Science of the Total Environment 536 (2015) 116–122

Contents lists available at ScienceDirect

Science of the Total Environment

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

Sulfate reducing bacteria and their activities in oil sands process-affected water biofilm

Hong Liu, Tong Yu *, Yang Liu *

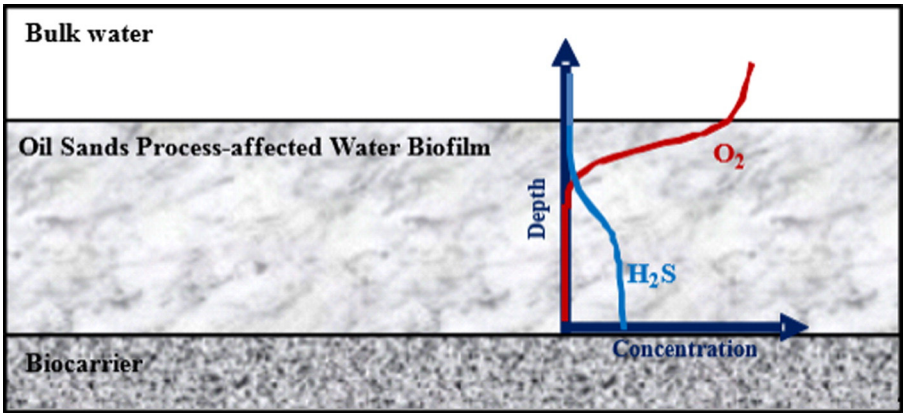
Department of Civil and Environmental Engineering, University of Alberta, Edmonton T6G 2W2, Canada

HIGHLIGHTS

- Biofilm in oil sands wastewater was developed on engineered biocarriers.
- Bacterial community and *in situ* activity of SRB were studied in the biofilm.
- Sulfate reduction activity was observed in the deeper zone of the biofilm.
- The biofilm could simultaneously remove sulfate, organic carbon, and nitrogen.

GRAPHICAL ABSTRACT

The development of sulfate reducing bacteria (SRB) within Oil Sands Process-affected Water (OSPW) biofilm and the biofilm treatment of OSPW were evaluated by Liu and coworkers. Combined microsensor and molecular biology techniques were utilized in this study. Their results demonstrated that multispecies biofilm with a thickness of 1000 μm was successfully developed on engineered biocarriers. H_2S production was observed in the deeper anoxic zone of the biofilm from around 750 μm to 1000 μm below the bulk water-biofilm interface, revealing sulfate reduction in the deeper zone of the biofilm. The biofilm removed chemical oxygen demand (COD), sulfate, and nitrogen.



ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form 29 June 2015
Accepted 29 June 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Sulfate reducing bacteria
Oil sands process-affected water
Biofilm reactor
Microsensor
Molecular biology

ABSTRACT

Biofilm reactors were constructed to grow stratified multispecies biofilm in oil sands process-affected water (OSPW) supplemented with growth medium. The development of sulfate reducing bacteria (SRB) within the biofilm and the biofilm treatment of OSPW were evaluated. The community structure and potential activity of SRB in the biofilm were investigated with H_2S microsensor measurements, *dsrB* gene-based denaturing gradient gel electrophoresis (DGGE), and the real time quantitative polymerase chain reaction (qPCR). Multispecies biofilm with a thickness of 1000 μm was successfully developed on engineered biocarriers. H_2S production was observed in the deeper anoxic zone of the biofilm from around 750 μm to 1000 μm below the bulk water-biofilm interface, revealing sulfate reduction in the deeper zone of the stratified biofilm. The biofilm removed chemical oxygen demand (COD), sulfate, and nitrogen. The study expands current knowledge of biofilm treatment of OSPW and the function of anaerobic SRB in OSPW biofilm, and thus provides information for future bioreactor development in the reclamation of OSPW.

* Corresponding authors.
E-mail addresses: tong.yu@ualberta.ca (T. Yu), yang.liu@ualberta.ca (Y. Liu).

http://dx.doi.org/10.1016/j.scitotenv.2015.06.135
0048-9697/

1. Introduction

Bitumen extraction from one cubic meter of the Alberta oil sands requires up to three cubic meters of hot caustic water, and produces about four cubic meters of slurry wastes containing sand, clay, unrecovered bitumen, dissolved inorganic and organic compounds (Fedorak et al., 2002). Under the zero discharge policy for the oil sands, the slurry wastes released in the oil sands mining operation have to be stored in tailings ponds onsite. The volume of slurry wastes are continuously accumulating, and it is estimated that over 1 billion m^3 of tailings pond water will be produced by 2025 (Quagraine et al., 2005). The oil sands process-affected water (OSPW) released from the densification of slurry wastes is recycled to the bitumen extraction operation. Continuous recycling of tailings pond water concentrates the organic and inorganic compounds in the water (Allen, 2008). Consequently, treatment of OSPW is a critical challenge for the oil sands industry.

Biofilm treatment of wastewater has been demonstrated to be cost effective and environmentally friendly compared to chemical treatments. Biofilm, a thin layer of microorganisms adhering to a structure, can be used to break down unwanted compounds in wastewater (Lewandowski and Boltz, 2011). Researchers have characterized microbial communities within biofilm and demonstrated that these microorganisms successfully in adapted to OSPW and were potentially capable of *in situ* bioremediation. For instance, Golby et al. (2011) demonstrated that 1–3 cell-layer thick biofilm was formed within one week in the Calgary Biofilm Device by seeding with oil sands' indigenous microorganisms. Islam et al. (2014) used a fluidized bed biofilm reactor (FBBR) with granular activated carbon (GAC) as support media to treat OSPW. After 120 days of OSPW treatment, biofilm with 30–40 μm thickness contained *Polaromonas jejuensis*, *Algoriphagus* sp., *Chelatococcus* sp., and *Methylobacterium fujisawaense*. Biofilm of ~30 μm thickness containing *Flavobacterium*, rhizosphere soil bacteria, *Rhizobium*, *Azoarcus*, *Stigmatella aurantiaca*, *Actinobacterium*, and *Sulfuritalea hydrogenivorans* was obtained in 28 days of OSPW treatment in batch and continuous biofilm reactors by Choi et al. (2014).

However, the biofilms in these studies were thin (<50 μm). Typical wastewater biofilms can be as thick as 1000 μm (Li and Bishop, 2004). A thicker biofilm will contain oxic and anoxic zones, and the anoxic strata of biofilm provide a good environment for the growth of sulfate-reducing bacteria (SRB) which play a significant role in sulfate reduction activities in wastewater biofilms (Santegoeds et al., 1998; Okabe et al., 1999; Ito et al., 2002). The sulfate reduction accounts for the degradation of organic compounds; however, the hydrogen sulfide (H_2S) generated during sulfate reduction is an air pollutant, and its invasion of the atmosphere with a noxious odor makes the atmosphere unpleasant to humans. In addition, hydrogen sulfide inhibited the growth of various bacteria (Reis et al., 1992) by linking to metal ions that might interfere in the normal cell chemistry of microorganisms.

Microsensors are innovative experimental tools for the study of complex structure and functions of biofilms. The tiny needle type microsensor measurements could provide potential activity local chemistries in that the measurements were conducted without destroying biofilm. Both the bacteria community and the community activity were maintained and the microsensor signals reflected the in-place condition along the depth of the biofilm; while molecular techniques provide a way to investigate the presence and functional diversity of specific populations without isolation.

The aim of this study was to investigate the presence, functional diversity, and potential activity of SRB in biofilms grown in OSPW using combined techniques of dissimilatory sulfite reductase subunit B (dsrB) gene based polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE); qPCR; and microsensor measurements of H_2S, pH, oxidation-reduction potential (ORP), and dissolved oxygen (O_2). We believe that these studies will provide information on biofilm formation in engineered biofilm reactors for OSPW treatment. We also hypothesize that biofilms can be used to remove organic contaminants from OSPW. Our study demonstrates that biofilm as thick as 1000 μm can be cultured under proper operational conditions. As expected, the biofilm displayed capability for Chemical Oxidation Demand (COD), nitrogen, and sulfate removal in the OSPW. The information obtained will guide biofilm reactor design and improve biological OSPW treatment.

2. Material and methods

2.1. Biofilm reactor design

Two identical 1 L glass jars (Catalog No: 02-912-313, Fisher Scientific) 9 cm in diameter and 15 cm in height were used as the reactor vessels. A schematic drawing of the reactor is shown in Fig. 1. Four biocarriers for biofilm attachment were strung together on each of four steel rods that were fastened at one end to the lid of the bioreactor. Two of the rods held biocarriers that are commonly used in moving bed biofilm reactors (MBBRs), and two rods held modified MBBR biocarriers. The modified biocarriers were prepared by trimming the inside of a normal MBBR biocarrier and inserting a flat substratum with more roughness on one side. Both reactors were run at room temperature on a shaker with a speed of 150 rpm.

2.2. Biofilm reactor operation

One reactor (R1) contained 600 mL OSPW; the other reactor (R2) contained 300 mL OSPW supplemented with 300 mL external growth medium R2B containing 0.525 g/L peptone, 0.35 g/L yeast extract, 0.35 g/L dextrose, 0.35 g/L starch, 0.21 g/L K_2HPO_4, 0.21 g/L sodium pyruvate, 0.175 g/L tryptone, and 0.0168 g/L $MgSO_4$. The growth medium was selected based on Golby et al. study (2011) in order to facilitate the growth of microorganisms.

A tailings sludge sample with approximately 80–85% solids from a tailings pond in northern Alberta was collected and put into a sealed 2 L sample jar. Ten commercial biocarriers (Active Cell™ media 515, Headworks BIO™) were added in the glass jar. A layer of biofilm of several hundred micrometers thickness was formed on the biocarriers surface after six months of culturing. The biofilms formed on the biocarriers were used as inoculum to seed the biofilm reactor in this research. Reactor operation was started by immersion in the water of two biocarriers; under shaking condition, the microorganisms attached to the biocarriers will transfer to the fixed biocarriers in the reactor and form a biofilm layer.

Reactors R1 and R2 were operated in batch mode. The chemical properties including NH_4^+–N, NO_3^-–N, TN, SO_4^{2-}, and COD of the water supplied to R1 and R2 were listed in Table 1. NO_2^-–N was absent in water supplied to R1 and R2. Water in both reactors was changed periodically. Initially, water in both reactors was replaced once per week, after six weeks of operation, water in both reactors was replaced every 2–3 weeks, after five water changes, water in both reactors was replaced once per month with two water changes. The reactor was running for 184 days. During water change periods from day 14 to day 22, day 31 to day 38, day 58 to day 70, day 85 to day 106, day 126 to day 154, and day 154 day to 184 (we defined these six periods as P1, P2, P3, P4, P5, and P6, respectively), samples were taken from the influent and the effluent of both reactors in order to monitor sulfate change. In addition, samples were taken during P6 from influent and effluent to monitor NH_4^+–N, NO_2^-–N, NO_3^-–N, and total nitrogen (TN).

2.3. Chemical analysis

Before draining the water from the reactors, 50 mL samples were filtered with 0.22 μm membrane and preserved at 4 °C for chemical analysis. The chemical parameters COD, sulfate, ammonium, nitrate, and total nitrogen were determined. COD was measured according to standard methods (American Public Health Association, 2005). Concentrations of sulfate, ammonium, and nitrate were determined using ion