Transfer up to 750 µL of supernatant to a clean **2 mL collection tube** (provided)
    a. *This pellet contains non-DNA organic and inorganic material*
15. Shake to mix **Solution C4** before use. Add 1.2 mL of **Solution C4** to the supernatant (be careful that the solution doesn't exceed the rim of the tube) and vortex for 5 seconds
    a. *This solution is a high concentration salt solution. Since the DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that still may be present at low levels to the Spin Filters.*
16. Load approximately 675 µL onto a **Spin Filter** and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the **Spin Filter** and centrifuge at 10,000 x g for 1 minute at room temperature.
    a. A total of **three loads** for each sample processed are required
    b. *The contaminants will pass through the filter membrane, leaving only the DNA bound to the membrane*
17. Add 500 µL of **Solution C5** and centrifuge at room temperature for 30 seconds at 10,000 x g
    a. *This solution is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter.*
18. Discard the flow through from the **2 mL collection tube**
19. Centrifuge at room temperature for 1 minute at 10,000 x g
    a. *This second spin removes residual Solution C5 since the ethanol can interfere will PCR*
20. Carefully place the **Spin Filter** in a clean **2 mL collection tube** (provided). Avoid splashing any C5 onto the **Spin Filter**
21. Add 100 µL of **Solution C6** to the center of the white filter membrane
    a. *This will make sure that the entire membrane is wetted. As the solution (elution buffer) passes through h the silica membrane, the DNA that was bound in the presence of high salt is selectively released which lacks salt.*
    b. Sterile DNA-free PCR grade water may also be used for this step
22. Centrifuge at room temperature for 30 seconds at 10,000 x g
23. Discard the **Spin Filter**. The DNA in the tube is now ready for any downstream application. No further steps are required.

24. Store DNA in the freezer (-20 to -80^{o}C)

Appendix B: Schematic of monitoring wells