Science of the Total Environment 521–522 (2015) 59–67

Contents lists available at ScienceDirect

Science of the Total Environment

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

Pseudomonads biodegradation of aromatic compounds in oil sands process-affected water

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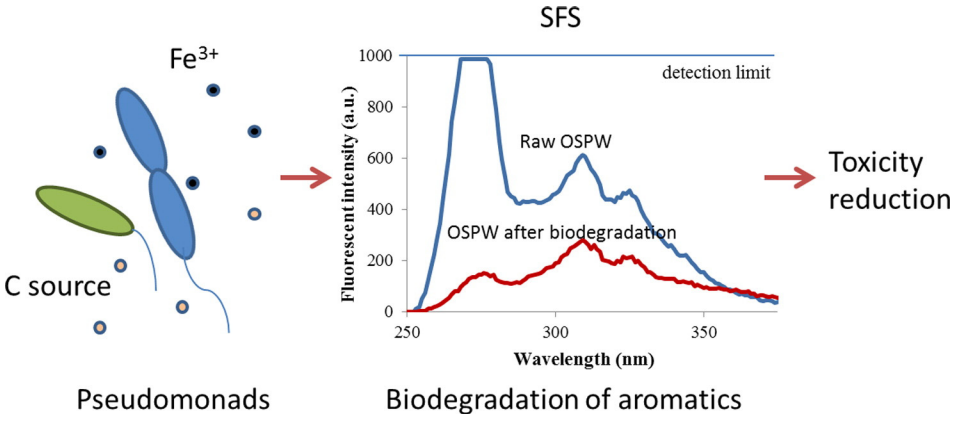
HIGHLIGHTS

- Pseudomonads are capable of biodegrading OSPW aromatic compounds.
- The toxicity of OSPW was reduced significantly after biodegradation.
- The external carbon source played an important role in ring cleavage.
- The combined ozonation and biodegradation approach is promising for OSPW treatment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form 17 March 2015
Accepted 17 March 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Oil sands process-affected water
Pseudomonads
Toxicity
Aromatics
Biodegradation

ABSTRACT

Aromatic naphthenic acids (NAs) have been shown to be more toxic than the classical NAs found in oil sands process-affected water (OSPW). To reduce this toxicity, *Pseudomonas fluorescens* and *Pseudomonas putida* were used to determine their ability to biodegrade aromatic compounds including treatments considering the impacts of external carbon and iron addition. Results showed that with added carbon *P. fluorescens* and *P. putida* have the capability of biodegrading these aromatics. In the presence of external carbon, gene expression of a functional PAH-ring hydroxylating dioxygenase (PAH-RHDα) was determined through reverse transcription real-time PCR, suggesting active degradation of OSPW aromatic compounds. Although no significant classical NAs removal was observed during this process, toxicity was reduced by 49.3% under optimal conditions. OSPW toxicity was eliminated with the combination of ozonation at a dose of 80 mg/L followed by biodegradation, indicating that it is a promising combined OSPW treatment approach for the safe discharge to the aquatic environment.

1. Introduction

The potential environmental impact of oil sands process-affected water (OSPW), contained in storage ponds until eventual release after treatment, has received extensive attention in recent years. Large amounts of OSPW are generated through the bitumen extraction

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http://dx.doi.org/10.1016/j.scitotenv.2015.03.068
0048-9697/

process of the oil sands in the Athabascan region of northern Alberta, Canada. The OSPW contains a complex, alkaline mixture of organic and inorganic compounds, which have shown toxicity to aquatic organisms (He et al., 2012), bacteria (Gamal El-Din et al., 2011), benthic invertebrates (Anderson et al., 2012), and mammalian species (Garcia-Garcia et al., 2011). Given this toxicity, appropriate remediation approaches are urgently needed for safe discharge of treated OSPW to the receiving environments.

Conventionally, naphthenic acids (NAs) have been considered to be the primary toxic constituents of OSPW (Anderson et al., 2012) with previous toxicity studies relevant to OSPW mainly focusing on the classical NAs. These classical NAs are complex mixtures of cycloaliphatic and alkyl-substituted acyclic carboxylic acids with a general chemical formula $C_nH_{2n+Z}O_2$, where n is the carbon number, and Z is zero or a negative even integer related to a hydrogen deficiency due to ring formation. In addition, the OSPW NAs also include smaller quantities of other compounds such as oxidized-NAs ($C_nH_{2n+Z}O_x$, where $x = 3$ to 5), nitrogen and sulfur species (Barrow et al., 2010), and other aromatic species (Jones et al., 2012; Rowland et al., 2011). Each of these groups may contribute to the toxicity, making the investigation of each of them toxicologically important to help in determining which groups are most responsible for OSPW toxicity. Furthermore, other organic compounds may also play a role in toxicity including priority pollutants such as the polycyclic aromatic hydrocarbons (PAHs) (Kavanagh et al., 2009).

Recently, the presence and toxicological impacts of the OSPW aromatic compounds have been frequently investigated. Synchronous fluorescence spectroscopy (SFS) and ultraviolet absorption spectrophotometry (UV) results have suggested the presence of aromatic compounds in OSPW extracts (Kavanagh et al., 2009; Rowland et al., 2011). Gas chromatography–mass spectrometry analysis has demonstrated that oil sands development contributes a significant amount of PAHs to the Athabasca River (Kelly et al., 2009). Examinations of OSPW acid-extractable organic matter by electrospray ionization high and low resolution mass spectrometry (Barrow et al., 2010; Martin et al., 2008) have shown that they contain compounds with four or more double bond equivalents (DBE), indicating the existence of aromatic species with alkyl branch or alicyclic rings. Recently, numerous aromatic acids in OSPW have been tentatively identified by comprehensive two dimensional gas chromatography–mass spectrometry of the methyl esters (GC × GC–MS) (Jones et al., 2012; Reinardy et al., 2013; Scarlett et al., 2013). Although PAHs and aromatic NAs make up a small percentage of OSPW compounds, they may contribute disproportionately to its toxicity. For example, a recent OSPW study showed that 13.1 mg/L of classical NAs caused 50% mortality (LC_{50}), whereas only 8.1 mg/L of an aromatic NA (dehydroabietic acid) caused the same mortality (Scarlett et al., 2013). Moreover, since the aromatic alkanoic NAs have similar structures to estrone and estradiol, they may account for part of the environmental estrogenic activity reported in OSPW (Rowland et al., 2011). This possible estrogenic activity has been confirmed using computer modeling of some tentatively identified OSPW aromatic NAs (Scarlett et al., 2012). Clearly, further assessment of the OSPW aromatic compounds is needed to better assess their possible role in overall toxicity and estrogenicity.

Biodegradation is potentially an economical, energy-efficient and environmentally sound approach for tailings water reclamation. However, previous studies have shown that oil sands classical NAs are persistent toward biodegradation because of their extensive cyclical molecular structures (Scott et al., 2005). Batch and continuous biofilm bioreactor studies have been used to determine the biodegradation of classical NAs by endogenous OSPW bacteria (Choi et al., 2014; Hwang et al., 2013), however, the study of biodegradation of the other aromatic compounds in OSPW is still only in its early stages. The bacteria *Pseudomonas fluorescens* and *Pseudomonas putida* have a demonstrated capacity to degrade commercial NAs, however, showed limited removal of OSPW NAs due to the recalcitrance of their branched alkyl chains (Del Rio et al., 2006). Aromatic OSPW NAs also have highly branched alkyl chains, in addition to aromatic rings, which may further limit their biodegradability. *Pseudomonas* species have been shown to metabolize a wide variety of aromatic substrates (Jiménez et al., 2002) and their diverse metabolisms make them an excellent potential candidate species for the degradation of these recalcitrant aromatic compounds in OSPW.

The objective of the current study was to determine the potential biodegradation of aromatic compounds in OSPW by *P. fluorescens* and *P. putida*. This degradation was monitored by assessing the SFS spectrum before and after treatment to determine the biodegradation of aromatic compounds as described previously (Kavanagh et al., 2009). Due to the recalcitrance of organic compounds in OSPW, an external carbon source was added to provide adequate bacterial nutrition. In addition, various concentrations of this external carbon source, and an external iron source, on biodegradation were evaluated. Moreover, during biodegradation ring cleavage for aromatic compounds in OSPW was assessed by the measurement of enzyme activity and the gene expression of PAH-ring hydroxylating dioxygenases. Furthermore, the role of the Pseudomonads in removing the residual toxicity of ozonated OSPW was also investigated.

2. Materials and methods

2.1. Source of OSPW and bacterial strains

Process water from an individual OSPW recycle pond site in Fort McMurray, AB, Canada was sampled and shipped in October 2012. The raw OSPW with a pH of 8.4 was received in a 200 L barrel and preserved at 4 °C in a cold storage room before use. The chemical oxygen demand (COD), acid extractable organic fraction (AEF) and NA concentration of the raw OSPW were 215 ± 3 mg/L, 71 ± 4 mg/L and 20.7 ± 1.2 mg/L, respectively ($n = 3$). In the raw OSPW, the concentrations of BTEX (benzene, toluene, ethylbenzene, and xylenes) and 23 species of PAHs (polycyclic aromatic hydrocarbons) were below detection limits while 0.28 mg/L of the petroleum hydrocarbon (PHC) fraction F2 (C_{10}–C_{16}) was found (Table S1).

P. fluorescens and P. putida have a demonstrated capacity to degrade various aromatic compounds (Haritash and Kaushik, 2009; Samanta et al., 2002; Shim et al., 2005). For example, the catabolic ability of *P. putida* in degrading alkyl branched aromatic NAs was recently reported (Johnson et al., 2013). Therefore, in this study, the bacteria *P. fluorescens* (ATCC 13525) and *P. putida* (ATCC 12633) were used as bacterial inoculums for the degradation of aromatics in OSPW. Before inoculation, they were grown individually in a Luria-Bertani (LB) media for 16 h on a rotary incubator at 30 °C and 150 rpm. A 100 μL aliquot of the overnight bacterial culture was washed with a phosphate buffer solution (PBS) three times to remove the residual LB media before being resuspended in OSPW for the batch studies.

2.2. Experiment design

The bioreactors were set up using autoclaved 500 mL Erlenmeyer flasks containing 300 mL of OSPW filtered with a 0.22 μm nylon membrane (Millipore, USA) to remove endogenous microorganisms prior to inoculation of the Pseudomonads. Aliquots of sodium acetate, ammonium sulfate and sodium dihydrogen phosphate were added as carbon, nitrogen and phosphate sources at final concentrations of 5000, 142 and 23 mg/L, respectively. The reactors were operated at room temperature (~20 °C) and were rotated at 150 rpm on an orbital shaker. Each day bacterial cell number in each reactor was enumerated by agar plate method to evaluate the bacterial growth. As well, water samples were centrifuged at 10,000 ×*g* for 10 min to remove bacteria prior to analysis of organic compounds, COD, acid extractable organic fraction, synchronous fluorescence spectroscopy (SFS), and toxicity tests.