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Exploring the capacity for anaerobic biodegradation of polycyclic aromatic hydrocarbons and naphthenic acids by microbes from oil-sands-process-affected waters

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ABSTRACT

Both polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids (NAs) are natural components of fossil fuels, but they are also widespread toxic and environmentally persistent pollutants. They are the major cause of environmental toxicity in oil-sands-process waters (OSPW). This study aimed to investigate the anaerobic biodegradation of the PAHs pyrene and 2-methylnaphthalene, and the NAs adamantane-1-carboxylic acid and a "natural" NA mixture (i.e., acid-extractable NAs from OSPW) under sulfate-reducing and methanogenic conditions by a microbial community derived from an oil sands tailings pond. Using gas-chromatography mass spectrometry (GC–MS), the rate of biodegradation was measured in relation to changes in bacterial community composition. Only 2-methylnaphthalene was significantly degraded after 260 days, with significantly more degradation under sulfate-reducing (40%) than methanogenic conditions (25%). During 2-methylnaphthalene biodegradation, a major metabolite was produced and tentatively identified as 2-naphthoic acid. Denaturing gradient gel electrophoresis (DGGE) demonstrated an increase in intensity of bands during the anaerobic biodegradation of 2-methylnaphalene, which derived from species of the genera *Fusibacter*, *Alkaliphilus*, *Desulfobacterium*, *Variovorax*, *Thaurea*, and *Hydrogenophaga*. Despite the biodegradation of 2-methylnaphthalene, this study demonstrates that, under anaerobic conditions, NAs and high-molecular-weight PAHs are the predominant molecules likely to persist in OSPW. Therefore alternative remediation strategies are required.

Introduction

Oil sands operations in Canada produce more than 200 million barrels of crude oil per year (Del Rio et al., 2006). During oil-sand refining, vast quantities of wastewaters known as tailings are generated that have to be stored indefinitely in settling basins or ponds until strategies for reclamation are devised and approved. Tailings are composed of solids (e.g., sand, silt) and oil-sands-process-affected water (OSPW) that contains complex mixtures of toxic carboxylic acids known as naphthenic acids (NAs). It has been estimated that in Canada alone >840 million m^3 of OSPW has accumulated and is being stored in tailings ponds (Siddique et al., 2011). There have been very few studies on anaerobic NA biodegradation despite the fact that, as most tailings ponds mature, they become anoxic (Whitby, 2010); and in sulfate-depleted ponds, methane is produced in very large quantities (Holowenko et al., 2000; Fedorak et al., 2002; Clemente and Fedorak, 2005). For example, methane released from Syncrude's largest tailings pond, the Mildred Lake Settling Basin, has been estimated to be at levels up to 43,000 m^3 day^{-1} (Holowenko et al., 2000; Siddique et al., 2006). It is not understood whether NAs present in tailings ponds can act as substrates in methane biogenesis in situ. Holowenko et al. (2001) demonstrated that methanogenesis was stimulated by the model NA compounds 3-cyclohexylpropanoic acid and 4-cyclohexanepentanoic acid in microcosms containing tailings pond water (Holowenko et al., 2001). Furthermore, Siddique et al. (2006, 2007, 2011) have shown that short-chain alkanes, BTEX compounds (benzene,

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toluene, ethylbenzene, and xylenes) and longer-chain alkanes (up to C_{18}) are degraded under methanogenic conditions in OSPW (Siddique et al., 2011). Additionally, to date, only a limited number of hydrocarbons have been shown definitively to biodegrade anaerobically in other environments (reviewed by Widdel et al., 2010). This has been shown using either pure cultures or in microcosms (containing soil, river sediment, aquifer material, or marine sediment) under methanogenic conditions or using nitrate, iron III, manganese IV, sulfate, or carbon dioxide as terminal electron acceptors (Widdel et al., 2010). In addition to the environmental problems caused by NAs, recalcitrant polycyclic aromatic hydrocarbons (PAHs) have been found in environments surrounding the oil sands developments in Alberta, Canada (Kelly et al., 2009; Timoney and Lee, 2009). Detailed information on anaerobic degradation of high-molecular-weight PAHs (HMW-PAHs) is scarce, and there is debate on whether PAHs having three or more rings can support growth under anoxic conditions or whether they are only partially oxidized through co-metabolism with growth substrates such as lower-molecular-weight hydrocarbons (Meckenstock et al., 2004; Ambrosoli et al., 2005).

Addition of sulfate to tailings inhibits methanogenesis by stimulating bacterial competition (Holowenko et al., 2000). Therefore, if NAs or other hydrocarbons in OSPW, such as HMW-PAHs, can be degraded under sulfate-reducing conditions, it will not only contribute to the bioremediation of these toxic, recalcitrant pollutants, but it may also reduce the amount of methane produced by OSPW stored in tailings ponds. The aim of the present study was to investigate the anaerobic biodegradation of HMW-PAH, pyrene (Pyr), adamantane-1-carboxylic acid (A1CA, a NA), and a "natural" NA mixture, (i.e., acid-extractable NAs from OSPW) compared to the more readily degradable low-molecular-weight PAH (LMW-PAH) 2-methylnaphthalene (2-MN), under sulfate-reducing and methanogenic conditions. The rate of biodegradation was measured in relation to changes in bacterial community composition.

Materials and methods

Environmental sample

The tailings pond water sample used in this study, was supplied by L. Gieg (of the University of Calgary, Alberta, Canada). The sample was collected in the summer of 2010 at a depth of 20 m from a Suncor tailings pond, in Alberta and designated 20 m. The sample was maintained at 4 °C prior to use.

PAH and NA compounds

Pyrene (Pyr), 2-methylnaphthalene (2-MN), and adamantane-1-carboxylic acid (A1CA) were obtained from Sigma–Aldrich, Gillingham, UK, at >98% purity. The "natural" NA mixture was the acid-extractable fraction from a Suncor OSPW sample and was supplied by L. Gieg (University of Calgary). The OSPW samples were acidified to pH 2 and NAs were extracted using dichloromethane according to the method of Holowenko et al. (2002).

Biodegradation experiment

The basal medium for all anaerobic cultures contained, per liter of anaerobic water: K_2HPO_4, 0.652 g; $NaH_2PO_4.H_2O$, 0.173 g; NH_4HCO_3, 0.443 g; $NaHCO_3$, 3.73 g; and 1 ml Resazurin solution (0.5 g l^{-1}). The basal medium for sulfate reducers was made as described above with the addition of $NaSO_4$ at 4 g l^{-1}. Basal medium (92.5 ml) was dispensed into serum bottles that were closed with butyl rubber stoppers and crimp-sealed. The gas phase was replaced with H_2/CO_2 (80%/20%) (in order to initiate anaerobic growth) to a pressure of 1.5 bar, and autoclaved. Thereafter, 2.5 ml each of three filter-sterilized solutions (A, B, and C), were added by syringe to each bottle. Solution A contained: 1 ml each of four vitamin solutions, 1 ml of trace elements solution, and 1 ml of amino acid solution added to 20 ml of sterile anaerobic water (see Supplementary information for details). Solution B contained 2.402 g Na_2S per liter of anaerobic water. Solution C contained 4.410 g $CaCl_2.2H_2O$ and 4.066 g $MgCl_2.6H_2O$ per liter of anaerobic distilled water. Individual cultures were amended with either Pyr, A1CA, "natural" NAs, or 2-MN (5 mg l^{-1} final concentration) as the sole carbon and energy source as described in Johnson et al. (2011). Bottles were inoculated with 2% (v/v) of the tailing pond water sample (denoted 2 m). Abiotic controls containing either Pyr, A1CA, "natural" NAs, or 2-MN (5 mg l^{-1} final concentration) and anaerobic medium were also prepared. Procedural blanks containing the inoculum (denoted 2 m) and anaerobic media only were also prepared. All bottles were incubated statically in the dark at 20 °C for 260 days. Sampling of triplicate bottles was carried out at 0, 130, and 260 days. After 130 days the gas phase was changed to N_2/CO_2 (80%/20%). At 0, 130, and 260 days a 30-ml sub-sample was removed, centrifuged at 3435 × *g* for 10 min, and acidified to pH 2 with HCl for ethyl acetate extraction according to the method of Johnson et al. (2011).

Solvent extraction, GC–MS, and analysis

To remove hydrocarbon contamination all glassware was soaked overnight in Decon^{90} (Decon), rinsed three times with distilled water, baked until dry, and rinsed three times with acetone (Fisher) (Johnson et al., 2011). The internal standard 4-phenylbutanoic acid (Acros Organics; final concentration 2 mg l^{-1}) was used for all NA samples and 2-MN, while the internal standard 2-MN (Acros Organics; final concentration 2 mg l^{-1}) was used for all pyrene-amended samples. Each NA from the supernatants was extracted by acidifying to pH 2 (using 2 drops of concentrated HCl) and extracted three times with 15 ml of HPLC-grade ethyl acetate (Fisher Scientific) using a separating funnel. Each HMW-PAH was extracted as described above except samples were extracted with HPLC-grade acetone (Fisher). Solvent extracts were pooled, then dried with 5–10 g anhydrous Na_2SO_4 (Fisher Scientific), and the organic acids were concentrated by rotary evaporation (Buchi) at 40 °C. Samples were transferred to a gas chromatography vial (Chromacol) and stored at −20 °C. Prior to analysis, all samples were reduced to dryness under a gentle stream of nitrogen at 40 °C and reacted with N,O-bis(trimethylsilyl)trifluoroacetamide (Supelco, Bellefonte, PA, USA) at 60 °C for 20 min to form trimethylsilyl derivatives. Derivatized samples were resuspended in 1 ml dichloromethane (HPLC, Acros Organics). Samples were separated by gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890 GC interfaced with an Agilent 5975C MS. Samples were injected with a 1 μl splitless injection (injector temperature 250 °C) onto a 30 m × 250 μm × 0.25 μm Rtx − 1MS column using helium as the carrier gas at a constant flow of 1 ml min^{-1}. Oven temperatures were programmed with an initial increase from 40 °C to 250 °C at 10 °C min^{-1} and a final hold at 250 °C for 10 min. For PAHs, oven temperatures were programmed with an initial increase from 40 °C to 300 °C at 10 °C min^{-1} and a final hold at 300 °C for 10 min. The transfer line was held at 230 °C onto a source for the MS that was in full scan mode (scan range 50–550 Da). Data were analyzed and integrated with Agilent GC Chemstation. Agilent GC Chemstation was used to tentatively identify metabolites through mass spectral analysis.

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