Microbial Community Dynamics during the Biochemical Treatment of Acid Mine Drainage under three different Hydraulic Retention Times

Yaneth Vasquez[1], María C. Escobar[1], Carmen M. Neculita[2], Ziv Arbeli[3], Fabio Roldan[3]

[1]*Departamento de Ciencias Naturales, Universidad Central, Bogotá, Colombia, ovasquezo@ucentral.edu.co*
[2] *RIME - Research Institute on Mines and Environment, UQAT - University of Quebec in Abitibi-Temiscamingue, QC, Canada, Carmen-Mihaela.Neculita@uqat.ca*
[3]*Unidad de Saneamiento y Biotecnología Ambiental (USBA), Departamento de Biología, Pontificia Universidad Javeriana, Bogotá, Colombia, fabio.roldan@javeriana.edu.co*

Abstract

In the Zipaquirá Mining District of Colombia, there are about 600 coal mines that generate 70,400 m^3/month of acid mine drainage. A sustainable approach to remediate AMD is to use biochemical passive reactors. However, limited data is available on the dynamics (temporal and spatial) of microbial community and their activity under different hydraulic retention times, in the long-term operation of BPR. Seven 5L biochemical passive reactors (73 × 10 cm) were operated during 36 weeks, under three different hydraulic retention time (1, 2, and 4 days). The reactors were sacrificed on 8, 17 and 36 weeks, and the reactive mixture was sampled at the bottom, middle, and top layers. The microbial community of the post-treatment reactive mixtures was monitored by sequencing (Illumina MiSeq) and correlated with physicochemical parameters. The result showed that operation time, location and hydraulic retention time had significant effects on physicochemical changes of the reactive mixture and it is rather the combination of factors affect diversity during the AMD treatment. In addition, the microbial community analysis resulted in the identification of specialized groups related to cellulose degraders and fermentative bacteria that work in synergy for degrading substrate make the organic material available to sulfate-reducing bacteria. This microbial community analysis provides a base line for future studies in the BPR

Key words: acid mine drainage; microbial diversity; Illumina; biochemical passive reactors

Introduction

Biochemical passive reactors (BPR) is a successful acid mine drainage (AMD) treatment technology with potential advantages such as low costs, few site visits required, ability to work in remote areas, opportunities to use recycled or waste materials, and natural appearance (Doshi 2006). In the Zipaquirá Mining District of Colombia, there are about 600 coal mines that generate ~70,400 m^3/month of drainages and in this region a sustainable approach to remediate AMD is to use BPR. The most efficient reactive mixture for increasing pH and alkalinity, as well as promoting sulfate reduction and metal removal during AMD treatment in Zipaquirá Mining District was selected (Vasquez et al 2016a). In addition, the effect (temporal and spatial) of hydraulic retention time (HRT) (1, 2 and 4 days) on the efficiency of BPRs and microbial activity was also evaluated (Vasquez et al 2016b). However, the microbial community dynamics of this system has not been characterized despite its importance for BPR.

The HRT is a crucial design parameter, which influences the overall performance of BPR during AMD treatment (Neculita et al 2008a). Nevertheless, little is known about how the HRT affects microbial communities during operation time in BPR. In this context, the objective of the present study was to assess the impacts of HRT, location in the reactor and operation time on the microbial community involved in the synthetic AMD remediation under a column study.

Materials and methods

BPR design and AMD characteristics

Seven up-flows BPR were constructed using acrylic columns (73 × 10 cm) and operated for 36 weeks treating synthetic AMD, characterized by high sulfate concentrations, and low metal loading (mg L^{-1} 201 ± 44 Fe^{2+}; 30 ± 2 Mn^{2+}; 19 ± 2 Zn^{2+}; 215 ± 11 Ca^{2+}; 128 ± 13 Mg^{2+} and 2,500 ± 105 SO_4^{2-}, at pH 3.0 – 3.7). The AMD was prepared according the information collected at five active mine sites in the Zipaquirá Mining District, Colombia. The columns were filled with the same reactive mixture (15% cow manure, 10% mushroom compost, 25% sajo sawdust, 15% gravel, 20% limestone, and 15% wetland sediment as inoculum). Initially three BPR were operated with 2-days of HRT and four BPR with 4-days of HRT. After 17 weeks, a strong increase of soluble sulfide in treated effluents from the columns with 4-day HRT justified the decision to change one of the reactors of 4-day HRT to 1-day HRT (Vasquez et al 2016 a, b).

Column sampling

Columns were sacrificed throughout the study to monitor the changes in the post-treatment reactive mixture and microbial activity. Four columns, two of 2-day HRT and two of 4-day HRT, were sacrificed at week 8 and 17. The other three columns (1, 2, and 4-day HRT) were analyzed at the end of the study (36 weeks). The reactive mixture from the sacrificed columns was removed from different locations at the reactor. Three layers (20 × 10 cm), one from the bottom (at 0–20 cm), one from the middle (at 20–40 cm), and one from the top (at 40–60 cm) were taken. The samples were homogenized and refrigerated at 4 °C for physicochemical analyses and stored at − 80°C until DNA extraction.

Physicochemical analysis of reactive mixture post treatment

The pH was measured (Lab 870, Schott; Mainz, Germany) using method 4972–01 (ASTM 1995a) and a ratio of solid to de-ionized water of 1:1. The organic nitrogen (TKN) was measured by Method 4500–Norg (APHA 2005), and cellulose content was determined, according to Harper and Lynch (1981). The organic carbon (TOC) was analyzed by the Walkley–Black method (Schumacher 2002). Total metal concentrations immobilized in the reactive mixtures were determined by the digestion method described by Neculita et al (2008b) and dissolved metals (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}) were quantified by atomic absorption spectrometry (Varian 240 FS; Agilent Technologies; Santa Clara, CA), using method 7000B (USEPA 2007). Acid volatile sulfide (AVS) were separated by Brouwer & Murphy (1994) and soluble sulfate were extracted by Sobek et al (1978) and quantified by UV-VIS spectrophotometry (Genesys 10, Thermo Scientific; Waltham, MA) using method 4500–SO_4 (APHA 2005).

Nucleic acid extraction and sequence analysis

Genomic DNA was extracted from 22 samples of post-treatment reactive mixture and 1 sample of initial reactive mixture using the MoBio® PowerSoil DNA extraction kit (MoBio Laboratories, Solana Beach, CA). PCR amplification, purification, and sequencing for illumina MiSeq of a region V4 of the 16S rRNA gene were performed following the procedure described by Caporaso et al (2011). All extractions and amplifications were realized by triplicated. Sequencing was conducted using MiSeq Illumina (2×250 pb) technology at DNA Facilities (Iowa University). Total length of the Paired-end reads (250 bp) were assembled with the Fast Length Adjustment of Short Reads tool (Magoc and Salzberg 2011). QIIME v1.7 was used for all analysis and the sequences were aligned to the Greengenes reference alignment using PyNAST at the 97% confidence level. After the sequences were quality filtered and randomly in subsampled the 10000 sequences (this number was chosen by the minimum number on reads in a control sample) that were subsequently clustered into operational taxonomic units (OTUs). The relationships between dynamic of genera (relative abundance > 0.5%), physicochemical characteristics of BPR and samples were assessed by canonical correspondence analysis (CCA) using Conoco v4.5 for Windows package with Monte Carlo permutation test and Spearman correlation coefficients. The statistic difference of the relative abundance of the genera through the layers of the BPR was evaluated by *t* -test Welch's, adjusted for Benjamini Hochberg (p = 0.05), using STAMP 2.01 (Parcks et al 2010).

Results and discussion

The CCA (Fig. 1) reveled that samples were clustered in three subgroups corresponding to the operation time (8, 17 and 36 weeks) and that these subgroups were significantly different with respect to their physicochemical characteristics and microbial community. Besides, the Spearman correlation coefficients showed that operation time had negative correlation with pH, TKN and COT (–0.884, –0.845 and –0.812; p =0.00, respectively) and positive correlation with Zn, AVS, sulfate, Fe and Ca (0.817, 0.771, 0.742, 0.455, and 0.406; $p < 0.05$, respectively), indicating that organic components and pH decrease while the metal sulfides increase in reactive mixture over time. Operation time is one driver for shifting the physicochemical characteristics and microbial community composition. Previous studies have identified that lowering of the pH, re-oxidation of sulfide back to sulfate and a reduction in dissolved organic compounds contributed to change in the microbial community and decline in performance of the BPR (Baldwin et al 2015; Mirjafari et al 2011).

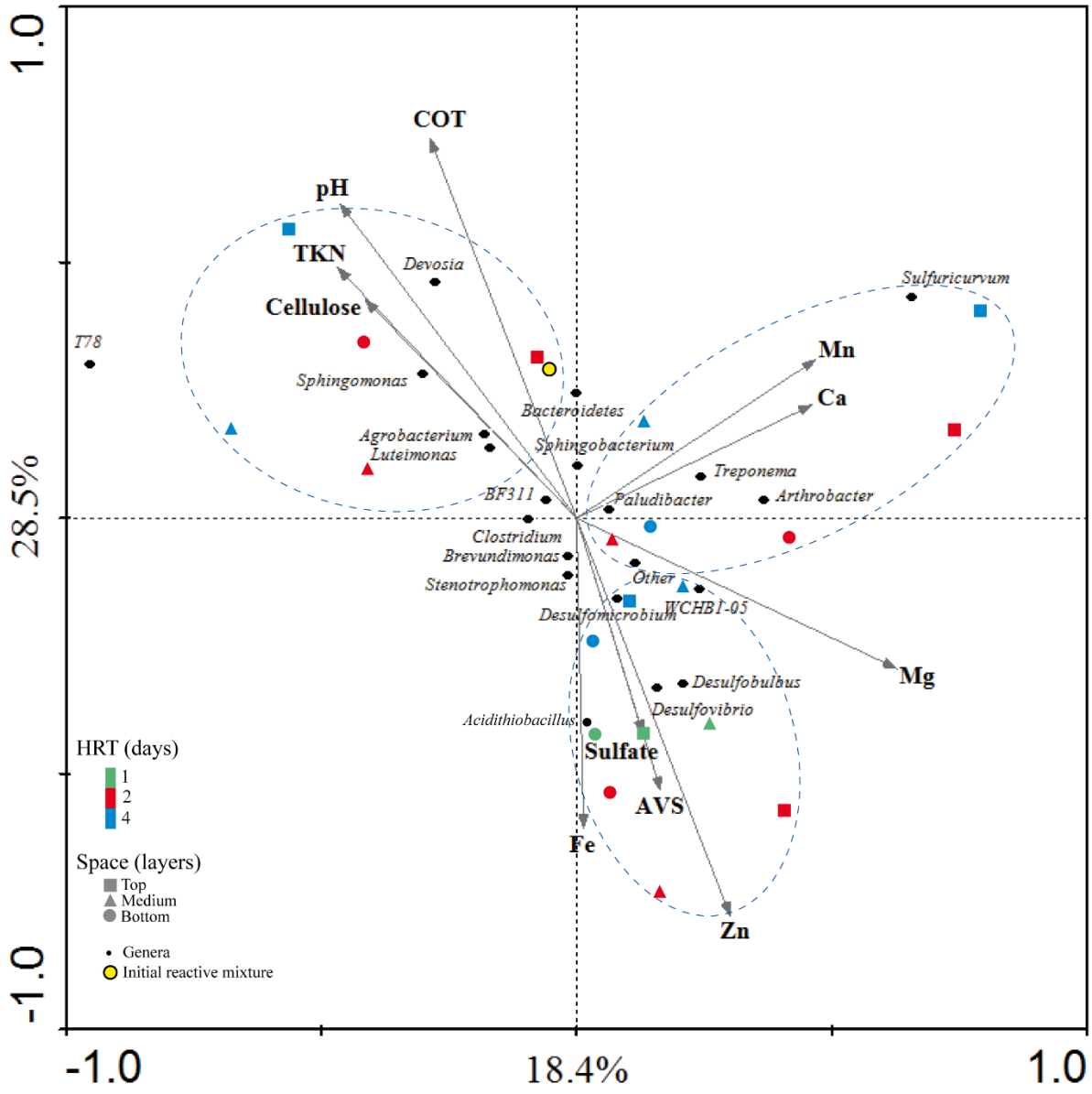

Figure 1 *Relation between the relative abundance of genera (>0.5%), physicochemical parameters and samples. CCA-triplot where the x-axis explains 18.4% and the y-axis explain 28.5% of the variation. The colors indicate samples with different HRT (1, 2 and 4 days) and its form (square, triangle and circle) show the location in BPR.*

The first group that emerge from the CCA correspond to the initial reactive mixture and 8 week samples. These samples were positively correlated (Spearman, $p < 0.05$) with pH (> 8.0) and as TKN, COT and cellulose (> 6.0, > 33.0 and > 50.7 % w/w, respectively) without difference significate between layers in the BPR. This group included the genera *Devosia* (4.6%), *Sphingobacterium* (4.2%), *Sphingomonas* (4.1%), *Agrobacterium* (3.8%), *Luteimonas* (2.0%) and *Bacteroides* (1.3 %). Members of these genera have been studied for their ability to degrade plant cell wall material and they utilize a wide variety of compounds as carbon and energy sources, including cellulose, hemicellulose, starch, and pectin. These genera have been reported in previous studies and its presence in the reactive mixture is considered an advantage during AMD treatment (Drennan et al 2015; Hiibel et al 2011). Other genera with low relative abundance (< 1.0%) were *Treponema*, *clostridium*, *Desulfovibrio*, *Desulfomicrobium* and *Desulfobacter*. The presence of SRB in the initial reactive mixture shortened the initial lag phase of the AMD treatment and contributes with low cost and improve the performance of BPR (Mirjafari et al*,* 2014).

Other group clustered in CCA correspond to samples extracted during the second sacrifice (17 week). In this group the genus more abundant was *Treponema* (10.8%) with significant abundance in medium layer for 4-day of HRT (t -test Welch's < 0.01). This genus has been observed to perform acetogenesis, carbon fixation and it is often associated with cellulose degradation (Do et al 2014; Sanchez-Andrea et al 2014). The second genus with the high abundance was *Sulfuricurvum* (6.4%, on average) with significant abundance in the top layer of columns with 2-day of HRT. These sulfur-oxidizing bacteria produces adverse effects in BPR because can oxidize the sulfide to sulfate (Zheng et al, 2014). Its presence possibility was due to change of pipeline in sampling ports located at the top cap of biorreactor allowing the formation of microaerobic regions. Other genera which also increased their abundance were *Desulfovibrio* (1.5%) and *Desulfomicrobium* (1.1%) with significant difference in BPR for 4-day of HRT. This increased of BSR favored that the concentration of sulfides (2,826 ± 185 mg H_2S L^1) was higher in this BPR because longer residence time allowed greater oxidation of available organic carbon and reduction of sulfate. In the week 17, the concentration of Ca (46 mg kg^{-1}) and Mn (< 1.0 mg kg^{-1}) in reactive mixture post treatment were the physicochemical parameter with higher effect on microbial community (0.406 and 0.502; $p < 0.05$, respectively). The effect of Ca could be related with the loss of nutrients in solution for formation of colloidal suspensions which precipitate making difficult the access for microorganism (Lindsay et al 2011). On the other hand, during 17 week the Mn presented low concentration in the reactive mixture post treatment and high concentration in the effluents (77 ± 4 mg L^{-1} for 4-day HRT and 60 ± 2 mg L^{-1} for 2-day HRT) which exceeded levels in the synthetic AMD (31 mg L^{-1}). This metal only was removed of the AMD at the beginning of the treatment when it was probably adsorbed on the reactive mixture but after it released causing toxicity on microbial community.

In the week 36, three bioreactors with different HRT (1, 2 and 4 d), were sacrificed. The genera most abundance in the columns with 2 and 4-day of HRT were *Treponema* (8.2% on average), and *Paludibacter* (3.2% on average). Previous studies demonstrated that *Paludibacter*, as fermentative bacteria, had appeared in enrichments of sulfate reduction systems in acidic condition (Zheng et al, 2014; Sánchez-Andrea et al, 2014). The *Paludibacter* was often accompanied by the SRB in sulfate reduction systems (Lindsay et al 2011). The presence of acid lactic producing bacteria genera as *Treponema* and *Paludibacter* suggests a potential for suitable SRB electron donor production in the system (Dennan et al 20165). In the column with 1-day of HRT, *Acidithiobacillus* (10.5% on average) was the genus with the most abundance and its presence was related with decline in pH (5.2), in the bottom layer of the reactive mixture. Members of these genera are frequently found in metal-rich acidic environments associated with metal sulfide leaching (Garcia-Moyano et al 2008). Acidophilic chemolithotrophic microorganisms play a key role maintaining a high concentration of ferric iron in AMD (Sánchez-Andrea et al 2014). In the week 36, the genera of SRB increased in the three bioreactors with significate difference in 4-day of HRT, with *Desulfovibrio* (1.5%) as most abundance follow by *Desulfomicrobium* (0.7%), *Desulfobulbus* (0.6%), and *Syntrophobacter* (0.5%). The CCA analysis showed correlation (Monte Carlo, $p < 0.05$) between sulfide, Fe, AVS and the genera *Desulfococcus*, *Desulfobulbus, Desulfomona*, *Desulfobacter* and *Desulfovibrio.* This genera of BSR were most abundance in bottom layer of BPR and its presence has been reported in rich environments with metal sulfides (Hao *et al*., 2014). Besides, this BSR have been found in BPR with low pH (< 5.0) and sediments from acid sites (Sánchez-Andrea et al 2014).

Conclusions

The findings in the present study provides critical information regarding dynamic of the microbial community present in BPR during the treatment of AMD. The operation time (8, 17 and 36 weeks), the space (top, medium and bottom) and the HRT (1, 2 and 4-day) had significant effects on physicochemical changes of the reactive mixture of BPR and these changes affected the diversity and the abundance relative (> 0.5%) of microbial community during AMD remediation. In addition, the microbial community analysis resulted in the identification of specialized groups related to cellulose degraders and fermentative bacteria that work in synergy for degrading substrate make the organic material available to sulfate-reducing bacteria. Finally, the microorganisms associated with metal-rich waters were identified with roles in the iron and sulfur cycles of AMD communities. This analysis provides a base line for future studies in field BPR.

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