Available online at www.sciencedirect.com

ScienceDirect

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

Biodegradation of a surrogate naphthenic acid under denitrifying conditions

Yetty Gunawan, Mehdi Nemati*, Ajay Dalai

Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon S7N5A9, Canada

ARTICLE INFO

Article history:
Received 4 August 2013
Received in revised form
27 November 2013
Accepted 10 December 2013
Available online 20 December 2013

Keywords:
Oil sand process water
Naphthenic acid
Anoxic biodegradation
Denitrification
CSTR
Biofilm reactor

ABSTRACT

Extraction of bitumen from the shallow oil sands generates extremely large volumes of waters contaminated by naphthenic acid which pose severe environmental and ecological risks. Aerobic biodegradation of NA in properly designed bioreactors has been investigated in our earlier works. In the present work, anoxic biodegradation of trans-4-methyl-1-cyclohexane carboxylic acid (trans-4MCHCA) coupled to denitrification was investigated as a potential ex situ approach for the treatment of oil sand process waters in bioreactors whereby excessive aeration cost could be eliminated, or as an in situ alternative for the treatment of these waters in anoxic stabilization ponds amended with nitrate. Using batch and continuous reactors (CSTR and biofilm), effects of NA concentration (100–750 mg L^{-1}), NA loading rate (up to 2607.9 mg L^{-1} h^{-1}) and temperature (10–35 °C) on biodegradation and denitrification processes were evaluated. In the batch system biodegradation of trans-4MCHCA coupled to denitrification occurred even at the highest concentration of 750 mg L^{-1}. Consistent with the patterns reported for aerobic biodegradation, increase in initial concentration of NA led to higher biodegradation and denitrification rates and the optimum temperature was determined as 23–24 °C. In the CSTR, NA removal and nitrate reduction rates passed through a maximum due to increases in NA loading rate. NA loading rate of 157.8 mg L^{-1} h^{-1} at which maximum anoxic NA and nitrate removal rates (105.3 mg L^{-1} h^{-1} and 144.5 mg L^{-1} h^{-1}, respectively) occurred was much higher than those reported for the aerobic alternative (NA loading and removal rates: 14.2 and 9.6 mg L^{-1} h^{-1}, respectively). In the anoxic biofilm reactor removal rates of NA and nitrate were dependent on NA loading rate in a linear fashion for the entire range of applied loading rates. The highest loading and removal rates for NA were 2607.9 and 2028.1 mg L^{-1} h^{-1}, respectively which were at least twofold higher than the values reported for the aerobic biofilm reactor. The highest nitrate removal rate coincided with maximum removal rate of NA and was 3164.7 mg L^{-1} h^{-1}.

1. Introduction

Extraction of bitumen and production of heavy oil from the Canadian oil sands are critical in the global energy supplies and represent a major thrust of the Canadian economy (Allen, 2008). Extraction of bitumen from the shallow oil sands in northern Alberta through open pit mining and a modified version of the Clark hot water process amounts to daily

* *Corresponding author*. Tel.: +1 (306) 966 4769; fax: +1 (306) 966 4777.
E-mail address: mehdi.nemati@usask.ca (M. Nemati).

0043-1354/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2013.12.016

production of ~1.31 million barrels of bitumen and generation of approximately 262,000 m^3 day^{-1} of tailings which consist of water, sand, clay, and unrecovered hydrocarbons, specially naphthenic acids (Siddique et al., 2011). As part of an effort to recover a portion of the water, tailing are transferred to sedimentation basins referred to as tailing ponds. Environmental and ecological concerns arising from the toxicity of the remaining waters as a result of naphthenic acids (NA) presence has led to implementation of zero discharge policy and storage of these contaminated waters in the tailing ponds which currently cover 170 km^2 and are expected to expand further rapidly (Siddique et al., 2011; Golby et al., 2012). Environmental and ecological challenges associated with the tailing ponds include changes in the natural landscape, detrimental effects on wild-life as a result of repeated exposures, and potential for the leakage and contamination of the nearby soil, ground and surface waters. Various physicochemical and biological techniques such as ozonation (Martin et al., 2010; Pérez-Estrada et al., 2011), advanced oxidation (Zhang et al., 2006; Liang et al., 2011), photolysis (McMartin et al., 2004; Drzewicz et al., 2010; Mishra et al., 2010; Afzal et al., 2012) and bioremediation (Biryukova et al., 2007; Smith et al., 2008; Quensel et al., 2011; Mahdavi et al., 2012), and combination of these (Martin et al., 2010) have been evaluated as potential approaches for the treatment of tailing waters. Among the various techniques mentioned above biological processes have been singled out as the most practical and economical options and the physicochemical techniques have been characterized as suitable pre- or post-treatment steps which could be used in conjunction with the biological process. Earlier attempts on biotreatment of NA-contaminated waters for the most parts have been conducted in small scale culture systems and have focused mainly on biodegradability of naphthenic acids, impacts of molecular structure on the extent of biodegradation, and identification of the pathways through which biodegradation occurs (Biryukova et al., 2007; Smith et al., 2008; Han et al., 2008; Johnson et al., 2012a,b) and lack of concerted effort in investigating the engineering aspects of the biodegradation process is clear. Moreover, the majority of these works, if not all, have been carried out in aerobic systems and very little information regarding the anoxic biodegradation of naphthenic acids exists.

Aerobic biodegradation of surrogate naphthenic acids (octanoic acid, trans-4-methyl-1-cyclohexane carboxylic acid, and cis- and trans-4-methyl-1-cyclohexane acetic acids) individually and in mixtures has been investigated in our earlier works using batch system, continuous stirred tank reactor (CSTR), packed-bed biofilm reactors, and circulating packed-bed reactors, with the aim of comparing the performance of various bioreactor designs and assessing the impacts of naphthenic acids molecular structure such as linearity and cyclicity, carbon number, presence of functional groups and their spatial arrangements on the process of aerobic biodegradation (Paslawski et al., 2009a,b; Huang et al., 2012). Furthermore, potential for improving the biodegradation of recalcitrant NAs through co-biodegradation with linear NAs which are more amenable to biodegradation has been investigated (D'Souza et al., 2013). Effective aerobic biodegradation of naphthenic acids requires intense aeration to promote the microbial activity and biodegradation of NAs. Anoxic biodegradation, if successful in achieving biodegradation rates comparable to that of aerobic option, could be an attractive option in which technical challenges and excessive cost associated with the effective aeration are eliminated. Moreover, anoxic biodegradation could serve as an alternative approach for the treatment of NA-contaminated waters in anoxic stabilization ponds supplied with a proper electron acceptor (e.g. nitrate). In fact, in situ bio-oxidation of hydrogen sulphide through injection of nitrate (coupling of sulphide bio-oxidation and nitrate reduction) has been proven as a successful strategy in control of souring in oil reservoirs subjected to water flooding (Nemati et al., 2001a,b). In the work presented here, anoxic biodegradation of a surrogate naphthenic acid (trans-4-methyl-1-cyclohexane carboxylic acid referred to as trans-4MCHCA for the rest of the article) has been investigated under denitrifying conditions in batch and continuous reactors (both CSTR and biofilm reactor). Effects of NA concentration, temperature and loading rate on biodegradation and denitrification processes have been evaluated. Finally, the generated data have been contrasted with those obtained with the same surrogate naphthenic acids in similar systems under aerobic conditions.

2. Materials and methods

2.1. Surrogate naphthenic acid, microbial culture and medium

Surrogate naphthenic acid used in this study was analytical grade trans-4-methyl-1-cyclohexane carboxylic acid (trans-4MCHCA, $C_8H_{14}O_2$, CAS NO. 13064-83-0; Sigma–Aldrich Canada Ltd., Ontario). Availability of extensive biodegradation data under aerobic conditions (Paslawski et al., 2009a,b) which allowed comparison of the aerobic and anoxic processes was the rational for selecting trans-4MCHCA as the surrogate naphthenic acid.

The microbial culture was a mixed culture, enriched in our laboratory and has been used extensively for aerobic biodegradation of naphthenic acids in our earlier works (Paslawski et al., 2009a,b; Huang et al., 2012). The culture was acclimated to anoxic biodegradation of trans-4MCHCA, using modified McKinney's medium with 100 mg L^{-1} trans-4MCHCA and 620 mg L^{-1} nitrate as the terminal electron acceptor. Modified McKinney's medium contained per litre: 840 mg KH_2PO_4, 750 mg K_2HPO_4, 474 mg $(NH_4)_2SO_4$, 60 mg NaCl, 60 mg$CaCl_2$, 60 mg$MgSO_4 \cdot 7H_2O$,20 mg $Fe(NH_4)_2SO_4 \cdot 6H_2O$ and 1 mL of micronutrient solution. The micronutrient solution contained per liter: 600 mg H_3BO_3, 400 mg $CoCl_3$, 200 mg $ZnSO_4 \cdot 7H_2O$, 60 mg $MnCl_2$, 60 mg $NaMoO_4 \cdot 2H_2O$, 40 mg$NiCl_2$, 20 mg $CuCl_2$ (Paslawski et al., 2009a,b). Acclimation was carried out in 150 mL serum bottles containing sterilized medium which had been purged with nitrogen gas for 15 min to ensure the absence of oxygen. The microbial culture grown previously under aerobic conditions was used as the initial inoculum (10% v/v). Inoculated serum bottles were maintained at room temperature ($24 \pm 2\,^{\circ}C$). Following the complete biodegradation of trans-4MCHCA (approximately two weeks after inoculation) which was associated with the reduction of