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Microbial degradation of Cold Lake Blend and Western Canadian select dilbits by freshwater enrichments

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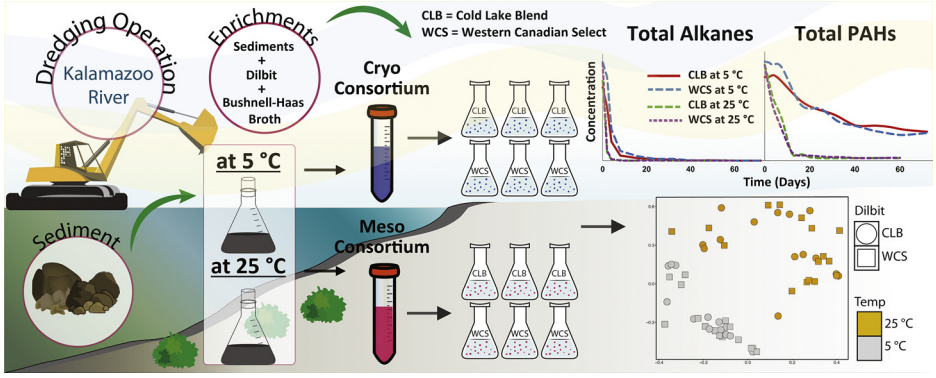
GRAPHICAL ABSTRACT



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ABSTRACT

Treatability experiments were conducted to determine the biodegradation of diluted bitumen (dilbit) at 5 and 25 °C for 72 and 60 days, respectively. Microbial consortia obtained from the Kalamazoo River Enbridge Energy spill site were enriched on dilbit at both 5 (cryo) and 25 (meso) °C. On every sampling day, triplicates were sacrificed and residual hydrocarbon concentrations (alkanes and polycyclic aromatic hydrocarbons) were determined by GC–MS/MS. The composition and relative abundance of different bacterial groups were identified by 16S rRNA gene sequencing analysis. While some physicochemical differences were observed between the two dilbits, their biodegradation profiles were similar. The rates and extent of degradation were greater at 25 °C. Both consortia metabolized 99.9% of alkanes; however, the meso consortium was more effective at removing aromatics than the cryo consortium (97.5 vs 70%). Known hydrocarbon-degrading bacteria were present in both consortia (*Pseudomonas*, *Rhodococcus*, *Hydrogenophaga*, *Parvibaculum*, *Arthrobacter*, *Acidovorax*), although their relative abundances depended on the temperatures at which they were enriched. Regardless of the dilbit type, the microbial community structure significantly changed as a response to the diminishing hydrocarbon load. Our results demonstrate that dilbit can be effectively degraded by autochthonous microbial consortia from sites with recent exposure to dilbit contamination.

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1. Introduction

Among the known oil sand deposits distributed worldwide, reservoirs found in Alberta (Canada) are the largest and associated with the highest production. Those deposits are concentrated in the northeastern part and are found primarily in Athabasca, Cold Lake, and Peace River regions [1,2]. Oil sands are a combination of sand, water, and bitumen, the most viscous, heavily biodegraded form of petroleum crude. For transportation purposes, bitumen is blended with either synthetic crude oil (synbit), or diluents such as natural gas condensate, naphtha, or a mixture of light hydrocarbons (dilbit) [3]. Although dilbit may resemble conventional heavy crude oil, there are significant differences in terms of physical and chemical properties. Dilbit has a greater proportion of resins, asphaltenes, sulfur, and metals in addition to having a higher acid number [4,5]. The composition of bitumen varies across as well as within reservoirs [6]. The variety of diluents used for blending results in inherently heterogeneous dilbit products.

The commercial levels of oil sand and derived products are escalating due to technological developments in oil recovery and demand for unconventional hydrocarbon sources [7]. According to the Canadian Association of Petroleum Producers [8], production will rise from 2.4 million barrels/day in 2015 to about 3.7 million barrels/day in 2030. Such an increase in production implies a much higher transport demand and as a result a higher risk for the occurrence of accidental spills. In recent years, three major dilbit spills were reported: Kinder Morgan spill in Burnaby, Canada (2007), Kalamazoo River Enbridge spill in Michigan, USA (2010), and ExxonMobil's spill in Mayflower, Arkansas, USA (2013). Along with the preventive measures, preparedness to manage such incidents is equally important. Depending on the degradation rates of the hydrocarbon, bioremediation may be an important remediation practice. For the latter to be a viable in situ remediation option in dilbit spills, knowledge on the fate of dilbit under different environmental conditions is necessary. When compared to conventional crude oil, dilbit shows several distinct properties and while these differences may impact microbial activity, relatively limited information exists regarding the ease and extent of its biodegradability. More importantly, some of the studies have reached different conclusions as far as the levels and rate of dilbit degradation. For instance, Cobanli et al. [9] and King et al. [10] reported biodegradation of dilbit, which is in contrast with others that suggested limited degradation [3,11–13]. Although differences in bacterial community structure could in part explain differences in the biodegradation patterns observed thus far, none of these studies have identified the bacterial composition of their microbial enrichments.

To address these issues, the main objectives of this work were to gain insight into the biodegradability of diluted bitumen at different temperatures and to identity bacterial groups potentially linked to dilbit degradation. The two types of dilbit implicated in 2010 Kalamazoo (Michigan, USA) spill were chosen for this study (i.e., Cold Lake Blend, CLB and Western Canadian Select, WCS). These products are commonly transported within the United States and gaining insight into their behavior when exposed to microbial activity would be useful to inform future spill planning [14]. The experiments were carried out in a synthetic freshwater medium with enriched microbial consortia obtained from the dredging operations after the Kalamazoo River Enbridge spill. Since temperature can play a crucial role in biodegradation, two sets of experiments were run at 5 and 25 °C to study degradation in cryogenic and mesophilic conditions. Microbial composition during the course of the experiment was determined for each microcosm using 16S rRNA gene sequencing analyses.

2. Materials and methods

2.1. Chemicals and reagents

The U.S. Environmental Protection Agency provided non-weathered CLB and WCS dilbits. These were stored in closed amber glass container with Teflon lid in a well-ventilated area according to MSDS requirements [15]. Mineral salts, dichloromethane (DCM), and hexane were obtained from Fisher Scientific (Pittsburg, PA, USA).

2.2. Media

Bushnell-Haas broth was used as medium for microbial support in the enrichments and treatability tests [16]. This medium is commonly used to enrich hydrocarbon degrading bacteria and has been used in many crude oil degradation studies [17,18]. It contains excess of nitrogen and phosphate, sufficient to support microbial growth when using hydrocarbons as sole carbon source. It should be noted that nitrogen and phosphate are among the environmental limiting factors in hydrocarbon biodegradation in situ [19]. The broth was prepared by dissolving magnesium sulfate (0.2 g/L), calcium chloride (0.02 g/L), monopotassium phosphate (1.0 g/L), dipotassium phosphate (1.0 g/L), ammonium nitrate (1.0 g/L), and ferric chloride (0.05 g/L) in distilled water. This broth was autoclaved at 120 °C for 15 min in batches of 1 L.

2.3. Microbial enrichment

The original mixed consortium was obtained from dredged sediment following the Kalamazoo River Enbridge Energy spill (2010). Two separate enrichments were grown on Cold Lake Blend dilbit as the carbon source in Bushnell-Haas broth at 5 (cryo) and 25 °C (meso). These temperatures are representative of the temperatures of winter and summer of the contaminated site from which the sediments samples were originally collected. Then enrichments were washed, concentrated tenfold, and stored frozen on 10% glycerol at −80 °C until ready to use in the degradation experiments.

2.4. Experimental setup and procedure

The degradation of the two different dilbits (WCS or CLB) was tested at 5 (cryo) and 25 °C (meso). The experimental design layout for this treatability study is summarized in Table A1. Each dilbit was individually tested and samples were collected in triplicates for the chemical and bacterial analyses. For the 25 °C setup, 12 sampling events were conducted at 0, 2, 4, 8, 12, 16, 20, 28, 35, 42, 54, and 60 d, while sampling events at 5 °C were on days 0, 2, 4, 8, 16, 24, 32, 40, 48, 56, 62, and, 72. To account for abiotic losses, 0.5 mL of sodium azide (500 mg/L stock solution) was added to a separate set of flasks or killed controls (KC), which were run in triplicate for each treatment and sampled at the final sampling day. Aliquots (75 µL) of either WCS or CLB were dispensed into all different shake flasks (including KC flasks) containing 100 mL of sterile broth, and then inoculated with 0.5 mL of the meso or the cryo enrichment for the experimental setup at 25 and 5 °C, respectively. Flasks were mixed (200 rpm) with rotary shakers in temperature-controlled rooms. At a given sampling day, three flasks were sacrificed per dilbit and temperature treatments. Bacterial activity was stopped by adding 0.5 mL of the sterilant to the sample flasks prior to analysis. To separate media and remaining dilbit, liquid-liquid extractions were carried out with DCM. Each replicate was extracted with 60 mL of DCM. Oil extracts were then filtered through anhydrous sodium sulfate to remove any water and concentrated down to 1 mL under nitrogen gas. Solvent exchange was performed by adding 9 mL of hexane to precipitate asphaltenes. These extracts were then analyzed to measure residual alkanes and polycyclic aromatic hydrocarbons (PAHs).

2.5. Hydrocarbon analysis

2.5.1. Laboratory methodology

Residual alkane and PAH concentrations were quantified with an Agilent 7890 A gas chromatograph and an Agilent 7000 mass selective