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Effect of hydraulic retention time on microbial community in biochemical passive reactors during treatment of acid mine drainage

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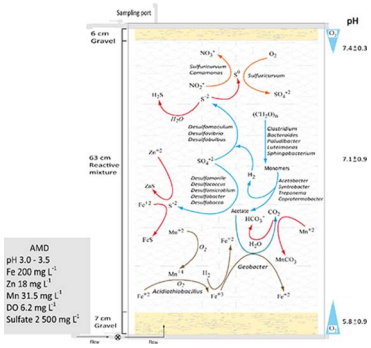
GRAPHICAL ABSTRACT



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ABSTRACT

The effect of hydraulic retention time (HRT) on the microbial community during acid mine drainage (AMD) treatment was investigated. Physicochemical and molecular (illumina and qPCR) analyses were performed on reactive mixtures collected from seven bioreactors in three-operation period (8, 17 and 36 weeks). Long HRT (4 day) favored the relative abundance of SRB, causing the increase of residual sulfides and short HRT (1 day) affected the anaerobic conditions of the bioreactors and favored the presence the acidophilic chemolithotrophic microorganisms. Besides qPCR indicated that genes related to cellulose degradation were present in low copy numbers and were affected by the HRT. Finally, environmental factors (pH, organic source, metal sulfides, and sulfate concentrations) had significant impact on relative abundance of the phylogenetic lineages, rather than the types of lineages present in the reactive mixture. The findings of this study indicate that HRT affects the stability of passive bioreactors and their microbial communities.

1. Introduction

Acid mine drainage (AMD) is a major type of contaminated effluent generated by the mining and metallurgical industry. Biochemical passive reactors (BPRs) are a common AMD treatment technology, which relies on the biological, chemical, and physical processes in natural environment systems (Skousen et al., 2017). BPRs are systems packed with mixtures of organic and inorganic substrates referred to as

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"reactive mixtures". During the operation of BPRs, members of the microbial communities, such as cellulose degraders and fermentative bacteria, degrade complex substrate into simple carbon compounds. The organic substrate enables gradual release of electron donors and carbon sources to drive microbial, specifically sulfides produced by sulfate-reducing bacteria (SRB), and chemical reactions to reduce acidity and concentrations of metals and sulfate in AMD (Drennan et al., 2016; Mirjafari and Baldwin, 2016).

Previous studies have confirmed that performance of BPRs depend on several factors, such as the type of carbon source, AMD chemistry, and reactor configuration (Vasquez et al., 2016a; Zheng et al., 2014). Since this is a microbiologically driven process (Baldwin et al., 2016; Mirjafari and Baldwin, 2016), understanding the microbial community is crucial to improve efficiency and process stability of BPRs. One of the major challenges during the study of BPRs is the difficulty in predicting which factors affect the association between the microbial community's structure and reactor performance. For this reason, the investigation of microbial community in BPRs has attracted increasing attention in recent years (Drennan et al., 2016). Many attempts have been made to correlate composition and dynamics of microbial community with substrate type used in the reactive mixture, temperature variations, and operation period after the installation of BPRs (Mirjafari and Baldwin, 2016). Few studies are available of the relationship between the microbial community and operation parameters, such as HRT, AMD flow direction, operation period and location in the BPR has been done.

One of the most important operation parameters of BPRs is the HRT, since it influences hydraulic conditions in the reactor and the contact between the AMD and the microorganisms in BPRs. The effect of the HRT has been studied with a focus on the efficiency of BPRs for metal and sulfate removing, changes in composition of the reactive mixture and microbial activity (Aoyagi et al., 2017; Vasquez et al., 2016a). However, the relation between HRT and microbial community structure during the treatment of AMD, characterized by high sulfate concentrations with moderate metal loading, has not yet been addressed.

This study was developed in three parts. The first part (Vasquez et al., 2016b) provides details on the physicochemical characterization AMD from the Zipaquirá Mining District (Colombia) and the selection of reactive mixtures for BPRs, based on their composition, efficiency, and permeability. The second part (Vasquez et al., 2016a) determined the effect of three hydraulic retention times (1, 2, and 4 day) on changes in system efficiency during AMD treatment, composition of the reactive mixture and enzymatic activity in bioreactors under upward flow conditions. Finally, in the present study, the main goal was to assess the impact of HRT (1, 2, and 4 day) on microbial community during the treatment of AMD (with low metal but high sulfate concentrations) in BPRs. This study determined the dynamics of cellulose degraders, fermentative bacteria, and SRB, which could contribute to opening the "black box" of bioremediation of AMD. To quantify and reveal the community dynamics in the PBRs, Illumina high-throughput sequencing of *16S rRNA* gene and quantitative PCR (qPCR) of three functional genes (*cel48*, *hydA*, and *dsrA*) were used.

2. Materials and methods

2.1. Design, set-up, and operation of biochemical passive reactors

Artificial AMD, characterized by high sulfate concentrations ($2500 \pm 105\ mg\ L^{-1}$ SO_4^{2-}) and moderate metal loading ($201 \pm 44\ mg\ L^{-1}$ Fe^{2+}; $30 \pm 2\ mg\ L^{-1}$ Mn^{2+}; $19 \pm 2\ mg\ L^{-1}$ Zn^{2+}; $215 \pm 11\ mg\ L^{-1}$ Ca^{2+}, and $128 \pm 13\ mg\ L^{-1}$ Mg^{2+}), at pH 3.0–3.7, were treated in seven 5 L column BPRs (73 × 10 cm). The AMD parameters were selected to simulate real AMD quality, according to data collected at five active coal mines in the Zipaquirá Mining District of Colombia (Vasquez et al., 2016b). All BPRs were filled with the same reactive mixture (15% cow manure, 10% mushroom compost, 25% sajo sawdust, 15% gravel, 20% limestone, and 15% sediment of artificial wetland as inoculum), as previously defined in batch tests for 45 days. Initially, three BPRs were operated with 2 day HRT and four BPRs with 4 day HRT. After 17 weeks, a strong increase in concentration of soluble sulfides in treated effluents from the columns with 4 day HRT justified the decision to change one of these reactors to 1 day HRT. During the 36 weeks of the experiment, 50 mL of treated effluent was sampled weekly and analyzed for physicochemical parameters (pH, redox potential (ORP), alkalinity, sulfate, sulfides, and dissolved metals) to determine the efficiency of the BPRs in AMD treatment (Vasquez et al., 2016a).

2.2. Sampling of reactive mixture

The reactors were sacrificed during the study to monitor physicochemical changes and microbial community dynamics in the reactive mixture. Four reactors, two of 2 day HRT and two of 4 day HRT, were sacrificed at weeks 8 and 17. The other three reactors (one each of 1, 2, and 4 day HRT) were sacrificed at the end of the study (36 weeks). The reactive mixture was sampled at three places: bottom (0–20 cm), middle (20–40 cm), and top (40–60 cm). The reactive mixture of each layer was homogenized and divided into three replicates. Samples were kept at 4 °C, prior to physicochemical analyses, while the fractions used for microbial community analysis were stored at −80 °C until DNA extraction.

2.3. Physicochemical analysis of reactive mixture

The pH was immediately measured with a multiparameter probe (HI 9828, Hanna Instruments; Woonsocket, RI) using the method 4972-01 (APHA, 2005). The organic nitrogen (TKN) was measured by method 4500–Norg (Schumacher, 2002), the total organic carbon (TOC) was analyzed by titration (APHA, 2005), and cellulose content was determined by the extraction method (Harper and Lynch, 1981). Metal concentrations were analyzed after the digestion and dissolved species quantified by atomic absorption spectrometry (AAS Varian 240FS; Agilent Technologies; Santa Clara, CA), using method 7000B (USEPA, 2007). Acid volatile sulfides (AVS) were separated (Brouwer and Murphy, 1994) and analyzed with a UV spectrometer (Genesys 10, Thermo Scientific; Waltham, MA) by method 4500D (APHA, 2005). Finally, soluble sulfate was analyzed in the HCl extract (40%, v/v) (Sobek et al., 1978).

2.4. DNA extraction, PCR amplification, and sequence analysis

Metagenomic DNA was extracted from 66 samples: 3 initial reactive mixtures and 63 post-treatments reactive mixtures (7 BPR × 3 cores × 3 replicates), using the PowerMaxSoil DNA kit (MoBio Laboratories; Solana Beach, CA). Amplicons of region V4 of the *16S rRNA* were sequenced on an Illumina MiSeq with a 2 × 250 paired-end at the University of Iowa (Caporaso et al., 2012). Quantitative Insights into Microbial Ecology (QIIME) v 1.8 software was used for all sequence analyses. Sequences were assembled, filtered, and clustered to OTUs at 97% identity level, using UCLUST (Edgar, 2010), and then assigned, based on the Greengenes 13_8 databases (DeSantis et al., 2006). A threshold of 0.005% of the total sequences was defined to keep an operational taxonomic unit (OTU).

2.5. Quantification of functional genes and biomass

The *cel48* (cellulose degradation), *hydA* (fermentation), *dsrA* (sulfate reduction), and *16S rRNA* (indicator of bacterial abundance) genes were quantified by qPCR. Quantification was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad Technology; Hercules, CA), using SsoAdvanced SYBR®Green Supermix and primers described in previous studies (Ben-Dov et al., 2007; Pereyra et al., 2010; Suzuki et al., 2000; Wagner et al., 1998). Standard curves were