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Assessing the bioremediation potential of algal species indigenous to oil sands process-affected waters on mixtures of oil sands acid extractable organics

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ABSTRACT

Surface mining extraction of bitumen from oil sand in Alberta, Canada results in the accumulation of oil sands process-affected water (OSPW). In attempts to maximize water recycling, and because its constituents are recognized as being toxic, OSPW is retained in settling basins. Consequently, research efforts are currently focused on developing remediation strategies capable of detoxifying OSPW to allow for eventual release. One potential bioremediation strategy proposes to utilize phytoplankton native to the Alberta oil sand region to sequester, break down, or modify the complex oil sands acid extractable organic (AEO) mixtures in OSPW. Preliminary attempts to quantify changes in total oil sands AEO concentration in test solutions by ESI-MS following a 14-day algal remediation period revealed the presence of unknown organic acids in control samples, likely released by the phytoplankton strains and often of the same atomic mass range as the oil sands AEO under investigation. To address the presence of these "biogenic" organic acids in test samples, ESI-MS in MRM mode was utilized to identify oil sands AEO "marker ions" that were a) present within the tested oil sands AEO extract and b) unique to the oil sands AEO extract only (e.g. atomic masses different from biogenic organic acids). Using this approach, one of the 21 tested algal strains, *Stichococcus sp.* 1, proved capable of significantly reducing the AEO marker ion concentration at test concentrations of 10, 30, and 100 mg L^{-1}. This result, along with the accelerated growth rate and recalcitrance of this algal strain with exposure to oil sands AEO, suggests the strong potential for the use of the isolated *Stichococcus sp.* 1 as a candidate for bioremediation strategies.
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1. Introduction

Bitumen influenced waters within Alberta's oil sands region are home to a variety of phytoplankton (Woodworth et al., 2012), yet few studies have focused on the potential for these native algal species for use in the remediation of oil sands process-affected water (OSPW). However, research has shown that algae may be useful in bioremediation for a range of compounds, including heavy metals (Wilde and Benemann, 1993) , polycyclic aromatic hydrocarbons (PAH) (Chan et al., 2006) and model naphthenic acids (NA) (Quesnel et al., 2011). (Headley et al., 2008) identified algal strains that were able to take up a model naphthenic acid compound (4-methycyclohexaneacetic acid), exemplifying the species-specific nature of biodegradation, as well as the influence of NA structure on biodegradability. These studies highlight the potential feasibility of using microalgae as a bioremediation process for Alberta oil sands tailings.

Containing an estimated 167.1 billion barrels of recoverable bitumen (Energy Resources Conservation Board, 2014), the Alberta oil sands deposits represent the third largest proven global oil reserves, trailing only Saudi Arabia and Venezuela in estimated crude reserves (Canadian Association of Petroleum Producers, 2012). Surface mining of oil sands, focused primarily within the Athabasca oil sands deposit (Royal Society of Canada Expert Panel, 2010), utilizes the Clark hot water extraction process to separate bitumen from oil sands (FTFC, 1995). To maximize recycling in the extraction process, and due to

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the known toxicity of OSPW to many aquatic organisms [reviewed by Brown and Ulrich (2015)], OSPW is contained within tailings basins, with a previously estimated volume of 840 million m^3 contained within the mining areas (Energy Resources Conservation Board, 2010). Investigation into OSPW has identified the organic acid fraction, including NAs, as the principal toxic constituents (MacKinnon and Boerger, 1986) contributing to both acute and sub-acute toxicity (Brown and Ulrich, 2015; Clemente and Fedorak, 2005; Headley and McMartin, 2004).

Naphthenic acids are classically defined as a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the chemical formula of $C_nH_{2n+z}O_2$, where n indicates the carbon number and z (zero or negative) represents the hydrogen deficiency associated with the number of rings and double bonds (Clemente and Fedorak, 2005). While comprehensive multi-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOF/MS) has identified NAs in OSPW fitting the classical formula (Hao et al., 2005) and more specifically, tricyclic adamantane-type acids (Rowland et al., 2011a), pentacyclic diamantane-type acids, and tetracyclic acids with cage-like structures (Rowland et al., 2011b; West et al., 2013), recent research has demonstrated that this definition is not sufficient to describe the complexity of structures observed within the organic acid fraction of OSPW. High resolution mass spectrometry (HRMS), has demonstrated recently that for acid extractable organics (AEO) in OSPW, the mixture also includes multiple oxygenated acid species and sulfur- and nitrogen-containing compounds (Barrow et al., 2009; Bataineh et al., 2006; Headley et al., 2011, 2013a, 2013b; Smith et al., 2008), as well as compounds containing aromatic rings (Frank et al., 2009; Jones et al., 2012; Kavanagh et al., 2009), bicyclic acids (Wilde and Rowland, 2015), and multiple carboxyl moieties (Frank et al., 2009).

While intensive research efforts are underway to better characterize the chemistry and toxicology of OSPW (Morandi et al., 2015), concurrent remediation and treatment efforts to address the still-growing reserves of OSPW are also under investigation. Ozonation is a relatively quick and effective method for reducing the AEO concentration and associated toxicity of OSPW (Anderson et al., 2012; He et al., 2010; Scott et al., 2008), however phytoplankton could become an alternative or potential complement to ozonation, due to their ancillary benefits. Subsequent to remediation, the accumulated algal biomass could be used in reclamation efforts as a soil amendment, or as biomass for biodiesel production (Chisti, 2007).

Although OSPW is known to be toxic to many forms of aquatic biota, several strains of microalgae are present in OSPW (Leung et al., 2001, 2003; Woodworth et al., 2012). The potential for AEO uptake by algal strains was assessed previously (Headley et al., 2008) by exposing several laboratory strains of algae to a model AEO surrogate as well as an AEO mixture extracted from OSPW, reporting two strains that appeared capable of degrading or removing the model AEO throughout the course of the experiment. However in this previous work no strains appeared to be able to reduce the concentration of the more environmentally relevant AEO mixture extract. Recent research has identified algal species which are able to degrade model NAs, such as *Rhodococcus* spp. (Demeter et al., 2015), and NAs in tailings water, such as *Dunaliella tertiolecta* (Quesnel et al., 2011). In addition, when a single species native to OSPW such as *Rhodococcus* spp. was compared to an OSPW algal community, the community degraded a greater degree of NAs (Demeter et al., 2015). Notably, the efficacy of an alga to remediate wastewater is dependent on the algal genus and species, exemplified by the variation in biodegradation between *Chlorella vulgaris* and *Emiliania huxleyi* resulting in partial and near-complete degradation of 1 mg L^{-1} *tert*-BPBA, respectively (Beddow et al., 2016). A 2012 study investigated the growth potential of 21 algal strains isolated from the Athabasca region following exposure to a range of OSPW derived AEO concentrations (Woodworth et al., 2012), revealing several strains that exhibited high growth rate and tolerance to AEO. The current investigation assessed the ability of these same 21 algal strains to naturally remove or break down AEO mixtures following a two-week exposure. These results, concomitant with the algal toxicity and growth rate results (Woodworth et al., 2012) for each species, indicate the most promising candidates for future assessment of bioremediation potential.

2. Materials and methods

2.1. Collection and purification of an acid extractable organics mixture

A 3000 L sample of fresh OSPW was collected at the inflow of West In-pit settling basin at Syncrude Canada Ltd. in Fort McMurray, Alberta, Canada in June 2008. The AEO fraction, including NAs, was extracted from the OSPW and partially purified as described previously (Frank et al., 2006). In brief, fresh OSPW was acidified and centrifuged, and the resulting organic acid precipitate was collected and stored in aqueous base. Diethylaminoethyl (DEAE) cellulose (Sigma Chemical Co., St. Louis, MO) was used to remove humic-like compounds, a solvent wash of dichloromethane (DCM, Caledon Laboratories Ltd., Georgetown, ON) was used to remove neutral organic compounds such as PAHs, and several acidification and filtering steps were applied, resulting in a final mixture that was comprised of the AEO fraction. Once the extraction and purification of the AEO mixture was complete, the final product was reconstituted in 0.05 N NaOH (VWR International, LLC, Ontario, Canada) at approximately pH 12 to ensure that the organic acids were in a water-soluble ionic state. The AEO mixture was then combined in 25-L polyethylene carboys (Fisher Scientific, Whitby, ON), dispensed into 1-L amber glass bottles with PTFE lids (Fisher Scientific), and stored at 4 °C. A solvent control of 0.05 N NaOH was tested to investigate the impact on algal growth due to limited nutrients in the highest test concentration.

2.2. Collection, isolation, and identification of algal strains indigenous to OSPW

Algal samples were collected using a 20 μm phytoplankton net from 21 sites located within and near the oil sands leases north of Fort McMurray, Alberta and stored at 5 °C until isolation was performed. Individual phytoplankton cells were then isolated from these samples and repeatedly washed using sterile micropipetting techniques. A total of 21 cultures resulting from the isolated algal cells were maintained in a modified cyanobacterial growth media (Jüttner, 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 $CoSO_4$ (Sigma-Aldrich Canada Co, Ontario, Canada) and 0.6 mM Na_2SiO_3. All chemicals used in this medium were purchased from VWR International (Ontario, Canada), unless otherwise stated. Cultures were grown under axenic conditions in a plant diurnal growth chamber (Model E-36HO, DiaMed Lab Supplies Inc., Mississauga, ON) (Woodworth et al., 2012). The incubator was kept at a carbon dioxide concentration of 1250 ppm and cultures were shaken manually twice per day. The growth conditions selected were used to approximate conditions in Fort McMurray, Alberta. Growth conditions included a light intensity of 46,000 cd sr m^{-2}, and 17 h:7 h (light: dark) light cycle, during which the temperature was 19.5 °C during the light period and 15.5 °C during the dark period. These conditions were

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