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Biodegradation of naphthenic acids in oils sands process waters in an immobilized soil/sediment bioreactor

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HIGHLIGHTS

- Naphthenic acids in oil sands process water were degraded by biofilm consortium.
- High biological removal rate of naphthenic acids (2.32 mg L^{-1} d^{-1}) was achieved.
- Ion Torrent sequencing showed a dominance of ammonium and nitrite oxidizing bacteria.
- Species of *Truepera*, *Flexibacter* and *Saprospiraceae* may degrade more recalcitrant NAs.

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ABSTRACT

Aqueous extraction of bitumen in the Alberta oil sands industry produces large volumes of oil sands process water (OSPW) containing naphthenic acids (NAs), a complex mixture of carboxylic acids that are acutely toxic to aquatic organisms. Although aerobic biodegradation reduces NA concentrations and OSPW toxicity, treatment times are long, however, immobilized cell reactors have the potential to improve NA removal rates. In this study, two immobilized soil/sediment bioreactors (ISBRs) operating in series were evaluated for treatment of NAs in OSPW. A biofilm was established from microorganisms associated with sediment particles from an OSPW contaminated wetland on a non-woven textile. At 16 months of continuous operation with OSPW as the sole source of carbon and energy, 38 ± 7% NA removal was consistently achieved at a residence time of 160 h at a removal rate of 2.32 mg NAs L^{-1} d^{-1}. The change in NA profile measured by gas chromatography–mass spectrometry indicated that biodegradability decreased with increasing cyclicity. These results indicate that such treatment can significantly reduce NA removal rates compared to most studies, and the treatment of native process water in a bioreactor has been demonstrated. Amplification of bacterial 16S rRNA genes and sequencing using Ion Torrent sequencing characterized the reactors' biofilm populations and found as many as 235 and 198 distinct genera in the first and second bioreactor, respectively, with significant populations of ammonium- and nitrite-oxidizers.

1. Introduction

The Athabasca oil sands of Northern Alberta is one of the world's largest petroleum reserves containing ~168 billion recoverable barrels of bitumen (ERCB, 2013). In 2012, bitumen production exceeded 302 000 m^3 d^{-1} (1.9 million barrels per day (bbl d^{-1})) and is expected to reach 604 000 m^3 d^{-1} (3.8 million bbl d^{-1}) by 2022 (ERCB, 2013). The aqueous hot water extraction process to recover bitumen from surface mined ore produces large quantities of aqueous tailings, known as oil sands process water (OSPW) which contains sand, clay fines, silts, dissolved ions, heavy metals and organic compounds. OSPW has demonstrated toxicity to a number of species (MacKinnon and Boerger, 1986; Rogers et al., 2002; Nero et al., 2006; Armstrong et al., 2009) and cannot be discharged to ground or surface waters under Alberta's Environmental Protection and Enhancement Act. Consequently, it is held in tailing and settling ponds, the total volume of which exceeded 720 million m^3 in 2011 (ERCB, 2011).

MacKinnon and Boerger (1986) first attributed the toxicity of OSPW to naphthenic acids (NAs), a persistent group of aliphatic and cyclic organic acids having the general form $C_nH_{2n+Z}O_2$, where

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n is the carbon number and Z is zero or a negative, even integer representing the hydrogen deficiency due to rings or unsaturated bonds. However, the acid-extractable organic fraction of OSPW has been found to contain additional components that are not consistent with this definition, including "oxy-NAs" of the form $C_nH_{2n+Z}O_x$, where $x = 2–5$, and heteroatomic species (Grewer et al., 2010), which may also contribute to toxicity. In this work, the term NAs refers to the total acid extractable organic (AEO) portion of OSPW.

The biodegradability of model NAs (Herman et al, 1993; Smith et al., 2008; Paslawski et al., 2009; Johnson et al., 2011) and commercially available NAs (Clemente et al., 2004; del Rio et al., 2006; Biryukova et al., 2007) is well established. However, OSPW NAs are less biodegradable than commercial NAs (Scott et al., 2005), and often cultures that degraded commercial NAs could not reduce NA concentrations in OSPW (Bataineh et al., 2006; Headley et al., 2010). The ability of microorganisms to reduce OSPW NA concentrations and toxicity has been established in shake flask (Herman et al., 1994; Scott et al., 2005; Han et al., 2008) and in simulated wetlands (Toor et al., 2012), however, treatment times are long, with half-lives of 44–240 d in shake flask studies (Han et al., 2008), and of 11–13 y for natural attenuation in tailings ponds (Han et al., 2009).

A potential strategy to reduce the treatment time is to use a bioreactor in which the degrading population is immobilized, such as in an immobilized soil/sediment bioreactor (ISBR). The ISBR has been used to treat pollutants such as pentachlorophenol (Karamanev et al., 1997), trichloroethylene (Ramsay et al., 2001) and petroleum hydrocarbons (Kermanshahi et al., 2005; Jajuee et al., 2006), but not NAs in OSPW. The reactor is inoculated with contaminated soil containing microorganisms that have been naturally enriched to degrade the contaminant and immobilization allows retention of slow growing cells and attainment of higher volumetric productivities (Karamanev and Samson, 1998). Treatment of a model NA (*trans*-4-methyl-1-cyclohexane carboxylic acid) in an immobilized cell, packed bed reactor achieved a removal rate almost two orders of magnitude higher than in continuous suspension culture (Paslawski et al., 2009). Huang et al. (2013) achieved 18.5% removal of classic NAs in 19 h using a continuous flow biofilm reactor. Compared to planktonic cells, biofilms are believed to be less susceptible to environmental stresses such as heavy metals and hydrocarbons (Hall-Stoodley et al., 2004) and will better survive system perturbations.

The aim of this study was to evaluate the potential of two ISBRs connected in series to remove NAs from OSPW. The hypothesis was that using an immobilized population in the ISBRs would decrease the treatment time required to reduce the NA concentration. Over 16 months of continuous operation, typical NA removal levels were established and the change in the NA profile with ISBR treatment determined. Operating and monitoring concerns associated with OSPW treatment were identified. Additionally, the microbial communities in the biofilm were characterized using metagenomics method with Ion Torrent sequencing.

2. Materials and methods

2.1. Materials

OSPW was obtained from the West-In-Pit (WIP), an active settling basin on the Syncrude Canada site in Fort McMurray. Water was stored in sealed 20 L carboys at room temperature, with periodic measurement to ensure that NA concentrations were constant.

A mixture of sediments from a natural wetland, a consolidated tailings wetland, and a high sulfate wetland was used as the inoculum for the ISBRs. The sediments were mixed with water to form a slurry that was poured onto the polyester fiber matting used as an inert surface for biofilm formation. The OSPW fed to the ISBRs was not autoclaved and can also be considered a source of NA-degrading organisms.

2.2. Reactor set-up and operation

A two-stage ISBR system should allow the development of two NA-degrading populations, one that can degrade the easier to degrade NAs in the first reactor, and with the more recalcitrant NAs feeding into the second ISBR, a second population that can degrade the more recalcitrant NAs should develop. The 1st stage ISBR (R1) set-up is shown in Fig. 1, and is identical to the 2nd stage ISBR except for the nutrient and OSPW feed. Each ISBR was a cylindrical glass vessel with an internal diameter of 16.3 cm, a height of 30.5 cm, and a working volume of ~4.8 L, enclosed on the top and bottom by stainless steel plates secured with flanges. In the center of the cylinder are two stainless steel screens (16.3 cm × 23 cm) with a 1 cm mesh, supporting a 3 cm thick piece of polyester fiber. Reactors were operated in a continuous flow regime with separate OSPW and mineral salts medium (MSM) feeds to R1 via peristaltic pumps using Masterflex Tygon tubing (Cole-Parmer Canada, Montreal, QC) at flow rates of 48 mL h^{-1} and 12 mL h^{-1}, respectively, resulting in an 80 h residence time in each reactor. The MSM contained (per Liter) 1.10 g $(NH_4)_2SO_4$, 1.33 g Na_2HPO_4, 0.13 g KH_2PO_4, 1.77 mg $MgSO_4{\cdot}7H_2O$, and 1.33 mg $CaCl_2{\cdot}2H_2O$. R1 effluent was fed directly to Reactor 2 (R2). R2 nutrients were provided by the effluent of R1, with the exception of an independent feed of 15.53 g L^{-1} $NaNO_3$ at a rate of 0.006 L h^{-1}, providing 1.03 g L^{-1} NO_3^- to R2. Reactors were operated at room temperature, 22 ± 2 °C.

The pH in each reactor was measured by a Cole-Parmer (Montreal, QC) high-pH tolerant, double junction pH electrode, attached to an αlpha (Eutech Instruments, Vernon Hills, IL) pH800 pH/ORP controller. The low pH set point was 8.00 with a hysteresis band of 0.1 and 2 M KOH was used for pH adjustment. Fresh air was added to each reactor at a rate of 20 mL min^{-1}, controlled by an Omega (Stamford, CT) FMA-2600A series mass flow controller and distributed with a fish tank sparger. The headspace gas was recirculated at a flow rate of ~150 mL min^{-1} by a diaphragm pump in the CO_2 sensor. Moisture was removed from the air with a Drierite (Xenia, OH) column prior to measuring CO_2 in the headspace with a Topac (Cohasset, MA) Guardian CO_2 monitor. O_2 in the headspace gas was monitored during experimentation with a differential oxygen analyzer (DOX) (S104 DOX system, Qubit Systems, Kingston, ON). The DOX was calibrated prior to each use and headspace oxygen monitored for a minimum of 3 h, with measurements recorded every 90 s. Dissolved oxygen (DO) was measured with a Sensorex (Garden Grove, CA) 12 mm DO probe. pH, DO, and CO_2 were constantly monitored. Instrumentation output signals were collected by an ADR 2000 board (Ontrak Control Systems Inc., Sudbury, ON) and recorded with Labview 6 (National Instruments, Vaudreuil-Dorion, QC). Measurements were recorded every 90 s.

2.3. Sample collection and analyses

Untreated OSPW and aqueous reactor samples were centrifuged at 14475*g* for 20 min to remove suspended sediment and microorganisms. The supernatant was collected and stored at −20 °C in 100 mL glass vials until analysis. Biofilm samples were recovered from the geotextile membrane by vortexing a 1 g sample of biofilm/support with 30 mL of phosphate saline buffer and 3 g of glass beads. The biomass was recovered after centrifugation at 8000*g* for 10 min and 0.7 mL of suspended cells were mixed with

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