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Toxicity assessment of collected fractions from an extracted naphthenic acid mixture

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ABSTRACT

Recent expansion within the oil sands industry of the Athabasca Basin of Alberta, Canada has led to increased concern regarding process-affected wastewaters produced during bitumen extraction. Naphthenic acids (NAs) have been identified as the primary toxic constituents of oil sands process-affected waters (OSPW) and studies have shown that with time, microbial degradation of lower molecular weight NAs has led to a decrease in observed toxicity. As earlier studies identified the need for an "unequivocal demonstration" of lower molecular weight NAs being the primary contributors to mixture toxicity, a study was initiated to fractionate an extracted NA mixture by molecular weight and to assess each fraction's toxicity. Successful molecular weight fractionation of a methylated NA mixture was achieved using a Kugelrohr distillation apparatus, in which fractions collected at higher boiling points contained NAs with greater total carbon content as well as greater degree of cyclicity. Assays with *Vibrio fischeri* bioluminescence (via Microtox assay) revealed that the lowest molecular weight NAs collected had higher potency (EC_{50}: 41.9 ± 2.8 mg l^{-1}) than the highest molecular weight NAs collected (EC_{50}: 64.9 ± 7.4 mg l^{-1}). Although these results support field observations of microbial degradation of low molecular weight NAs decreasing OSPW toxicity, it is not clear why larger NAs, given their greater hydrophobicity, would be less toxic.

1. Introduction

The oil sands industry of north-eastern Alberta, Canada, has experienced rapid development and growth throughout the past decade. In 2000, non-conventional oil production methods (oil sands) approximately equalled conventional oil production methods, with non-conventional methods totalling 604 700 barrels day^{-1} (1 barrel = 159 L), and conventional methods totalling 591 000 barrels day^{-1} (Alberta Energy and Utilities Board, 2004–2005). In 2007, non-conventional methods increased to an average of 1 130 000 barrels day^{-1} while conventional methods dropped to an average of 543 000 barrels day^{-1} (Alberta Energy and Utilities Board, 2006–2007). In addition to increased production, oil sand royalties have steadily increased due to steady increases in the average cost of oil. The average cost (USD) for a barrel of oil in 2003 was $29.13 (Alberta Energy and Utilities Board, 2003–2004), $64.90 in 2007 (Alberta Energy and Utilities Board, 2006–2007), and $105.62 in March 2008 (Williams, 2008). As the costs associated with oil sands refining decrease due to technological advances, and global conventional oil supplies decline, the oil sands projects of the Athabasca Basin will continue to expand (Williams, 2003).

Most oil sands refining processes involve the Clark hot water extraction method in which large volumes of water are used to extract bitumen (FTFC, 1995a). The oil sands process-affected water (OSPW) produced by this method is comprised primarily of sand, clay, and unrecoverable bitumen and hydrocarbons, and is contained in ponds on site as part of a zero discharge policy (FTFC, 1995b). Investigations into the toxicity of OSPW showed that aquatic organisms such as *Salmo gairdneri* and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) were sensitive to exposure at concentrations present in oil sands tailings ponds (MacKinnon and Boerger, 1986; Warith and Yong, 1994). Further studies have identified naphthenic acids (NAs) and their sodium naphthenate salts as the primary toxic components (Dokholyan and Magomedov, 1983; MacKinnon and Boerger, 1986).

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Naphthenic acids (Fig. 1) are a diverse group of acyclic, monocyclic, and polycyclic carboxylic acids, all with the general formula of $C_nH_{2n+z}O_2$, where n represents the carbon number and z is the homologous group series number related to the number of five- or six-membered carbon rings within the structure. NAs are natural components of petroleum and their presence in the Athabasca oil sands is thought to be due to the biodegradation of mature petroleum (Clemente and Fedorak, 2005). In addition to being released into OSPW through the extraction of bitumen from the Athabasca oil sands deposit (FTFC, 1995b), NAs may also enter surface water systems through natural groundwater mixing and erosion of riverbank oil deposits (Headley and McMartin, 2004).

Field studies have demonstrated that the acute toxicity of OSPW decreases with time (MacKinnon and Boerger, 1986). Fathead minnows exposed to OSPW that had been degraded for four weeks in 4 l Erlenmeyer flasks experienced a decrease in toxicity compared to fresh tailings (Lai et al., 1996), and *Vibrio fischeri* (via Microtox assay) exposed to commercial NA mixtures that had undergone microbial degradation in 500 ml Erlenmeyer flasks demonstrated complete removal of toxicity following several weeks (Clemente et al., 2004). This decrease in toxicity has been correlated with an increase in the proportion of NAs with $\geqslant$22 carbons (the C_{22+} NAs) (Holowenko et al., 2002), suggesting that OSPW NA mixture toxicity is mainly influenced by lower molecular weight constituents. It is suspected that since indigenous microbial populations are better able to degrade NAs with <22 carbons (Clemente et al., 2004; Biryukova et al., 2007), the proportion of C_{22+} NAs increases with aging (Quagraine et al., 2005). Recent analysis using capillary HPLC/QTOF-MS to characterize NA mixtures suggests that C_{22+} NAs may not be present within OSPW and previous detection was likely due to the double-derivatization of hydroxylated NAs (Bataineh et al., 2006). Although NAs containing greater than 22 carbons may not be abundant, microbial populations responsible for the biodegradation of NAs are more effective at removing lower MW compounds, and the shift in composition of the NA mixture to higher MW compounds over time has led to decreased toxicity of OSPW.

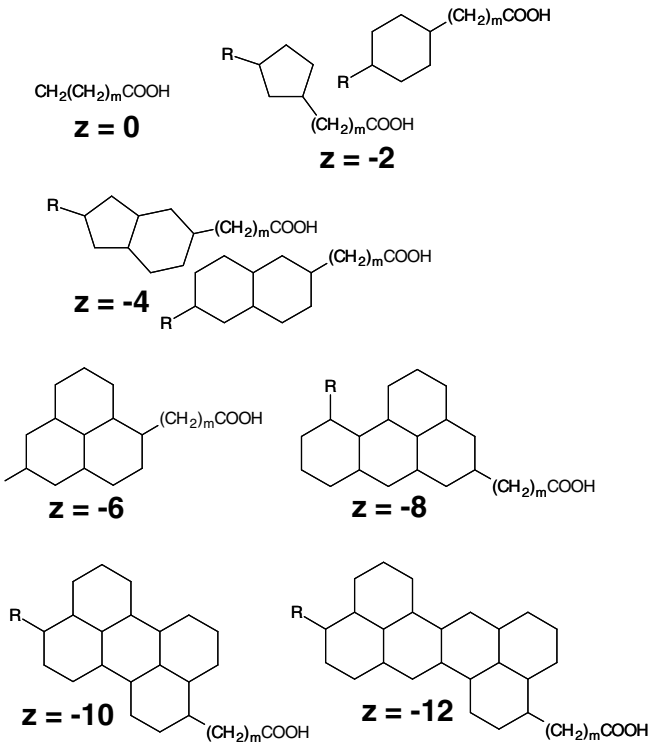

Fig. 1. Examples of naphthenic acid structures for various z-series. R represents an alkyl group and m represents the length of the alkyl chain.

Naphthenic acids most likely act as surfactants due to the presence of hydrophobic alkyl groups and a hydrophilic carboxylic moiety (Clemente and Fedorak, 2005), and a probable primary mode of action for acute toxicity of NAs is narcosis. Narcosis, also known as membrane disruption, is a non-specific mode of action recognized as the disruption of a cell membrane through the presence of a hydrophobic compound in the lipid bilayer (Klopman et al., 1999; Konemann, 1981). The accumulation of a xenobiotic agent in the lipid bilayer affects membrane fluidity, thickness, and surface tension (Schultz, 1989), all of which contribute to alterations in membrane function and can ultimately result in cell death. The narcotic effect of a molecule is correlated with its size and lipophilicity, due to the ability of lipophilic compounds to enter the lipid bilayer and for larger molecules to cause greater disruption and damage to the structure of a cell membrane (Protic and Sabljic, 1989; Schultz, 1989). Molecules up to approximately 1000 Da in size are considered small enough to be capable of permeating a cellular membrane (Sanderson et al., 2004). As the MWs of OSPW NAs $\leqslant$600 Da (Holowenko et al., 2002), it would be expected that all NAs are bioavailable and that their toxicity should be correlated with increasing molecular size. However, as discussed previously, field studies indicate that the toxicity of NA mixtures is driven primarily by the smaller compounds.

The objective of this study was to determine the relationship between the molecular weight of NAs found in OSPW and their toxicity. To accomplish this goal, a NA extract from fresh OSPW was fractionated by Kugelrohr distillation to generate fractions of different molecular sizes. The toxicity of these fractions was then assessed using the Microtox assay which is based on bioluminescence of the marine bacterium, *V. fischeri*.

2. Materials and methods

2.1. Collection, extraction, and purification of a naphthenic acid extract

A 3000 l sample of OSPW was collected from the input of West End pit settling basin of Syncrude Canada Ltd. in Fort McMurray, Alberta, Canada in June 2005. NAs were extracted from the OSPW and partially purified as previously described (Frank et al., 2006). In brief, the extraction and purification process included a dichloromethane (DCM, Caledon, Georgetown, ON) wash as well as several acidification and filtering steps, creating a final mixture that was comprised primarily of acidic compounds (e.g., NAs). Once the extraction and purification of NAs was complete, the final product was combined in 20 l polyethylene carboys (Fisher Scientific, Whitby, ON), then dispensed into 1 l amber glass bottles (Fisher Scientific), and stored at 4 °C.

2.2. Preparation of NA stock for methylation

To enable fractional distillation, all NAs were converted to methyl esters. A one-l amber glass NA stock bottle was sonicated in warm water for 5 min (Ultrasonic cleaner model FS30, 130 W, Fisher Scientific) to dissolve any NAs that may have precipitated out of solution while in storage. A 500 ml aliquot of NA stock solution was removed and acidified to pH 2 with 12 M hydrochloric acid (HCl). The NA precipitate that formed was added to a vacuum filtration unit (90 mm glass holder, Advantec MFS, Inc., Dublin, CA) fitted with a 0.2 μm pore size PTFE filter (Sartorius, Fisher Scientific) set at 330 mm Hg. The NA precipitate was solubilised by sonicating the PTFE filter paper in a beaker containing 100 ml of