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Characterization of microbial communities during start-up of integrated fixed-film activated sludge (IFAS) systems for the treatment of oil sands process-affected water (OSPW)

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ABSTRACT

In this study, integrated fixed-film activated sludge (IFAS) microbial development and degradation efficiency were investigated for oil sands process-affected water (OSPW) remediation. IFAS microbial community was characterized using 454 high-throughput *16S rRNA* gene pyrosequencing that revealed that the IFAS seed sludge (activated sludge [AS] from the Gold Bar Wastewater Treatment Plant [GBWTP]) showed the greatest richness and evenness of bacterial community, as compare to other biomass samples. The Chao 1 value and the Shannon diversity index showed that the bacterial richness and microbial diversity in biofilms were significantly higher than those in flocs in both IFAS systems. *Proteobacteria*, *Nitrospirae*, *Acidobacteria*, and *Bacteroidetes* were dominant phyla in both flocs and biofilms in IFAS reactors. It is also noted that the phyla and class distributions of flocs and biofilms were significantly different. Principal coordinate analysis (PCoA) indicated that there were substantial differences between OSPW indigenous microbes and flocs and biofilm microbes in IFAS. Overall, a relatively low ozone dose (30 mg/L utilized) combined with IFAS reactor treatment significantly increased the organic contaminants removal. The combined ozonation and IFAS system showed the promise for OSPW treatment.

1. Introduction

Development and growth of the Athabasca oil sands industry in northern Alberta, Canada, has been rapid in recent decades [1]. Northern Alberta produces 1.3 million barrels of bitumen per day [2], and oil production is targeted to reach over 3.3 million barrels per day by 2020 [3]. Waste by-products of the oil sands industry are collected in large artificial settling ponds called tailings ponds [4]. The hot water extraction process was applied to separate bitumen from associated sands and clays produces large amounts of tailings in the water used for bitumen extraction; such water is known as oil sands process-affected water (OSPW). OSPW contains water, sand, clay, residual bitumen, heavy metals, naphtha diluents, and naphthenic acids (NAs), which are toxic to a wide range of aquatic organisms [5–8]. A limit to the extension of tailings ponds and aggressive timelines for OSPW reclamation had been mandated by the Alberta government [9]. The primary toxicity of OSPW to aquatic organisms is attributed to a group of low molecular weight organic acids known as naphthenic acids (NAs) [10], which constitute about 50% of the acid extractable fraction (AEF) in OSPW [11]. The concentration of classical NAs in OSPW has been reported to range from 20 to 120 mg/L [12]. Technologies to treat OSPW to allow it to be recycled or safely disposed are being studied. OSPW recycling would reduce the demand for fresh water intake from the Athabasca River.

Biodegradation techniques have been investigated for their ability to treat OSPW [13,14]. Such bioreactor technology is an economical, environmentally friendly, energy-efficient method for OSPW remediation [15]. Bioreactors employing suspended or attached aggregations (activated sludge flocs or attached biofilms) have been studied to prepare OSPW for recycling or safe disposal [1,16–19]. It has been reported that ozone treatment might be used as a pretreatment to break down recalcitrant NAs and improve the biodegradability of OSPW [17,20]. A low ozone dose could be employed to partially degrade target recalcitrant compounds in OSPW to benefit downstream bioreactor treatment [21]. When we treated OSPW with integrated fixed-film activated sludge (IFAS) with and without ozonation [19], it was observed that 12.1% of the AEF and 43.1% of the parent NAs were removed in the raw (nonozonated) OSPW IFAS, while 42.0% of the AEF and 80.2% of the parent NAs were removed in the ozonated OSPW IFAS. Therefore,

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microbial communities in the IFAS systems need to be characterized to investigate the complex interaction between suspended and attached biomass and to improve the design and understanding of IFAS reactor operation.

Among the microorganisms in bioreactors, including bacteria, eukaryotes, archaea, and viruses [22], bacteria play an important role in wastewater treatment [23]. The bacterial community composition is very sensitive to changes in operational parameters and the nutrient composition of the bioreactor. Since microbiological parameters indicate stress or change in the system, characterization of microbial community composition in a bioreactor is critical to assessment of biological limiting factors related to the removal efficiency of contaminants and improvement in bioreactor performance [24]. Molecular biological techniques commonly used in wastewater treatment include denatured gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), the quantitative polymerase chain reaction (q-PCR), and fluorescence in situ hybridization (FISH) [25]. However, conventional molecular biology approaches cannot provide sufficient sequences to capture comprehensive information about diverse bacterial communities [26,27]. The main limitation of PCR-based fingerprinting techniques (DGGE and T-RFLP) is their low resolution [28]. More sensitive techniques are necessary to achieve a more precise and comprehensive characterization of microbial communities in IFAS systems.

In recent years, high-throughput sequencing technologies have been used to study microbial communities in soil [29], raw sewage [30], activated sludge [31], and bioreactors [1,32]. These technologies can generate hundreds of thousands of short sequences from hypervariable regions in rRNA genes [33,34]. This powerful technique has the potential to obtain a comprehensive coverage of microbial communities [35].

The present study focuses on the utilization of ozonation combined with IFAS to treat OSPW. Through applying 454 high-throughput pyrosequencing techniques, this study aims (i) to monitor the microbial changes of suspended flocs and attached biofilms during the start-up period of IFAS systems, (ii) to investigate the biodiversity in dominant bacterial communities of activated sludge flocs and attached biofilms in IFAS systems, and (iii) to identify differences in microbial community composition between tailing ponds microorganisms, aerobic activated sludge from the Gold Bar Wastewater Treatment Plant (GBWTP) (Edmonton, Canada), suspended flocs, and attached biofilms in IFAS systems. This is the first report of high-throughput pyrosequencing of microbial communities in IFAS reactors treating oil sands tailings water.

2. Materials and methods

2.1. Source water and ozonation

Fresh OSPW was collected from tailings ponds in Northern Alberta in September 2013 and stored in a cold room (4 °C) prior to use. An ozone dose of 30 mg/L was applied for OSPW pretreatment based on our previous studies [20]. Ozonated OSPW was produced using an AGSO 30 Effizon ozone generator (WEDECO AG Water Technology, Herford, Germany). A stable concentration of ozone gas from the ozone generator was introduced into the liquid phase through a ceramic gas bubble diffuser at the bottom of the container. Two ozone monitors (HC500, WEDECO, Charlotte, NC, USA) were applied to monitor the ozone concentration in the feed-gas and the off-gas; the residual ozone concentration in the container was monitored using the indigo method [36]. Ozonated OSPW was purged with nitrogen for 10 min to strip oxygen and residual ozone.

2.2. IFAS system operation

Two parallel laboratory-scale IFAS reactors [19] provided by Napier-Reid Ltd. (Markham, Canada)—one for raw OSPW treatment and the other for ozonated OSPW treatment—was initiated by inoculating the reactors with 2 L of activated sludge from the GBWTP. A 60% volume fraction of polyethylene (PE) carriers (Bioflow 9, Rauschert, Steinwiessen, Germany) with specific biofilm growth areas of 800 m^2/m^3 were applied in both IFAS reactors. During the reactor start-up stage, we increased the volume ratio of raw and ozonated OSPW step by step (from 10% to 100%). From 10% phase to 60% phase, commercial NAs (30 mg/L COD) (naphthenic acid, SIGMA-ALDRICH, Belgium) were provided to encourage the growth of NA degraders in the IFAS systems. During the entire start-up stage, carbon (sodium acetate), nitrogen (NH_4Cl, 30.0 ± 3.0 mg N/L), and phosphorus (KH_2PO_4, 3.0 ± 0.2 mg P/L) were provided to maintain the growth of biomass in the IFAS systems [19]. The hydraulic retention time (HRT) of the two IFAS reactors was 48 h. The suspended solid retention time (SRT) was maintained for 43 days by manually discharging sludge from the clarifier. The dissolved oxygen (DO) concentration was in the range of 6–7 mg/L during IFAS operation. The detailed start-up conditions are listed in Table 1.

2.3. DNA extraction

Genomic DNA was extracted from activated sludge flocs and biofilms in raw and ozonated OSPW IFAS systems. The total DNA was extracted from 5 mL of activated sludge flocs using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's protocol. To obtain microorganisms from the biofilms formed on the carriers, 5 carriers from each reactor were first immersed in phosphate buffered saline (PBS) to remove nonadherent bacteria, then placed in a 20 mL sterile glass bottle (Falcon, CA, USA) with 5 mL sterile PBS, and sonicated for 10 min. The glass bottles were then vortexed for 1 min until the residual biofilm was completely removed from the carrier. The mixed liquid in the glass bottle was used for biofilm DNA extraction. DNA was extracted in duplicate from each sample.

2.4. 454 high-throughput 16S rRNA gene pyrosequencing

To determine the diversity and composition of the microbial communities in tailing ponds endogenous microorganisms (raw and ozonated OSPW), aerobic activated sludge from GBWTP (inocula), and suspended flocs and attached biofilms in the IFAS systems, DNA samples were extracted and paired-end sequencing was performed on a 454/Roche GS-FLX platform by the Research and Testing Laboratory (Lubbock, TX, USA). The V1-V3 regions of the *16S rRNA* genes were amplified by PCR using primer pairs of 28F (5′-GAGTTTGATCNTGGCTCAG-3′) and 519R (5′-GTNTTACNGCGGCKGCTG-3′) [37].

2.5. Data analysis

After read quality checking and denoising, a microbial diversity analysis was performed using the pipeline Quantitative Insights Into Microbial Ecology (QIIME), version 1.9.0 [38]. Raw reads were filtered by QIIME quality filters. Effective reads were normalized for the next analysis. Sequences with more than 97% similarity were clustered into the same operational taxonomic units (OTUs) using the UCLUST algorithm. Representative OTUs were selected based on the most abundant sequences, and taxonomic assignment was performed with a UCLUST consensus taxonomy classifier. OTU sequences were aligned with the Python Nearest Alignment Space Termination (PyNAST) tool, and the communities were summarized by taxonomic composition. To compute alpha diversity,