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Anoxic biodegradation of a surrogate naphthenic acid coupled to reduction of nitrite

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ABSTRACT

Large volumes of waters contaminated with naphthenic acids (NAs) are produced during the processing of oil sands. Anoxic biodegradation of *trans*-4-methyl-1-cyclohexane carboxylic acid (*trans*-4MCHCA) coupled to nitrate reduction was reported earlier. Given the generation of nitrite as an intermediate of nitrate reduction, its subsequent use as secondary electron acceptor and its potential inhibitory effect, biodegradation of *trans*-4MCHCA coupled to nitrite reduction was investigated in the current work.

Batch experiments revealed the optimum temperature as $24 \pm 2\,^{\circ}C$ and that the nitrite inhibitory effect was severe at 1150 mg nitrite L^{-1}. Maximum biodegradation rates in the stirred tank and biofilm reactors were 14.4 and 82.2 mg L^{-1} h^{-1}, and the corresponding nitrite reduction rates were 44.2 and 361.6 mg L^{-1} h^{-1}, respectively. Using the experimental data, maximum specific growth rate (μ_m), saturation constant (K_s), and yield (Y) were determined as 0.4 h^{-1}, 20.9 mg *trans*-4MCHCA L^{-1}, and 0.3 mg biomass (mg *trans*-4MCHCA)$^{-1}$, respectively. Comparison of anoxic biodegradation results with nitrate and nitrite demonstrated the superiority of nitrate as an electron acceptor for anoxic biodegradation of NAs.

1. Introduction

Oil sands of Northern Alberta and Saskatchewan, located in the Athabasca, Cold Lake, and Peace River regions, represent the largest Canadian oil reserves. Extraction of bitumen from oil sands for production of heavy oil is achieved by surface mining followed by the Clark hot water process for shallow reserves (*ex situ* process), or through *in situ* processes such as cyclic steam stimulation (CSS) and steam-assisted gravity drainage (SAGD) in case of deep reserves. [1,2] Bitumen recovery is a water intensive process and generates tailings that are comprised of water, sand, clay, and unrecovered hydrocarbons. Around 80–90% of water is recovered and recycled back to the extraction process using sedimentation basins referred to as tailing ponds. The remaining portion, known as oil sand process water (OSPW), is retrained in the tailing ponds due to their toxicity and a zero discharge policy implemented by the Government of Alberta. Naphthenic acids (NAs) that are transferred from the oil sand to the aqueous phase during the Clark hot water process are the main toxic constituents of OSPW and are known to impose severe adverse effects on a variety of mammals and aquatic life. [3–5] The oil sand tailing ponds currently cover a vast area and the volume of accumulated OSPWs is projected to increase 1 billion m^3 by 2025. [6] The concentration of NAs in OSPW which is currently ranging from 40 to 130 mg L^{-1} is expected to increase due to the recycling of water [6].

Given the need for the sustainable use of water in production of heavy oil and acute environmental challenges that are associated with the oil sand tailings, numerous research works aiming to develop technologies for the treatment of OSPW have been conducted. These efforts encompass a variety of physicochemical and biological techniques such as photocatalysis, [7,8] ozonation, [9–12] adsorption, [13,14] phytoremediation and treatment in wetlands, [2,15–19] as well as aerobic and anoxic biodegradations. [1,20–25] The technical challenges in large scale application and absence of feasible economics in case of physicochemical methods has singled out biotreatment or an integrated physicochemical and biological approach as practical strategies for the treatment of OSPW. While treatment of OSPW through aerobic biodegradation of naphthenic acids has attracted much attention, anoxic biodegradation has been mostly overlooked. The presence of anaerobic microorganism such as methanogens, sulphate and nitrate reducing bacteria and their role in different physical, chemical and biological processes occurring in the tailing ponds have been well documented. [26–29] Investigating the biodegradation of high molecular weight *n*-alkanes (C_{14}, C_{16} and C_{18}) with an oil sand indigenous methanogens, Siddique et al. [30]

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reported a long lag phase in methanogenic activity and a period of ~440 days for complete biodegradation of 400 mg L^{-1} of each individual *n*-alkane. Monitoring the biodegradation of *n*-alkanes (C11–C39) with oxygen and nitrate as terminal electron acceptors, Hasinger et al. [31] reported that aerobic biodegradation followed well-established patterns (i.e. faster biodegradation of low molecular weight alkanes), while under denitrifying conditions lower molecular weight alkanes were the recalcitrant constitutes. Misiti et al. [32] evaluated the inhibitory effect and biotransformation of a commercial naphthenic acid mixture on denitrification and methanogenesis processes and reported complete inhibition of methanogenesis by 80 mg L^{-1} naphthenic acids. The denitrification was not affected even with 400 mg L^{-1} naphthenic acids. [32] Interestingly biotransformation of naphthenic acids did not occur during the denitrification processes. Noah et al. [33] studied the fate of organic matter, cell abundances and microbial community compositions during the processing of oil sands, tailings management, and in reclamation sites. Root-bearing horizons on reclamation sites exhibited cell abundances and high diversity in the make-up of microbial community. These authors identified nitrate in the pore water and roots as the most important stimulants for microbial growth. Using a microbial culture derived from an oil sand tailing pond, Folwell et al. [34] investigated the anaerobic biodegradation of two model PAHs (pyrene and 2-methylnaphthalene) and two surrogate NAs (adamantane-1-carboxylic acid and an acid-extractable NAs from OSPW) under sulphate-reducing and methanogenic conditions. Of the tested compounds only 2-methylnaphthalene was significantly degraded after 260 days and the extent of biodegradation was higher under sulphate-reducing conditions. Results demonstrated that under anaerobic conditions, NAs and high-molecular-weight PAHs were the likely molecules to persist in OSPW.

The overview of the recent literature presented here reveals that the majority of earlier works have relied on aerobic biodegradation as the underlying process for the treatment of NA-contaminated waters and information on anaerobic (anoxic) alternatives is rather limited. A biotreatment process which relies on anoxic biodegradation could in fact eliminate the technical challenges and excessive cost associated with the aeration. Moreover, it could be utilized as an *in situ* treatment approach in anoxic stabilization ponds that are amended with a proper electron acceptor such as nitrate. Results of our earlier work on anoxic biodegradation of a surrogate naphthenic acid, *trans*-4-methyl-1-cyclohexane carboxylic acid (*trans*-4MCHCA), under denitrifying conditions has confirmed the effectiveness of this approach. [1] Specifically these results demonstrated that biodegradation of *trans*-4MCHCA was coupled to reduction of nitrate to nitrite and subsequent consumption of produced nitrite as a secondary electron acceptor. Interestingly, the anoxic biodegradation rates obtained in continuous stirred tank reactor (CSTR) and biofilm reactor were similar or higher than those obtained under aerobic conditions. [1,23] Considering the production of nitrite as an intermediate of nitrate reduction, its subsequent use as a secondary electron acceptor during the anoxic biodegradation, and the potential inhibitory effect that nitrite could impose on microbial activity, it is critical to develop a thorough understanding of biodegradation of NAs in the presence of nitrite. Therefore, in the present work anoxic biodegradation of *trans*-4MCHCA coupled to reduction of nitrite was investigated. Effects of NA concentration, temperature and the inhibitory effect of nitrite were examined in batch system. Biodegradation of *trans*-4MCHCA in the presence of nitrite was further evaluated in continuously operated stirred tank and biofilm reactors and the resulting data were compared with those obtained with nitrate in similar systems. Using the experimental data the biokinetic coefficients for anoxic biodegradation of *trans*-4MCHCA coupled to denitritation were also determined.

2. Materials and methods

2.1. Surrogate naphthenic acid, microbial culture and medium

Given the extensive information on aerobic and anoxic biodegradation of *trans*-4-methyl-1-cyclohexane carboxylic acid (*trans*-4MCHCA, $C_8H_{14}O_2$, CAS No.: 13064-83-0; Alfa Aesar) from our earlier works [1,6,22–25], this compound was used as the surrogate NA in the current study. This choice made it possible to compare the kinetics of NA biodegradation in the presence of various electron acceptors (i.e. oxygen, nitrate and nitrite).

The microbial culture used in this study was a mixed culture, enriched from the soil of an industrial site contaminated with heavy hydrocarbons. [22–25] This culture that has been characterized and used for aerobic biodegradation of naphthenic acids in earlier works, [22–25] was recently acclimated to anoxic conditions and used for biodegradation of *trans*-4MCHCA in the presence of nitrate. [1] Detailed characteristic and procedures for enrichment and acclimation have been described elsewhere [1,22–25]. In brief *Pseudomonas aeruginosa* and *Alcaligenes paradoxus* were the dominant species in this culture. Modified McKinney's medium containing 100.0 mg L^{-1} *trans*-4MCHCA and 460.0 mg L^{-1} nitrite ion (added as sodium nitrite) was used for the culture maintenance. The applied *trans*-4MCHCA concentration was consistent with NA concentration range encountered in the tailing ponds (40–130 mg L^{-1}) and similar to that used in our earlier work with nitrate. [1] Modified McKinney's medium contained per liter: 840.0 mg KH_2PO_4, 750.0 mg K_2HPO_4, 474.0 mg $(NH_4)_2SO_4$, 60.0 mg NaCl, 60.0 mg $CaCl_2$, 60.0 mg $MgSO_4{\cdot}7H_2O$, 20.0 mg $Fe(NH_4)_2SO_4{\cdot}6H_2O$ and 1.0 mL of micronutrient solution. The micronutrient solution contained per liter: 600.0 mg H_3BO_3, 400.0 mg $CoCl_3$, 200.0 mg $ZnSO_4{\cdot}7H_2O$, 60.0 mg $MnCl_2$, 60.0 mg $NaMoO_4{\cdot}2H_2O$, 40.0 mg $NiCl_2$, 20.0 mg $CuCl_2$ [25–27].

Cultures were prepared in serum bottles containing 90.0 mL sterilized modified McKinney's medium with 100.0 mg L^{-1} *trans*-4MCHCA, 460.0 mg L^{-1} nitrite ion and 10.0 mL of a seven day old culture as inoculum (10% v/v). It should pointed out that a seven day old culture is in the mid to late exponential phase of growth and has a high cell density. Our repeated tests under similar experimental conditions but with different inocula has demonstrated the reproducible activity of such culture. [1] Prior to inoculation, the medium was purged with filter sterilized nitrogen gas for 5–10 min to achieve anoxic conditions. Initially a culture grown on *trans*-4MCHCA and nitrate was used as the inoculum. The resulting culture was then used in subsequent culturing. Cultures were maintained at room temperature (24 ± 2 °C). Subculturing was carried out on a bi-weekly basis.

2.2. Anoxic biodegradation and denitritation in batch system

Effect of initial *trans*-4MCHCA concentration was investigated using 100.0 and 250.0 mg L^{-1} *trans*-4MCHCA with the corresponding nitrite concentrations of 460.0 and 828.0 mg L^{-1}, respectively. The required level of nitrite was determined through a number of preliminary experiments. These experiments also revealed that combination of NA at concentrations higher than 250.0 mg L^{-1} together with required nitrite (concentrations higher than 828.0 mg L^{-1}) hampered the microbial activity severely. Thus higher concentrations of *trans*-4MCHCA were not evaluated. In all cases serum bottles containing 90.0 mL sterilized modified McKinney's medium with designated *trans*-4MCHCA and nitrite concentrations was purged with filter sterilized nitrogen gas for 5–10 min and inoculated by a seven day old culture (10% v/v). Serum bottles were maintained at room temperature (24 ± 2 °C) and sampled regularly. The optical density, residual concentrations of *trans*-4MCHCA and nitrite were determined in these samples. To