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Effect of reactor configuration and microbial characteristics on biofilm reactors for oil sands process-affected water treatment

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ABSTRACT

Batch and continuous biofilm reactors (BBR and CBR) were operated to treat raw and ozone-treated oil sands process-affected water (OSPW). In raw OSPW, the BBR removed less (24% vs. 29%) chemical oxygen demand (COD) than did the CBR. The CBR removed 14% of the acid-extractable fraction (AEF) from raw OSPW and 51% from ozonated OSPW, whereas the BBR had lower AEF removal efficiencies of 6.2% and 37% for raw and ozonated OSPW, respectively. NAs with low molecular weight were preferentially degraded over those with high molecular weight, and classical NA degradation was more effective in the CBR than in the BBR. CBR operation with ozone-treated OSPW allowed a favorable growth of bacteria due to the high bioavailability of low molecular weight compounds. Scanning electron micrographs showed that distinct biofilm and extracellular polymeric substances (EPS) were formed under continuous flow conditions, and that the biofilm was thicker in the CBR than in the BBR. Microbial community analysis using denaturing gradient gel electrophoresis (DGGE) revealed more bands in BBR biomass samples; 23 strains were identified in the BBR compared to 21 in the CBR. The continuous flow mode removed organic compounds more favorably than the batch mode from raw and ozonated OSPW.

1. Introduction

Bitumen extraction from the Athabasca oil sands using a water-based extraction process produces 4 m^3 of polluted water containing mineral tailings for every 1 m^3 of bitumen extracted (Siddique et al., 2006). The used water is recycled in the bitumen extraction process until the pollution becomes intractable, then the used water is stored in a tailings pond on the industry site. Tailings ponds typically consist of 20–30 wt% solids (consisting largely of sands and clays), ~1 to 3 wt% residual bitumen, and slightly alkaline water (Chalaturnyk et al., 2002; Ramos-Padrón et al., 2011) containing naphthenic acids (NAs), benzene, toluene, and polycyclic aromatic hydrocarbons. Classical NAs are a broad group of alicyclic or noncyclic alkyl-substituted carboxylic acids having the general formula $C_nH_{2n+Z}O_2$, where n represents the number of carbon atoms and Z is zero or an even negative number referring to the number of saturated rings ($Z = 0$, no ring; $Z = -2$, one ring; $Z = -4$, two rings, etc.; however, Z may also be confounded by the presence of double bonds) (Headley and McMartin, 2004). Reported concentrations of classical NAs in oil sands process-affected water (OSPW) are in the range of 20–120 mg/L (Perez-Estrada et al., 2011; Toor et al., 2013), which is thought to cause considerable toxicity to the ecosystem (Hrudey et al., 2010).

Attempts to remove organic compounds such as NAs from OSPW have included membrane filtration, advanced oxidation processes, and biological treatments (Han et al., 2008, 2009; Smith et al., 2008; He et al., 2010; Martin et al., 2010; Kim et al., 2011, 2012). Ozonation of OSPW has been used to oxidize the most bio-persistent fraction of NAs to more easily biodegradable forms (Martin et al., 2010; Gamal El-Din et al., 2011; Hwang et al., 2013; Wang et al., 2013).

Biodegradation techniques have been widely studied for their ability to treat oil sands tailings (Foght et al., 1985; Herman et al., 1994; Holowenko et al., 2000; Fedorak et al., 2002; Del Rio et al., 2006; Penner and Foght, 2010; Golby et al., 2012; Johnson et al., 2013). Using reactors in batch-flow mode, Herman et al. (1994) reported that bacterial cultures enriched from oil sands tailings utilized both commercial NAs and NAs indigenous to OSPW as their carbon source. Batch experiments were performed to evaluate the capability of indigenous OSPW bacteria to remove NAs under methanogenic conditions (Siddique et al., 2006, 2007; Smith et al., 2008). Recently, Golby et al. (2012) showed that biofilm can be cultured in the Calgary Biofilm Device, a batch biofilm reactor (BBR), under aerobic and anaerobic growth conditions using oil sands' indigenous microorganisms.

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Despite the successful biotransformation of NAs under aerobic and anaerobic conditions in batch systems, the results of these studies may not be representative of NAs removal in engineered bioreactors having continuous inflow and outflow. Previous studies showed that biofilm formation can vary significantly depending on reactor configuration. For instance, static incubation can limit oxygen mass transfer to promote biosynthesis, thus, intermediate products are not generated and bacterial growth is restricted (Girguis et al., 2003). It has been reported that the reactor flow pattern (batch vs. continuous) influences not only sessile communities but also biofilm morphology (thickness, density, color) (Cao and Alerts, 1995). Since reactor type is an important determinant of the microbial ecology and the morphology of biofilms, it will also affect reactor performance and operation parameters. Previous work demonstrated that continuous flow bioreactors using biofilms (CBRs) and activated sludge effectively degraded three model NAs found in conventional oilfield water (Huang et al., 2012). Despite this, only three studies have been reported on the biological treatment of OSPW using continuous flow reactors (Huang et al., 2012; Hwang et al., 2013; Toor et al., 2013). Effective removal of oil sands-specific pollutants with continuous flow bioreactors requires further research as does the greater efficiency of the continuous flow bioreactor compared the batch system in the removal of such pollutants.

Biofilm reactors have been widely used for the treatment of domestic and industrial wastewaters in large and small scale systems (Li et al., 2012; Calderon et al., 2012; Zhang et al., 2012). Biofilm reactors are fixed growth systems where the microbial community is retained in the reactor as a biofilm; these reactors have low amounts of suspended solids and a limited biomass wash out. Biofilm systems have higher effluent quality and lower maintenance costs than suspended growth systems (MetCalf and Eddy, 2004). Other benefits of biofilm systems include a long sludge retention time and low sludge wasting (Anderottola et al., 2000). Biofilm enrichment is an important stage in the development of biofilm reactors. To achieve the removal of contaminants and encourage intercellular nutrient transfer, the biofilm should be relatively thick and evenly distributed on the biofilm carrier. Development of this morphology can be influenced by fluid turbulence and the microbial communities in the biofilm reactor (Purevdorj et al., 2002; Zhang et al., 2011).

This study compares the performance and microbial characteristics of the BBR and the CBR in the treatment of raw and ozone-treated OSPW. The main objective was to investigate and compare the removal of the acid-extractable fraction (AEF), representing NAs among other organic acids, from OSPW in bioreactors operated in batch and continuous flow modes. Characteristics of the biofilm structures and the microbial communities in the BBR and the CBR were examined using microscopy and DNA analysis.

2. Materials and methods

2.1. OSPW source and ozonation, and biofilm reactor operation

Raw and ozone-treated OSPW samples were used in these experiments. Raw OSPW was collected from an OSPW settling site in Fort McMurray, Alberta, Canada, and stored in a cold room (4 °C) prior to use. Raw OSPW was treated with ozone gas using an AGSO 30 Effizon ozone generator (WEDECO AG Water Technology, Herford, Germany). The ozone generator was allowed to stabilize for 10 min to obtain a stable ozone concentration in the feed gas. The feed ozone gas was added into the liquid phase (raw OSPW) through a ceramic fine bubble gas diffuser placed at the bottom of the 200 L reactor. During the ozonation process, two identical ozone monitors (HC-500, PCI-WEDECO) were used to control the ozone concentrations in the gas and off-gas lines continuously. The utilized ozone dose was 80 mg/L. Thereafter, the ozone-treated OSPW was purged with nitrogen for 10 min to remove residual ozone from the reactor.

Four biofilm reactors (Biosurface Technologies, Bozeman, MT, USA) were operated in batch or continuous modes to treat OSPW. Among the two reactors in each system (BBR and CBR), one reactor was operated using raw OSPW and the other was operated using ozonated OSPW. Each reactor contained biofilm-support media (Biosurface Technologies, Bozeman, MT, USA) which was immersed in 1–2% detergent (Sparkleen 1, Fisher Scientific) for 2 min, sonicated for 5 min, and then rinsed with tap water. The media were then sonicated again in deionized (DI) water for 5 min, fully wetted in 2 M HCl for 2 h, rinsed with DI water, and allowed to dry. Thereafter, the reactors were assembled, filled with 350 mL DI water, and autoclaved. To allow bacterial growth on the biofilm carriers, the reactors were all operated with 350 mL OSPW in a batch mode for the initial 24 h at room temperature (20 °C). Thereafter, each reactor was operated with continuous mixing at room temperature for six weeks. In the BBR, the influent was changed every seven days, whereas the CBR was operated with a continuous flow of 0.3 mL/min and a hydraulic residence time (HRT) of 19 h.

2.2. Analyses of water quality

The concentration of the acid extractable fraction (AEF) in OSPW was measured using Fourier transform infrared (FT-IR) spectroscopy (BioRad, FTS-6000, Cambridge, MA, USA). Filtered OSPW samples (50 mL) from the influent and effluent of each reactor were acidified to pH 2.0 and organics were extracted by liquid–liquid extraction with portions of dichloromethane (DCM) in a separation funnel. After the extraction, the DCM was evaporated and the AEF remained in the container. The extracted AEF was reconstituted with a known mass of DCM and examined using FT-IR spectral analysis. Absorbance was measured at wave numbers 1743 and 1706 cm^{-1}, representing the adsorption bands characteristic of monomeric and dimeric carboxylic groups, respectively (Clemente and Fedorak, 2005).

Classical NAs were separated by ultra performance liquid chromatography and measured by high resolution mass spectrometry (UPLC/HRMS) as previously described (Afzal et al., 2012). A 10 mL sample from each biofilm reactor was filtered (0.22 μm pore) (Millex GS, Millipore) and 500 μL of filtered sample was added to a 2 mL glass vial with 450 μL of methanol and 50 μL of an internal standard solution (tetradecanoic acid-1-^{13}C). Classical NAs were separated using a Water Acquity UPLC® System (Milford, MA, USA) and detected with a high resolution Synapt G2 HDMS mass spectrometer with the system controlled using MassLynx 4.1 software. Tuning and calibration were performed with a leucine enkephalin standard solution and sodium formate, respectively. TargetLynx 4.1 software was used for data analysis and the relative ratios of chromatographic peak areas compared to the internal standard were calculated for graphical analysis.

Ion chromatography (ICS-2000 and ICS-2500, Dionex, Sunnyvale, CA, USA) was employed to analyze cations (calcium, magnesium, sodium, potassium, ammonium) and anions (sulfate, chloride, fluoride, nitrate, nitrite, bromide, phosphate) in the OSPW. Chemical oxygen demand (COD) and total dissolved solids (TDS) were measured according to the protocol described in *Standard Methods for the Examination of Water and Wastewater*, 21 edn. (APHA, 2005).

2.3. Bacteria enumeration, staining, and imaging

A 100 μL sample was obtained from the bioreactors to complete heterotrophic plate counts (HPCs) using an R2A agar plating