Chemosphere 209 (2018) 551–559

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Indigenous microorganisms residing in oil sands tailings biodegrade residual bitumen

Xiaoxuan Yu [a], Korris Lee [a], Bin Ma [a], Evelyn Asiedu [b], Ania C. Ulrich [a, *]

[a] Department of Civil and Environmental Engineering, University of Alberta, Edmonton, Alberta, T6G 2W2, Canada
[b] Department of Laboratory Medicine & Pathology, University of Alberta, Edmonton, Alberta, T6G 2B7, Canada

HIGHLIGHTS

- Indigenous microorganisms removed PHCs (>58%) from bitumen.
- Bitumen addition increased tailings toxicity by 25 times.
- Acetate stimulated microbial growth and bitumen degradation.
- *Pseudomonas*, *Acidovorax*, and *Rhodoferax* were potential bitumen degraders.

ARTICLE INFO

Article history:
Received 22 March 2018
Received in revised form
22 May 2018
Accepted 18 June 2018
Available online 19 June 2018

Handling Editor: T Cutright

Keywords:
Oil sands end pit lakes
Bitumen biodegradation
Biostimulation
Petroleum hydrocarbon

ABSTRACT

The purpose of this study was to determine the capacity of indigenous microbes in tailings to degrade bitumen aerobically, and if acetate biostimulation further improved degradation. Fluid fine tailings, from Base Mine Lake (BML), were used as microbial inocula, and bitumen in the tailings served as a potential carbon source during the experiment. The tailings were capped with 0.22 μm-filtered BML surface water with or without BML bitumen and acetate addition and incubated for 100 days at 20 °C. CO_2 production and petroleum hydrocarbon reductions (50–70% for the biostimulation treatment) in the tailings were observed. DNA was extracted directly from the tailings, and increased bacterial density was observed by qPCR targeting the *rpoB* gene in the biostimulated group. 16 S rRNA sequencing was used to determine microbial composition profiles in each treatment group. The microbial communities indigenous to the tailings shifted after the bitumen was added. *Acidovorax*, *Rhodoferax*, *Pseudomonas* and *Pseudoxanthomonas* spp. significantly increased compared to the original microbial community and demonstrated tolerance to bitumen-based toxicity. The first three genera showed more potential for biostimulation treatment with acetate and may be important bitumen/hydrocarbon-degraders in an oil sands end pit lake environment.

1. Introduction

Due to the rapid development of the oil sands industry in northeastern Alberta, Canada, large quantities of tailings waste and oil sands process-affected water (OSPW) have been produced over the past five decades (Chalaturnyk et al., 2002; Percy et al., 2012). Tailings waste is often temporarily stored in tailings impoundments called tailings ponds. However, the ever-increasing geographical footprints and the urgent demand to reclaim the disturbed landscape has prompted the construction of oil sands end pit lakes (EPLs) (Hrynyshyn, 2012; Aubertin and McKenna, 2016). In EPLs, sand and clay tailings are placed in the bottom of mined-out pits and a water cap (made up of OSPW and fresh water from natural lakes or other healthy water bodies) is placed on top of the tailings (Hrynyshyn, 2012). The water cap is expected to develop into a thriving aquatic ecosystem capable of biodegrading chemicals of potential concern (Hrynyshyn, 2012). Base Mine Lake (BML) is the first commercial-scale demonstration EPL, which was commissioned by Syncrude Canada, Ltd. to support the development of this water capping technique for oil sands reclamation.

Efforts have been made to bioremediate organic compounds in

* Corresponding author. Department of Civil and Environmental Engineering, University of Alberta, 7-265 Donadeo Innovation Centre for Engineering, 9211 - 116 St NW, Edmonton, Alberta, T6G 1H9, Canada.
E-mail addresses: xyu2@ualberta.ca (X. Yu), korris@ualberta.ca (K. Lee), bma@zju.edu.cn (B. Ma), easiedu@ualberta.ca (E. Asiedu), aulrich@ualberta.ca (A.C. Ulrich).

https://doi.org/10.1016/j.chemosphere.2018.06.126
0045-6535/

OSPW and oil sands tailings, such as BTEX (benzene, toluene, ethylbenzene and xylene) and naphthenic acids (NAs). The biodegradation of BTEX, n-alkanes (C14–C18) and naphtha (C3–C14) has been confirmed under methanogenic conditions by oil sands tailings microorganisms (Siddique et al., 2011, 2007; Mohamad Shahimin and Siddique, 2017). NAs were known to be the major toxicity contributor in OSPW (Morandi et al., 2015), and its biodegradation tends to be more difficult, while the utilization of chemical pre-treatment, such as advanced oxidation, has shown some success (Brown et al., 2013; Brown and Ulrich, 2015; Zhang, 2016; Zhang et al., 2018). Gamma irradiation treatment was also reported to stimulate the hydrocarbon degraders from the tailings microbial community (VanMensel et al., 2017).

A less-studied aspect of EPLs is the bitumen in the tailings, left behind by the successive incomplete extraction processes. Bitumen remaining in the tailings after placement into the pit is carried upwards by biogenic gases (one of the proposed processes), and the viscous liquid spreads over the water cap (Darling, 2011). This bitumen acts as a hydrocarbon source: as it migrates through the water cap, the hydrocarbons are released and subsequently biodegraded, a process which consumes dissolved oxygen and prevents the establishment of a healthy lake ecosystem. If the bitumen cannot be further degraded or mineralized in situ, the hydrocarbons can contaminate the aqueous environment and the nearby littoral zone.

Hydrocarbon-degrading microbial isolates from sediments of the Athabasca River have been shown to grow on the lighter components of Athabasca bitumen (not on the recalcitrant asphaltene fraction) (Wyndham and Costerton, 1981). Microbial degradation of bitumen (up to 40% removal at 37 °C) was also reported in similarly polluted environments in other regions of the world (Wyndham and Costerton, 1981; Potter and Duval, 2001; Das and Chandran, 2011). Bitumen biodegradation can be enhanced by nutrient addition (nitrogen or phosphorus) and stimulated by addition of more easily biodegradable carbons (Das and Chandran, 2011). The most effective hydrocarbon degradation is usually accomplished under aerobic conditions, while nutrients and temperature are often the most important limiting factors of the process (Das and Chandran, 2011). Because bitumen is complex, and its biodegradation has been demonstrated to occur under various conditions, site-specific factors are important to the feasibility of in situ remediation.

Our previous research showed (Yu et al., 2018) that the addition of a proprietary blend of microbes, enzymes and organics to tailings resulted in significant reduction in the petroleum hydrocarbon fractions and tailings pore water toxicity. It was unclear whether these changes were caused by the indigenous microbial community or by the added microbes and organic carrier in the Cypher product. Therefore, the focus of this study was to investigate the ability of the microbial communities in BML to degrade bitumen, and the effectiveness of biostimulation with acetate. The changes in community composition and community responses were assessed by comparing 16 S rRNA gene sequence profiles.

2. Materials and methods

2.1. Materials

All samples were transported to the laboratory in sealed buckets and stored at 4 °C prior to use (bitumen samples were stored for 6 months; water and tailings samples were stored for less than one month). Fluid fine tailings (FFT) were provided by Syncrude Canada Ltd. FFT was sampled at the depth of 12 m below the sediment:-water interface at Platform 1 at BML. Extraction technique limitations cause unrecovered bitumen to end up in tailings; this residual bitumen can be observed upon commission of the BML (in this paper, 'bitumen' refers to the residual bitumen in the BML). BML bitumen used in this research was sampled directly from the BML surface. To eliminate moisture content, the bitumen was oven-dried at 105 °C overnight. However, this drying process sacrificed any volatile and semi-volatile hydrocarbon that may have resided within the bitumen. Clays, sands and other small particles were retained, but vegetation and large stones were manually removed. BML cap water used in this research was sampled from the surface of BML at Platform 1.

2.2. Chemical analysis

CO_2 was measured by a gas chromatography thermal conductivity detector. Dissolved organic carbon (DOC) was measured with a Shimadzu Model TOC-L_{CPH}. Acetate was measured by Ion Chromatography. NAs were measured by gas chromatography flame ionization detector (GC-FID) or by reversed-phase chromatography paired with a linear ion trap-Orbitrap mass spectrometer. Detailed procedures and machine conditions for all methods above can be found in the supplementary data: CO_2 measurement (Protocol S1), DOC measurement (Protocol S2), acetate measurement (Protocol S3), and NAs measurements (Protocol S4).

Petroleum hydrocarbons (PHC) are grouped into these fractions by using a Canada-Wide Standard: F1 (C6 – C10), F2 (C10 – C16), F3 (C16 – C34), F4 (C34 – C50), and F4G-SG (>C50) (CWS, 2003). F4 and F4G-SG fractions are classified into bitumen content in the Dean Stark extraction (industry accepted method) (Dean and Stark, 1920). F1 fractions were measured prior to submission to Maxxam Analytics and were non-detectable in all samples. All samples were mixed with organic solvent (toluene) and sonicated for greater homogeneity prior to submission to Maxxam. Maxxam then further homogenized the samples. One duplicate was submitted for analysis due to the sample size limitations.

2.3. Microbial analysis

2.3.1. Toxicity bioassay

The toxicity of aqueous samples was analyzed using the Microtox® bioassay. The 81.9% Basic Test protocol was followed (Microtox® 500 Analyzer, Azur Environmental) with an incubation time of 5 min (Anderson et al., 2011). Light emission was measured with MicrotoxOmni software to determine inhibitory concentration 20% (IC_{20}) or inhibitory concentration 50% (IC_{50}) values. Toxicity units, derived from IC_{50} ($TU = 100 \div IC_{50}$), was used to visualize high-level toxicity trends.

2.3.2. DNA extraction

DNA was isolated from the tailings phase using the FastDNA™ SPIN Kit for Soil (MP Biomedicals). Up to 500 mg of tailings were used per extraction, following the DNA isolation protocol suggested by the manufacturer.

2.3.3. Bacterial population by qPCR assay

The Bacterial population was determined by the qPCR amplification of the RNA polymerase beta subunit (*rpoB*) gene, utilizing *rpoB* 1698f (5′-AACATCGGTTTGCTCAAC-3′) and *rpoB* 2041r (5′-CGTTGCATGTTGGTACCCAT-3′) primers (Nava et al., 2011; Brown et al., 2013). The qPCR assay was performed using a Bio-Rad CFX96 optical reaction module conversion of the C1000 Touch thermal cycler. All samples and standards were completed in triplicate, and the amplification data were analyzed using Bio-Rad CFX Manager™ 3.0 software. The reaction followed the protocol suggested by the manufacturer (detailed description can be found