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Sequential biodegradation of complex naphtha hydrocarbons under methanogenic conditions in two different oil sands tailings☆

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ABSTRACT

Methane emissions in oil sands tailings ponds are sustained by anaerobic biodegradation of unrecovered hydrocarbons. Naphtha (primarily C_6–C_{10}; *n*- *iso*- and cycloalkanes) is commonly used as a solvent during bitumen extraction process and its residue escapes to tailings ponds during tailings deposition. To investigate biodegradability of hydrocarbons in naphtha, mature fine tailings (MFT) collected from Albian and CNRL tailings ponds were amended with CNRL naphtha at ~0.2 wt% (~2000 mg L^{-1}) and incubated under methanogenic conditions for ~1600 d. Microbial communities in both MFTs started metabolizing naphtha after a lag phase of ~100 d. Complete biodegradation/biotransformation of all *n*-alkanes (except partial biodegradation of *n*-octane in CNRL MFT) followed by major *iso*-alkanes (2-methylpentane, 3-methylhexane, 2- and 4-methylheptane, *iso*-nonanes and 2-methylnonane) and a few cycloalkanes (derivatives of cyclopentane and cyclohexane) was observed during the incubation. 16S rRNA gene pyrosequencing showed dominance of *Peptococcaceae* and *Anaerolineaceae* in Albian MFT and *Anaerolineaceae* and *Syntrophaceae* in CNRL MFT bacterial communities with co-domination of *Methanosaetaceae* and "*Candidatus* Methanoregula" in archaeal populations during active biodegradation of hydrocarbons. The findings extend the known range of hydrocarbons susceptible to methanogenic biodegradation in petroleum-impacted anaerobic environments and help refine existing kinetic model to predict greenhouse gas emissions from tailings ponds.

1. Introduction

Recent expansion in oil sands surface mining in northern Alberta, Canada, has resulted in increased volumes of fluid tailings that are generated during bitumen extraction from oil sands ores. Presently, settling basins (tailings ponds) contain ~1 billion m^3 of tailings that cover a total area of ~185 km^2 (http://osip.alberta.ca/map/). Besides important environmental issues such as large inventory, slow consolidation, and presence of organic (residual hydrocarbons, naphthenic acids) and inorganic (heavy metals) contaminants, deposited tailings emit significant quantities of methane (CH_4) and carbon dioxide (CO_2) to the atmosphere. Mildred Lake Settling Basin (MLSB), the largest and one of the oldest oil sands tailings ponds, has been estimated to emit ~43 million L CH_4 day^{-1} (Holowenko et al., 2000; Siddique et al., 2008). Studying the source of greenhouse gas emissions and mechanisms of natural attenuation of contaminants are important from global climate change and contaminant remediation perspectives.

Oil sands tailings deposited in tailings ponds contain residual concentrations of fugitive solvent hydrocarbons, which is the major substrate sustaining methanogenesis in tailings ponds (Siddique et al., 2007). Syncrude Canada Ltd. (Syncrude) along with other industrial operators such as Suncor and Canadian Natural Resources Ltd. (CNRL) use naphtha as a solvent for bitumen extraction, which comprises aliphatic and aromatic hydrocarbons (*n*-, *iso*- and cycloalkanes and alkylbenzenes) primarily in the range of C_6-C_{10}. Initial study conducted for a year using MFT retrieved from Syncrude's MLSB revealed that only labile fraction of naphtha (*n*-alkanes and alkylbenzenes) served as carbon source for methanogenesis because the other components such as *iso*- and cycloalkanes remained undegraded during a year-long incubation (Siddique et al., 2007). However, subsequent studies reported

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biodegradation (metabolically or cometabolically) of certain *iso*-alkanes in primary cultures and enrichment cultures derived from MLSB MFT and amended with *iso*-alkanes exclusively (Abu Laban et al., 2015; Siddique et al., 2015; Tan et al., 2015). Though these findings demonstrated that indigenous microbial communities in MFT were able to adapt to degrade certain groups of hydrocarbons in MLSB (Abu Laban et al., 2015; Siddique et al., 2012, 2011; Tan et al., 2015), no study has been conducted for extended period of time to comprehend biodegradability of different hydrocarbons (from labile to recalcitrant) when tailings ponds receive complex fugitive solvent like naphtha.

Most studies have been focused on Syncrude MLSB with little attention to other tailings ponds. Tailings from different operators are different in many respects such as age of deposited tailings, physicochemical properties and tailings management strategies. Compared to MLSB (~40 years old), Shell Albian Sands Inc. (Albian)'s Muskeg River Mine pond (~14 years old) and CNRL's Horizon pond (~7 years old) are much younger and receive tailings containing residual hydrocarbons from paraffinic solvent and naphtha, respectively. Albian amends tailings with trisodium citrate and organic polymer flocculants for consolidation before deposition (Li, 2010), whereas CNRL uses CO_2 to enhance consolidation of tailings in the pond (http://www.cnrl.com/). Consequently, the composition and catabolic abilities of the indigenous microbial communities in Albian and CNRL tailings ponds might be different. Our recent studies revealed different patterns of preferential biodegradation of *n*-alkanes in Albian and CNRL MFT (Mohamad Shahimin et al., 2016), and metabolism/cometabolism of certain *iso*-alkanes in Albian MFT (Siddique et al., 2015) when these MFT were amended with a mixture of *n*- or *iso*-alkanes. These observations raise question on the biodegradability of individual constituents when present in a complex solvent, because almost all previous studies tested biodegradation of individual hydrocarbon groups in tailings.

Therefore, in this study, we examined biodegradation of complex naphtha in MFTs retrieved from Albian and CNRL tailings ponds. The purpose of including Albian MFT for naphtha biodegradation was to examine the adaptability and flexibility of indigenous Albian microbial communities to biodegrade hydrocarbons that are different from the cognate hydrocarbons present in Albian tailings. This is the first study that provides insight into hydrocarbon biodegradation pattern under more realistic scenario and contributes to the overall understanding about the onset and duration of methanogenesis. The results broaden the range of hydrocarbons that are susceptible to methanogenic biodegradation that will help improve our existing kinetic model (Siddique et al., 2008) to predict greenhouse gas emissions from tailings ponds. The model estimates cumulative CH_4 production from the microbial degradation/metabolism of individual biodegradable hydrocarbons under methanogenic conditions using stoichiometric parameters and biodegradation rate constants calculated from the data acquired in our previous biodegradation studies (Siddique et al., 2006, 2007).

2. Materials and methods

2.1. Chemicals and materials

Samples of methanogenic MFT collected from tailings ponds were provided by Albian and CNRL. MFT is a thick water slurry of silt and clays (usually $\geq$ 25% solids), unextracted bitumen (~5 vol %) and unrecovered solvent ($\leq$1 vol%). Albian MFT was collected from the Muskeg River Mine Tailings Pond at 7 m below the water surface in 2008 (0465371E 6342304N) and stored in the dark at 4 °C until its use in 2011. Our numerous previous experiments showed that storage of MFT for several years under a cap of tailings pond water to maintain anaerobic conditions in the tailings sediments did not impair its ability to biodegrade hydrocarbons at a rate similar to that of fresh MFT samples. The CNRL MFT was collected from the Horizon tailings pond (446156E 6356933.1N) in 2011 and stored in the dark at 4 °C until used as inoculum in the same year. The physical and chemical characteristics of both MFTs have been described in our recent report (Mohamad Shahimin et al., 2016). The naphtha tested in this study was provided by CNRL.

2.2. Establishment of MFT cultures for naphtha biodegradation

The anaerobic microcosms were prepared using 50 mL methanogenic medium (Fedorak and Hrudey, 1984) mixed with 50 mL of either Albian or CNRL MFT in 158-mL serum bottles with a headspace of 30% CO_2 balance N_2 as previously described (Siddique et al., 2006). The microcosms were pre-incubated statically at room temperature in the dark for 2 weeks for microbial acclimation and consumption of residual hydrocarbons and any alternative electron acceptors in MFT (Siddique et al., 2006). Prior to amending the microcosms with naphtha, the headspace of all microcosms was flushed with 30% CO_2 balance N_2 to remove any CH_4 produced during pre-incubation. Both Albian and CNRL microcosms (in triplicate) were amended with ~0.2 wt% (final concentration: ~2000 mg L^{-1}) CNRL naphtha to achieve average naphtha concentration in tailings ponds. Duplicate autoclaved microcosm (abiotic controls) were prepared in parallel by autoclaving (121 °C, 20 psi, 60 min) for four consecutive days prior to hydrocarbon amendment to account for abiotic degradation. Duplicate unamended microcosms (viable baseline controls) were also prepared to account for CH_4 production from any residual endogenous substrates in the MFTs. Immediately after the amendment, samples were collected from all the microcosms to determine initial (day 0) status of MFTs for hydrocarbons and microbial community structures. The microcosms were incubated statically in the dark at room temperature and headspace analysis was performed periodically to monitor CH_4 production in the microcosms. MFT culture samples were also taken periodically from the microcosms to determine hydrocarbon concentrations and characterize microbial communities (see below).

2.3. Production of methane and depletion of naphtha (hydrocarbons)

CH_4 in the headspace of each microcosm was analyzed periodically as an indication of biodegradation of the added naphtha. Fifty microliter headspace gas was injected directly into a gas chromatograph equipped with flame ionization detector (GC-FID) as previously described (Holowenko et al., 2000).

For monitoring biodegradation during ~1600 d of incubation, two analytical approaches were employed to determine hydrocarbon concentrations in MFTs. Whole naphtha concentrations (F1 fraction; C_5-C_{10}) and identifiable labile hydrocarbons were quantified using GC-FID equipped with purge and trap system (P&T). Analyses were performed at 0, ~600 and ~1300 d (Table 1 and Fig. 2). Because naphtha is a complex mixture of hydrocarbons, PONAU (paraffins, olefins, naphthenes, aromatics, and unknown components) analysis was performed to resolve and identify all individual constituents of naphtha and estimate F1 fraction of naphtha at ~1600 d (Fig. 2 and Table 2 and S1). For determination of F1 fraction, each 1-mL culture sample retrieved from a hand-shaken microcosm was extracted with 10 mL methanol (Fisher Scientific) and analyzed by GC-FID P&T using the protocol previously described (Mohamad Shahimin et al., 2016; Siddique et al., 2007, 2006). Because most hydrocarbons in naphtha were within