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Toxic effects of oil sand naphthenic acids on the biomass accumulation of 21 potential phytoplankton remediation candidates ☆

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ABSTRACT

The oil sands of northern Alberta, Canada contain an estimated 170 billion barrels of crude oil. Extraction processes produce large amounts of liquid tailings known as oil sand process affected water (OSPW) that are toxic to aquatic organisms. Naphthenic acids (NAs), and their sodium salts, represent a significant contributor to the toxicity of these waters. Due to the recalcitrant nature of these compounds, an effective mode of remediation has yet to be established. This study investigates the suitability of the use of phytoplankton for remediation efforts based on two criteria: the ability of phytoplankton strains to withstand the toxic effects of NAs, and their rate of biomass accumulation. A total of 21 phytoplankton strains were isolated from waters containing NAs, cultured, and maintained under unialgal conditions. These strains were then exposed to NAs in concentrations ranging from 0 mg L^{-1} to 1000 mg L^{-1} over a 14 day period. Inhibition of growth was observed at 30 mg L^{-1} NA (one strain), 100 mg L^{-1} NA (one strain), 300 mg L^{-1} NA (six strains), and 1000 mg L^{-1} NA (six strains). Five strains failed to show any growth inhibition at any test concentration and two strains could not be analysed due to poor growth during the test period. Strains were then ranked based on their suitability for use in remediation efforts.

1. Introduction

The Athabasca oil sands of Alberta, Canada represent one of the largest deposits of oil worldwide. With proven reserves totalling approximately 170 billion barrels of bitumen, these deposits represent approximately 13% of all known crude oil reserves (RAMP, 2011). Production of synthetic crude oil from the Athabasca oil sands is a growing industry, with marketable bitumen and synthetic crude oil production from oil sand mining efforts averaging 1.35 million barrels per day in 2009, an increase of 160,000 barrels per day over the previous year (Alberta Energy and Utilities Board, 2010). Much of this production is based on the Clark hot water extraction method which utilizes hot (80 °C), caustic water to separate usable bitumen from sand and other waste materials (FTFC, 1995a). This process produces large amounts of liquid tailings, commonly referred to as oil sand process-affected water (OSPW), that are toxic to aquatic organisms (Dutka et al., 1995; Herman et al., 1994; Warith and Yong, 1994). As part of a governmental licensing agreement, the oil sand mining industry is obligated to return all oil sands and associated wetlands to an environmentally stable state once mining has ceased (FTFC, 1995b).

Principal toxic components of OSPW have been determined to be naphthenic acids (NAs) and their sodium salts (Dokholyan and Magomedov, 1983; MacKinnon and Boerger, 1986). NAs are natural constituents of oil sand ore and can comprise up to 2% by weight of total bitumen (Headley and McMartin, 2004). Consisting of a mixture of acyclic, monocyclic and polycyclic carboxylic acids, NAs have traditionally been assigned the general formula of $C_nH_{2n+z}O_2$, where n is the carbon number and z signifies hydrogen deficiency produced by ring formation (Rogers et al., 2002; Scott et al., 2008). However, recent analyses have revealed the presence of other chemical components and structural conformations (Bataineh et al., 2006; Frank et al., 2009; Headley et al., 2011; Rowland et al., 2011a, 2011b) leading to the suggestion that "naphthenic acids" likely define a class of chemicals far more complex than this traditional formula (Grewer et al., 2010). Due to their natural occurrence in oil sand, NAs can be observed in concentrations up to 2 mg L^{-1} in natural wetlands located throughout the oil sands (Allen, 2008). However, typical concentrations of NAs for water involved with the bitumen extraction process range from 3 to 68 mg L^{-1} for settling basins and tailings ponds up to 110 mg L^{-1} for fresh OSPW (Headley and McMartin, 2004). Due to the toxicity of these compounds with

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respect to aquatic organisms, methods for the reduction of NA concentrations and toxicity in OSPW have recently been the subject of much research. Ozonation and microbial degradation have both been proposed as possible methods for NA toxicity remediation of OSPW (Clemente and Fedorak, 2004; Martin et al., 2010; Scott et al., 2008). An alternative to these methods, phytoplankton assisted remediation, may reveal additional potential upon more thorough examination.

With an estimated 30,000–50,000 species of algae worldwide, phytoremediation using algae is a field that has yet to be explored in great detail. Although it has previously been shown that algal community structure in OSPW is dependent upon NA concentrations and salinity (Leung et al., 2001), it has also been demonstrated that strains of algae from a wide range of algal classes (Chlorophyceae, Cyanophyceae, Diatomophyceae, Dinophyceae, Euglenophyceae, Synurophyceae, Trebouxiophyceae) exist within waters contaminated with NAs in concentrations $>20\ mg\ L^{-1}$ (Leung et al., 2003). Due to their ability to exist in water containing elevated concentrations of NAs, algae provide an interesting potential platform for remediation efforts. Several species of microalgae have been observed to take up, and in some cases degrade, many types of toxic aquatic contaminants such as heavy metals (Wilde and Benemann, 1993) and polycyclic aromatic hydrocarbons (PAHs) (Chan et al., 2006; Lei et al., 2007, 2002). Headley et al. examined the phytodegradation potential of NAs by 12 different strains of phytoplankton (cyanobacteria, green algae and diatoms) and compared the uptake of a model NA compound (4-methycyclohexaneacetic acid) to that of a NA mixture extracted from OSPW (Headley et al., 2008). These authors demonstrated that one strain of *Selenastrum* sp. (Trebouxiophyceae) was potentially able to take up the oil sand extracted NA mixture and two strains of *Navicula* sp. (Diatomophyceae) were able to take up the model NA compound (Headley et al., 2008). This work not only demonstrates the potential of algal remediation efforts involving NAs, but also the degree of specificity of individual phytoplankton strains for impacting mixtures of NAs and naphthenic acid-like compounds.

Reduction of NA toxicity using phytoplankton has several other ancillary benefits that can increase this strategy's attractiveness as a cost-effective method for the removal of aquatic contaminants such as NAs. Among these benefits are the production of biomass that can be used as an active substrate for future remediation efforts, the potential for CO_2 mitigation (Yun et al., 1997), and the possibility for biodiesel production from algal oil (Chisti, 2007).

In order for the removal of NAs from OSPW by phytoplankton to be viable, several key criteria must be met: (i) the ideal algal strain candidate must have the ability to tolerate NA toxicity at concentrations at or exceeding the concentrations observed/present of NAs contained within fresh OSPW (approximately $100\ mg\ L^{-1}$) (Leung et al., 2003), (ii) the strain must possess a high growth rate in order to maintain production levels, and (iii) the ideal phytoplankton remediation candidate must possess the ability to reduce concentrations of NAs in OSPW, likely through uptake and/or degradation processes. This present study examines the feasibility of growing algae in water containing elevated concentrations of NAs and prioritizes potential phytoplankton remediation candidates based on their growth rates and response to a wide concentration range of NAs extracted from OSPW.

2. Materials and methods

2.1. Isolation and classification of algae species

Phytoplankton samples were collected from 21 sampling locations, from natural as well as lease sources near Fort McMurray, Alberta, Canada. Natural sampling locations included the Athabasca and Clearwater rivers, Gregoire Lake, Saline Lake and Poplar Creek reservoir. Lease sources included a number of test ponds located on oil sand leases. Samples were collected using a phytoplankton net with an effective pore size of 20 μm and maintained at 5 °C until culturing. Sterile micropipetting techniques (Andersen, 2005) were then used to isolate and repeatedly wash individual algal cells for culture. The resulting cultures were unialgal (containing only one algal strain as verified through compound microscopy). Throughout the course of this study cultures were stored in a diurnal plant growth chamber model #E-36HO (DiaMed Lab Supplies Inc., Mississauga, ON), with a photoperiod of 17 h, average temperatures of 19.5 °C during light period and 15.5 °C during dark period, an average illumination of approximately $46{,}000\ cd\ sr\ m^{-2}$ during light period, and carbon dioxide concentrations of 1250 ppm. With the exception of the increased carbon dioxide concentration, these conditions were intended to represent the average conditions present in Ft. McMurray, AB from June until August.

Isolated phytoplankton strains were identified to family, genus and, where possible, species level based on morphological characteristics (John et al., 2002; Wehr and Sheath, 2003) using a Nikon Labophot phase contrast compound stereo microscope (Nikon Instruments Inc., Melville, NY, USA). Further analyses using molecular phylogenetics are currently ongoing due to taxonomic uncertainty in many of these taxa.

2.2. NA extract

The NA extract used in this investigation was isolated from fresh OSPW collected prior to the point of discharge into the West In-Pit settling basin at Syncrude Canada Ltd., according to the methods described in Frank et al. (2006). In order to calculate the nominal test concentrations, the concentration of NAs in the extract stock solution was determined by electrospray ionization mass spectrometry (ESI-MS) at the National Water Research Institute in Saskatoon, SK using the method described by Headley et al. (2002).

2.3. Experimental setup

Sterilized glass culture tubes were inoculated with two millilitres of a single, axenic algal culture and eight millilitres of a mixture of phytoplankton culture media and NA stock solution. Each algal strain was subjected to a range of NA concentrations over the course of a 14-day period. Test treatments for this investigation were $0\ mg\ L^{-1}$ (control), $10\ mg\ L^{-1}$, $30\ mg\ L^{-1}$, $100\ mg\ L^{-1}$, $300\ mg\ L^{-1}$, and $1000\ mg\ L^{-1}$ total NAs, as well as a solvent control containing a volume of 0.05 N NaOH equal to that of the NA stock solution used in the $1000\ mg\ L^{-1}$ trials. As sampling was destructive, three replicates for each test concentration at each sampling date were created. The solvent control was included to assess the potential impact of nutrient deficiency caused by the decreased volume of media in the highest test concentration. The pH of the algal growth media was adjusted to 8.0 ± 0.1 in order to ensure that the NAs were in solution as naphthenate ions, representative of NAs in alkaline OSPW. Dry weight biomass was used as the endpoint for these experiments due to direct correlation with growth rate and for ease of sampling. Biomass sampling was destructive and was collected on pre-weighed 47 mm, 0.50 μm effective pore size glass fibre filters (Pall Corp., Port Washington, NY, USA) on days 0, 1, 2, 4, 7, and 14 via vacuum filtration, dried in a 40 °C drying oven, and weighed every 12 h until 3 consecutive readings provided a constant weight ± 0.002 g.

As naphthenic acid tolerance is one of the key criteria for the selection of a phytoplankton strain for NA remediation purposes, and due to the unknown nature of the toxic effects of NAs on the 21 isolated algal strains tested, this study was designed to determine at what critical concentration growth is affected. Following these tests, it can be established whether this effect concentration (EC) was near the concentration of NAs present in fresh OSPW. As such, test concentrations of a mixture of NAs extracted from fresh OSPW ranged from $0\ mg\ L^{-1}$ to $1000\ mg\ L^{-1}$. Consequently, precise EC values were not calculated for each tested phytoplankton strain. These results are summarized in Tables 1 and 2.

2.4. Growth conditions

All experiments were carried out in light intensity, photoperiod, and temperature conditions intended to represent conditions in Fort McMurray, AB from June until August. Conditions included a photoperiod of 17 h, average temperatures of 19.5 °C during light period and 15.5 °C during dark period, and an average illumination of approximately $46{,}000\ cd\ sr\ m^{-2}$ during light period. The growth media used for these experiments was selected based on a comparison of three media types (Bold's Basal media (Nichols and Bold, 1965), CHU-10 media (Stein, 1973), Cyano media (Juttner, 1983)). The media that displayed the highest average biomass accumulation across all strains was selected. A cyanobacterial media described by Juttner (1983) (Juttner, 1983) containing 0.6 mM $CaCl_2$, 8 mM $NaNO_3$, 0.8 mM K_2HPO_4, 0.4 mM $MgSO_4$, 10 μM NaFeEDTA, 10 μM $MnCl_2$, 2 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.2 μM $CoSO_4$, and 0.6 mM Na_2SiO_3 was used. This modified media was provided for all species at the start of the experiment. Although this media was originally intended for Cyanobacteria only, and was not specific to the