Microbial Communities Associated with Passive Treatment of Sulphate and Selenium Containing Water

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Abstract

Passive treatment is a method that is used in mining areas for treatment of mine drainage containing metals and sulphate. This method is based on the activity of sulphate reducing bacteria (SRB) respiring on sulphate while using fermentation products from decomposing complex organic materials. The product of this biological process is hydrogen sulphide, which reacts with metals in mine drainage and precipitates them as metal sulphides. The break down and decomposing of the complex organic materials occurs as a result of a synergy among various bacteria such as fermentative bacteria or cellulose degrading bacteria. There are not many studies on the characterization of the bacterial communities in passive treatment systems. Identifying the bacterial community and their changes with time will help us to understand the overall treatment mechanism. In this study, two bioreactors were set up with two different mixtures of hay, woodchips and cow manure. The bioreactors were inoculated with inocula that were prepared with mixtures of SRB cultures and cellulose-degrading bacteria (CDB) cultures. Each bioreactor was sacrificed after a certain time. DNA was extracted from the bioreactor contents and prepared by polymerase chain reactions (PCR) for pyro-sequencing. The paper presents and discusses the bacterial communities inferred from the sequences.

Key Words: mine drainage, organic carbon, bacteria, identification

Introduction

Mount Polley mine is a copper/gold mine located near the town of Likely in British Columbia. The water in the mine's tailing storage facility contains approximately 500 mg/L sulphate and 38 µg/L selenium. The BC Ministry of Environment (BC MOE) regulated concentrations are 100 mg/L (potential) and 2 µg/L for sulphate and selenium respectively. Therefore, Mount Polley mine needs to treat the tailing dam's water to meet the BC MOE's requirements if it is to discharge this water to the environment. One method of treatment for metal mine wastewater is passive treatment, and anaerobic subsurface flow bioreactors are by far the most frequent passive systems that have been in use (Calabrese *et al.* 2005). The objective of the project described in this paper was to develop a laboratory-based anaerobic passive treatment system for Mount Polley Mine tailings pond water that would be effective at removing sulphate and selenium.

To achieve this objective, tests were done with continuous-flow columns to define the start-up and operating conditions for successful performance of an anaerobic passive system for removing sulphate and selenium from simulated Mount Polley mine tailing pond water (Mirjafari *et al.* 2011). These treatment systems are based on the ability of sulphate-reducing bacteria (SRB) to reduce sulphate and generate hydrogen sulphide:

$$2\,CH_2O + SO_4^{2-} \longrightarrow 2HCO_3^- + H_2S \qquad (1)$$

where, CH_2O represents an organic carbon source that is required as an electron donor. Selenium is below sulphate on the periodic table and forms species (selenate and selenite) similar to sulphate and sulphite, thus some SRB are able to reduce these selenium compounds as well. Also there are bacteria that specifically respire on selenium (Fujita *et al.* 2002; Siddique *et al.* 2007). If other metals are present in the mine water, sulphide produced by SRB can react with these metals to form insoluble metal sulphide precipitates:

$$M^{2+} + H_2S \longrightarrow MS \qquad (2)$$

where, *M* is a cationic metal such as Fe, Mn, Cd or Zn .

Although the best organic carbon sources for SRB are low molecular weight compounds with simple structures such as ethanol, lactate and glucose (Postgate 1984), it is preferred to use complex organic materials that are available nearby, sometimes requiring only transportation costs to the mine site. Several studies have tested various materials such as wood chips, compost, leaf mulch and pulp and paper biosolids in an attempt to find the most suitable organic sources. They all concluded that a mixture of organic materials is more effective than using one particular type (Neculita *et al.* 2007; Zagury *et al.* 2006; Cocos *et al*. 2002). Sulphate-reducing bacteria cannot use complex organic compounds directly, and are reliant on hydrolytic and fermentative organisms to produce suitable electron donors. Figure 1 shows the relationship among various microorganisms in a passive treatment system. As this figure demonstrates, in the first step, the recalcitrant part of the complex organic material (lignin and cellulose) is broken down to their subunits by primary fermentative microorganisms. The secondary fermentative microorganisms convert the monomers as well as the water-soluble portion of the organic mixture into compounds such as organic acids, alcohols, hydrogen and CO_2. These products are used in sulphur, carbon, nitrogen and iron cycling mainly by SRB, methanogens, acetogenes, nitrifying bacteria and iron-oxidizing bacteria (Madigan *et al.* 2006).

One of the problems with passive treatment processes is that they are not always reliable and this makes it difficult for mining companies, regulators and the public to trust that the mine water will be treated effectively at all times. Because treatment mechanisms include many interacting biological and geochemical reactions, typical of a naturally based system, these passive processes are notoriously difficult to diagnose when they are not working properly. Since microbes are involved, methods are needed to define the microbial communities of both successfully operating and ineffective passive treatment systems to see if the presence or absence of specific groups of organisms can help to diagnose the causes of treatment difficulties. Thus, the objective of this study was to describe the microbes present

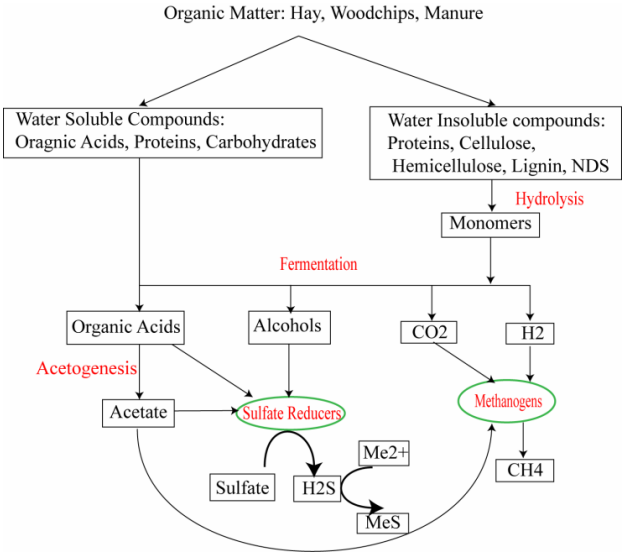

Figure 1. Microbial degradation pathway in a passive treatment system (adapted from Madigan *et al.* 2006)

in two laboratory-based treatment systems, one of which was successfully reducing sulphate and one that was less efficient at sulphate reduction.

Materials and Methods

Continuous flow column experiments, inoculation, start-up and operation

Two plexi-glass columns (I.D: 11.43 cm, length: 50.8 cm) were filled with sand, organics and gravel as shown in Figure 2. A previous survey found that wood chips, grass hay and cow manure were easily available to Mount Polley Mine. Therefore these three materials were used as the organics in the column bioreactors. The wood chips contained 50% fir, 30% balsam, 20 % pine and spruce and the cow manure was mixed with cow bedding material (saw dust and wood chips). Two mixtures of these organics were used: Mix1 contained 40%$_{dw}$ woodchips, 30%$_{dw}$ hay and 30%$_{dw}$ cow manure and Mix2 contained 20%$_{dw}$ woodchips, 50%$_{dw}$ hay and 30%$_{dw}$ cow manure.(%$_{dw}$ denotes weight percent based on dry weight). Mix1 was used in column 1 and Mix2 in column 2.

The details about the set-up, inoculation, start-up and operation of the columns can be found in Mirjafari *et al.* (2011). Table 1 summarizes the operational phases of the columns and the chemical composition of the feed can be found in Table 2.

During the operation of the columns, samples were taken from various side ports. These samples were tested for pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), conductivity, sulphate and sulphide, dissolved organic carbon (DOC), proteins and carbohydrates.

Sacrificing the columns

Since this was a proof-of-concept stage, both columns experienced different start-up procedures that included modifications to improve performance. Column 1 was the first column to be run and its performance was not optimal. Improvements of the start-up procedure lead to better performance of Column 2. Both columns were sacrificed so that the solid contents could be analyzed for their chemical composition as well as the bacterial communities present.

Columns were sacrificed either in the successful operation phase (sulphate concentration leaving the column < 100 mg/L) or the decline phase (sulphate concentration leaving the column >100 mg/L). The

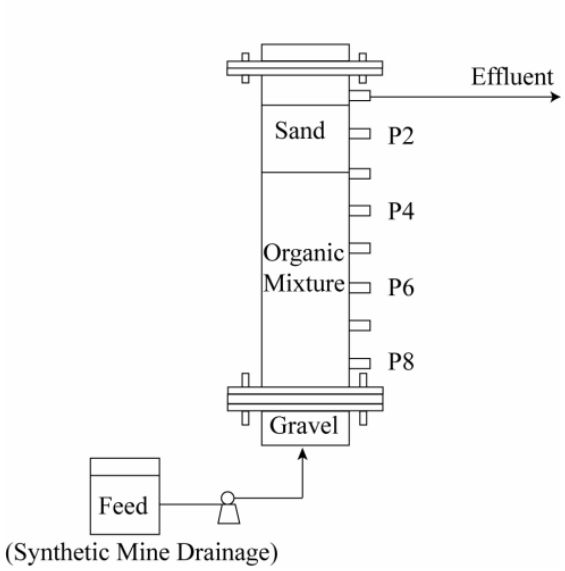

Figure 2. Schematic of a column used to study passive treatment of mine drainage

Table 1- Operational phases of Column 1 and Column 2

Column							
Column 1	**Mode**	Batch	Continuous Flow	Continuous Flow	Batch	Continuous Flow	Continuous Flow
	Duration	26 Days	50 Days	6 Days	7 Days	42 Days	151 Days
	Feed	SMD1	SMD1	Postgate B	Postgate B	SMD1	SMD1
	HRT		6 Days	6 Days		18 Days	36 Days
Column 2	**Mode**	Batch	Continuous Flow	Continuous Flow	Continuous Flow		
	Duration	26 Days	53 Days	48 Days	105 Days		
	Feed	Postgate B	Postgate B	SMD2	SMD3		
	HRT		40 Days	25 Days	25 Days		

Table 2- Composition of influent feed

Composition (g/L)	**SMD1**	**SMD2**	**SMD3**	**Postgate B**
SO_4^{2-}	0.5	0.5	0.6	1.650
$CaSO_4.2H_2O$	-	0.483	0.483	1.26
Na_2SO_4	0.739	0.253	0.253	-
$MgSO_4$	-	0.094	0.094	0.977
$FeSO_4.7H_2O$	-		0.25	0.5
KH_2PO_4	-	0.0475	0.0475	0.5
NH_4Cl	-	0.026	0.026	1
Sodium	-	-	-	3.5
Selenium	0.015	0.015	0.015	-

pH	7.5-8	7.5-8	7.5-8	7.5-8-

reason was to have a snapshot of the bacterial community in both operational phases. Column 1 was sacrificed in the decline phase, while Column 2 was sacrificed in the successful phase.

At the time of sacrificing, the flow to the columns was disconnected. Liquid samples were taken from various ports and were analyzed for pH, oxidation reduction potential (ORP), dissolved oxygen (DO) and conductivity. Then the liquid between every port or every other port was drained. The liquid was filtered with 0.45 µm filter paper. The filtered liquid was analyzed for sulphate, sulphide, carbohydrates, dissolved organic carbon (DOC) and proteins, while the filter paper was cut into small pieces and transferred to a sterile bag. The solid contents between the same ports were scooped out of the column with a clean and sterile scoop. These solids were transferred to the anaerobic hood in a clean and sterile container. They were mixed thoroughly. Some of the solids were transferred to a zip-lock bag for the chemical analysis. The other portion of the solids was transferred to a sterile bag and was stored in -80°C freezer. These solids were used for DNA extraction and genomic studies.

Analytical Methods

Sulphate was measured with the turbidimetric barium sulphate American Water Work Association method 4500-SO_4^{2-} (Eaton *et al*. 2005). Sulphide was measured with the methylene blue American Water Work Association 4500-S^{2-} method (Eaton *et al.* 2005). Dissolved organic carbon was measured using the Shimadzu Total Organic Carbon analyzer (Total Organic Carbon (TOC)-V_{cpH}). pH, ORP and conductivity were measured with a pH/Cond 7200 pH meter (WTW, Weilham, Germany). Dissolved oxygen (DO) was measured with a Symphony SP50D DO meter (VWR).

To study the bacterial community in the columns, samples were prepared for pyro-sequencing, which is a next generation sequencing technology (Metzker 2010; MacLean *et al.* 2009). To prepare samples, first the genomic deoxyribonucleic acids (DNA) of the solid samples were extracted using a MoBio Power Soil Isolation Kit (MO BIO Laboratories Incorporation, Carlsbad, CA, USA). Isolated DNA was subjected to polymerase chain reaction (PCR) amplification of 16S ribosomal DNA (16 s rDNA) genes. 12.5 µL of 2xPCR Master Mix (Fermentas Canada Incorporation, Burlington, ON), 10.5 µL of nuclease-free water (Fermentas), 1 µL of genomic DNA (2 ng), and 0.5 µL of FLX Titanium amplicon primers 454T- RA and 454T-FB (20 pmol/ µL) for a 25 µL PCR reaction was used. The primer sequences were 926f (aaactYaaaKgaattgacgg) and 1392r (acgggcggtgtgtRc) which amplify 16s ribosomal ribonucleic acid RNA(16s rRNA). Primer 454T-RA had a 25 nt A-adaptor (CGTATCGC- CTCCCTCGCGCCATCAG), whereas primer 454T-FB had a 25 nt B-adaptor sequence (CTATGCGCCTTGCCAGCCCGCT- CAG). Each sample contained a specific barcode, which consisted of 10 nucleotides in a row. PCR conditions were 95 °C for 3 min; 25 cycles of 95 °C; 30 s, 56 °C; 45 s, 72 °C; 90 s, 72 °C; 10 min; final hold at 4 °C. The PCR products were verified on a 1% agarose gel. PCR products (typically 30 µL of 50ng/µL) were sent to the Genome Quebec and McGill University Innovation Centre (Montreal, Canada) for 454 pyrotag sequencing.

Results and Discussion

Microbial community in the columns

For processing and analyzing the pyrotag sequences, scripts included in the Qiime package of commonly used bioinformatic programs was used (Caporaso *et al.* 2010). The pyrotag sequences were sorted into so-called operational taxonomic units (OTUs) based on their similarity. An OTU is what we think of as a species. In other words, all sequences that were more than 97% similar to each other would be assumed to come from the same species. The 97% cutoff is the currently accepted cutoff for the amount of sequence variation we expect within what we call a species. However, sequence variation within and between species is an active area of research.

Each sample from the columns produced between 6,585 and 8,622 sequences. In genomics parlance each one of these sequences is called a read. Combining all reads from all the samples and processing them through the bioinformatic pipeline Qiime produced a very large number of OTUs, i.e. 3,741. This means that there are this many species in the two columns, putatively. Thus, we can see that a very diverse microbial community exists in these systems, which is not surprising for an organic rich environment and similar studies of anaerobic digesters have revealed highly diverse communities as well. We assumed that those microbes that are most active in the columns would be present in larger amounts and therefore we would obtain more reads for them. Based on this assumption, we filtered out the OTUs represented by less than 200 reads, which reduced the number of predominant OTUs to 46. Figure 2 indicates how many reads of each of these OTUs were found in each of the samples and how the samples and OTUs, respectively, clustered together. In this Figure A01 to A04 represent samples from Column 1, port 8, port 6 through 8 (P68), port 4 through 6 (P46) and port 2 through 4 (P24) respectively. B01 to B05 represent samples from Column 2, P8, P78, P67, P56 and P45. The intensity of the green colour is proportional to the log to the base 2 of the number of reads.

The results indicate that at least six methanogen species were present in most samples. These microbes produce methane from acetate or H_2 and CO_2. Of the bacteria, some sequences were so novel that they could not be assigned to any phylum. Most of the bacteria species were from genera of microbes known to be involved in decomposition of complex organic matter, namely *Bacteroides*, *Parabacteroides*(Parabact), *Porphyromonas*(Porphyr), *Proteiniphilum*(Protein), *Fibrobacter*(F.Bacter), *Acetivibrio* (Acetiv) and

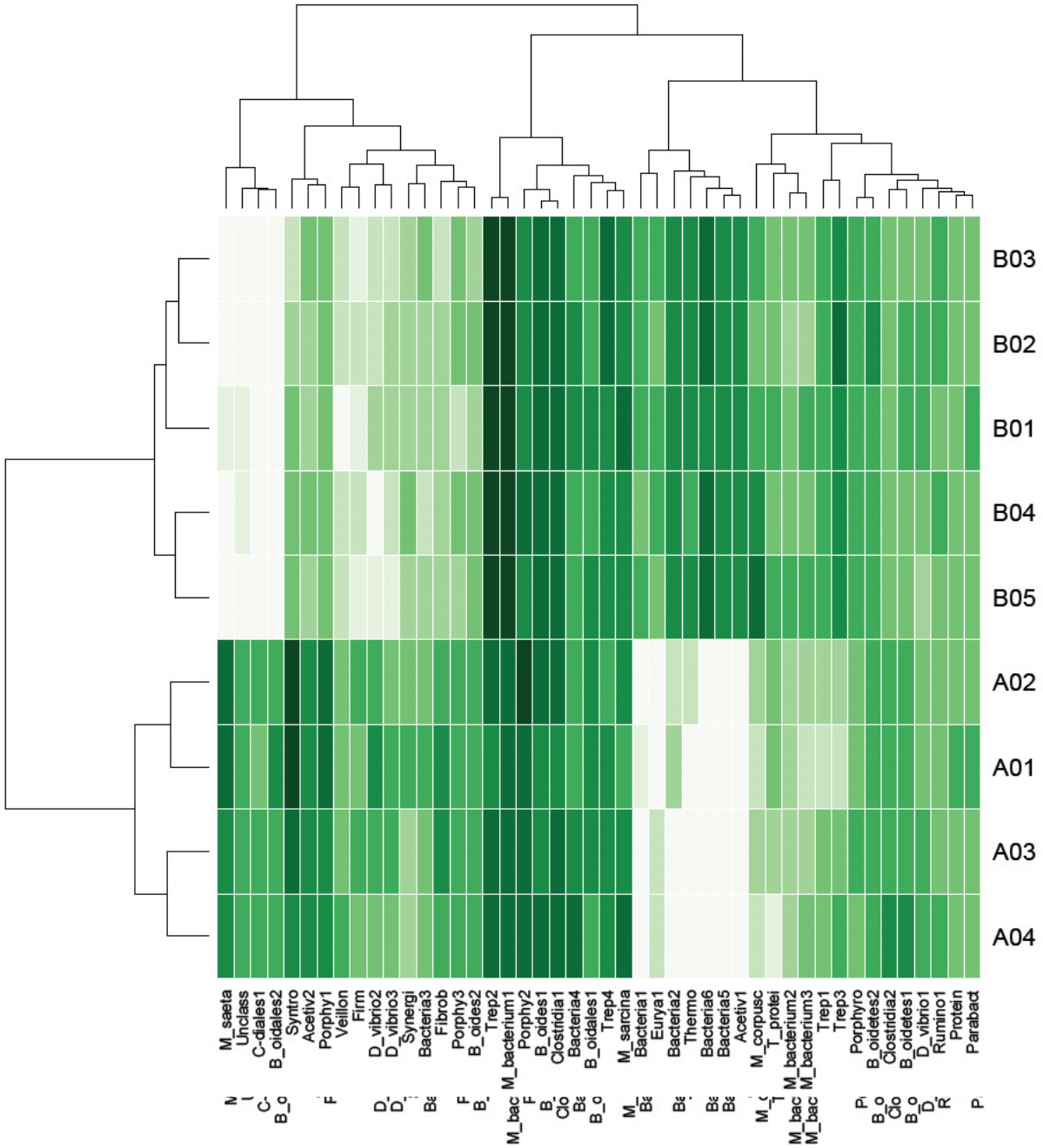

Figure 2. Heatmap of OTUs present in Column 1 and Column 2. The intensity of the green colour is proportional to the log to the base 2 of the number of total reads in that sample for the particular OTU.

Treponema. Three species from the sulphate-reducing genus of *Desulfovibrio* (DS.Vibrio) were found. Sulphate-reducing bacteria of this genus can use many different carbon sources and produce acetate as an end product. One bacteria species was related to the *Thermotogaceae* family, members of which are known to produce hydrogen.

Now that the predominant OTUs or species in the columns have been identified we can compare how the microbial community composition changes with position in the column, and compare the two columns with each other. Figure 2 shows that a *Treponema* and a *Methanobacterium* (M.Bacterium) OTU are clustered together and dominated most samples, but especially those in Column 2. Following these, another cluster of two OTUs, one from the *Synthrophoaceae* (Syntro) family and the other from the *Porphyromonadaceae* (Porphyr) family also were highly represented in Column 1, but not so much so in Column 2. Next OTUs present in high numbers are from *Clostridia* and *Bacteroides* families. They are both present in high numbers in Column 2. The *Clostridia* family related OTUs are also highly present in P24, P46 and P68 of Column 1, whereas the *Bacteroides* are high in ports P24 and P68 of Column 1. A high number of one *Acetovibrio* (Acetiv) species and OTUs from *Thermotogaceae* (Thermo) family and *Euryarchaeota* (Eurya) Phylum exist in Column 2 but they either are not presented or are present in very small numbers in Column 1. Two OTUs of *Desulfovibrio* along with two species from the *Firmicutes* phyla (*Veilon* and *Firm*) are highly represented in Column1 but are observed in very low numbers in Column 2. The substantial difference in the numbers of some of the species between the two columns can be attributed to the composition of organic mixture in the columns. Column 1 contained more wood and Column 2 more hay in their organic mixture. According to Schmidtova and Baldwin (2010), composition of the organic carbon source can affect the distribution of some of the microorganisms such as sulphate-reducing bacteria. In addition, some of these microorganisms are clustered together and occur at the same time in one sample or column. One explanation is that these species are dependent on one another for carbon and energy.

At the time of sacrificing the columns, liquid samples were taken from various ports and were analyzed for chemistry parameters such as pH, DO, ORP, conductivity, sulphate and sulphide. Figures 3 and 4 indicate the chemistry of pore water at different ports of the two columns.

To further compare the column sample microbial communities with respect to each other and to see if any of the environmental parameters measured can explain why their microbial communities are so different, a statistical method called detrended correspondence analysis was performed using the R-vegan package. This statistical method found that ORP and DO were the two measured chemical parameters that significantly affected the difference between the two column microbial communities ($p < 0.05$). The results are presented in Figures 5 and 6. Figure 5 compares the two columns regarding the ORP and DO values. As it is seen ORP and DO were both higher in Column 1. The ORP was above -280 mV in Column 1, whereas it was below -330 mV in Column 2. DO in Column 1 was between 1.2 mg/L and 1.6 mg/L but in column 2 it was less than 0.5 mg/L.

Figure 6 correlates the microbial species with the chemistry of the columns. As it can be seen in this Figure, *Methanobacterium*, *Treponema* and *Acetivibrio* related species were present in areas with high pH and conductivity and low ORP (<-340 mV) and DO (<0.3 mg/L). In contrast *Clostridia* were found at lower pH and conductivity. *Desulfovibrio* species were present in regions with ORP lower than -280 mV and DO less than 1.2 mg/L.

Figure 7 illustrates the change in sulphate concentration over time and across the columns during the columns' operation. Column 2 had a more successful performance regarding removal of sulphate to concentrations below 100 mg/L, especially in the beginning. The successful operation in this column lasted for 150 days, right before the column was sacrificed. But in Column 1 after 100 days, the sulphate concentration started to increase towards the top of the column. Figures 8a and 8b show the dominant bacterial species in these two columns at various ports. As these two figures show, *Treponema* and *Methanobacterium*, were the predominant groups in both columns with *Treponema* having the highest numbers. *Bacteroides*, *Acetovibrio* and *Desulfovibrio* related groups were the next dominant species respectively. In both columns, *Treponema* increased from Port 8 to Port 6 and then they decreased towards the top of the column, with the highest number of *Treponema* related sequenced detected midway through

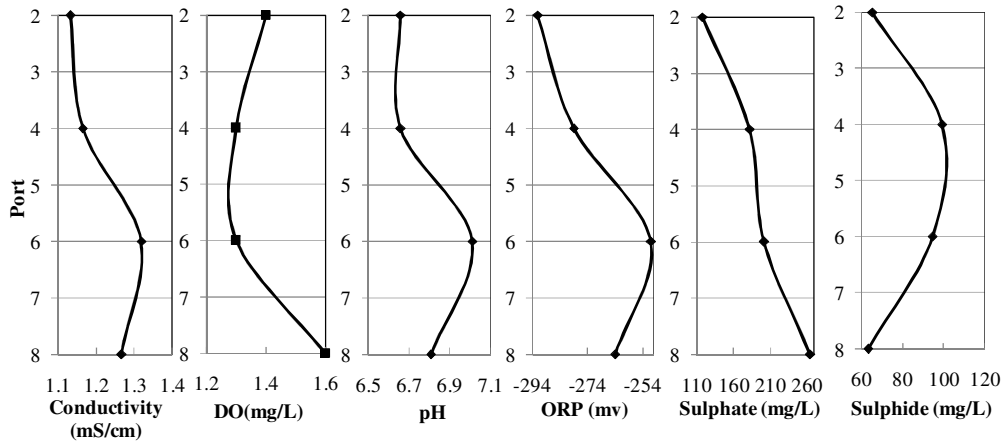

Figure 3. Chemistry of pore water in Column 1 at the time of sacrificing

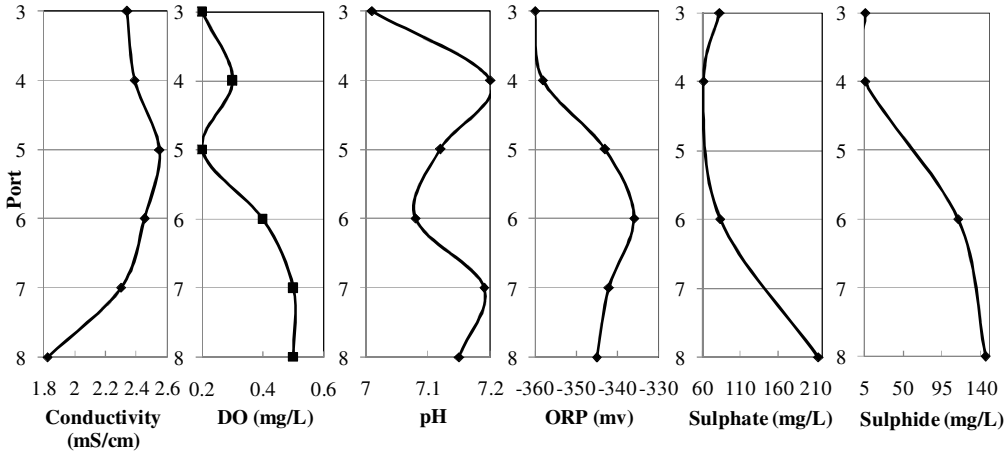

Figure 4. Chemistry of pore water in Column 2 at the time of sacrificing

the columns. This suggests that fermentation activity was higher in the middle of the columns. *Acetivibrio* related OTUs are likely cellulose degrading microorganisms in the columns and they were more prevalent at the bottom of the columns. It looks like that *Bacteroides*, which may also be involved in cellulose degradation, were constant throughout the columns. *Desulfovibrio* are present in a higher number in Column 1 than Column 2.

In both columns, the number of *Desulfovibrio* related sequences diminishes towards the top of the column. Sulphate concentration in Column 1 is higher comparing to Column 2 both during the operation and at the time of sacrificing (Figures 3, 4 and 7). Also, at the time of sacrificing concentration of sulphate decreases from Port 8 to Port 2 in Column 1 and Column 2. Therefore we conclude that the number of *Desulfovibrio* changed according to the concentration of sulphate. In other words, the higher the sulphate concentration the more *Desulfovibrio* were present. The reason is that at higher sulphate concentrations they have access to more sulphate ion for more rapid growth. In contrast to *Desulfovibrio*, *Methanobacterium* were present in lower numbers in Column 1. Methanogens and SRB compete for the same electron donors (Holmer and Kristensen 1994). Higher presence of *Desulfovibrio* in Column 1 means that they consume a large part of

the available electron donor. This results in lower methanogenic activity and reduces the presence of methanogens.

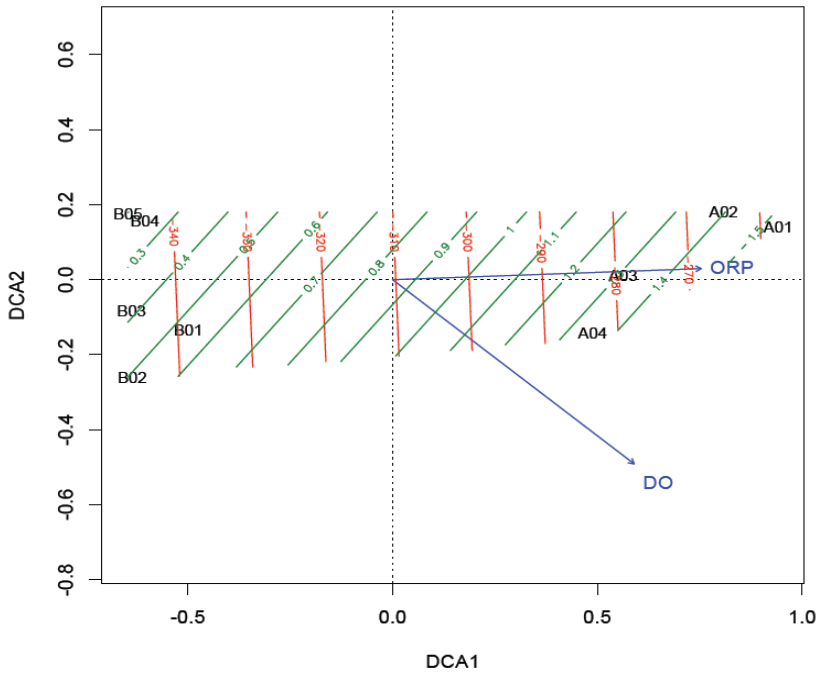

Figure 5. Results of detrended correspondence analysis, comparison of Column 1 and Column 2 regarding ORP and DO

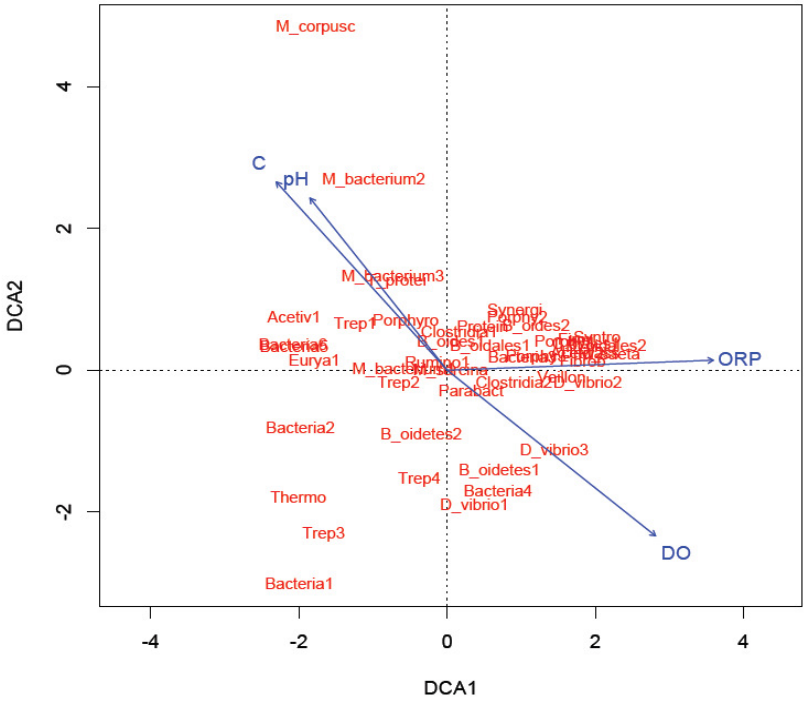

Figure 6. Results of detrended correspondence analysis, correlation of microbial species to the chemistry of the columns

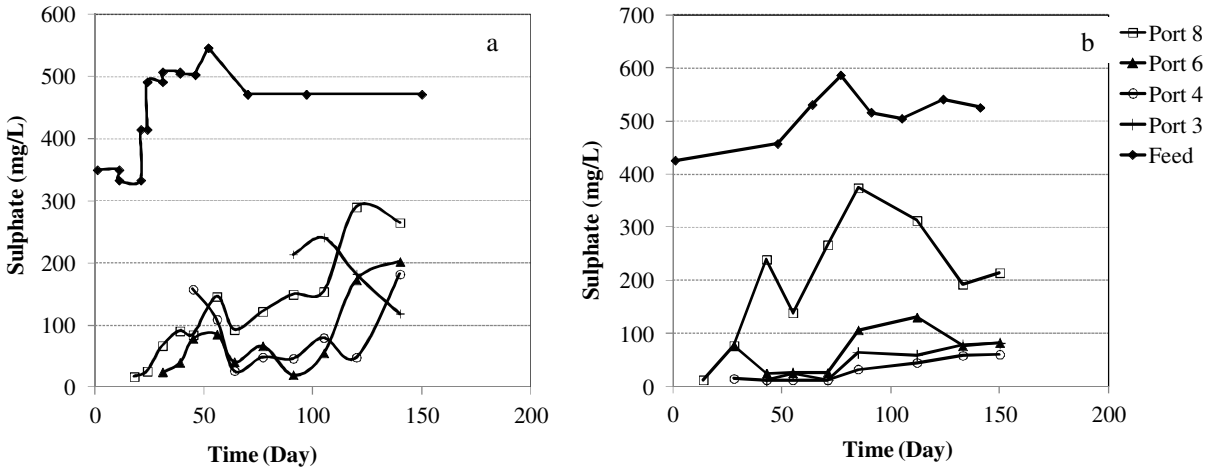

Figure 7. Sulphate concentration in a) Column 1 and b) Column 2 during the operation at different side ports

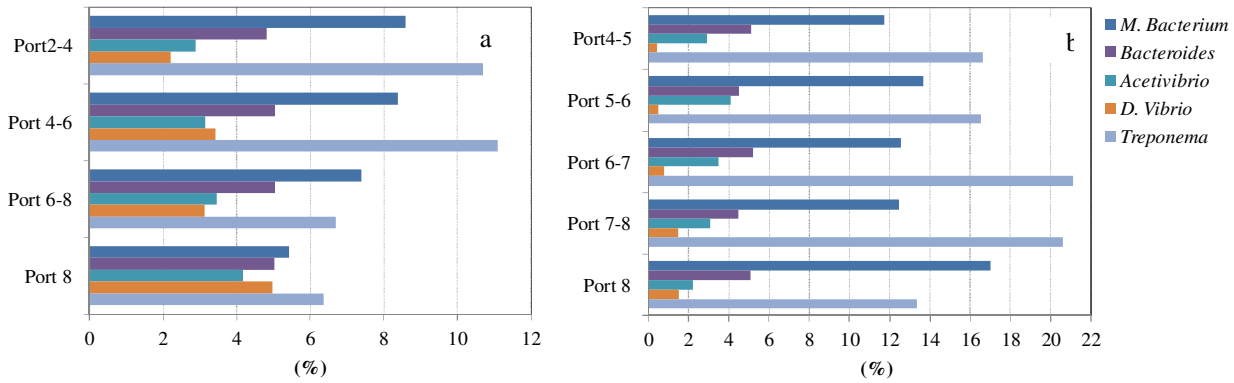

Figure 8. Dominant microbial species in a) Column 1 b) Column 2

In addition to sulphate, ORP has an important role in the change of number of microbial species in the columns. As ORP decreases in the columns the number of *Methanobacterium* increases while the *number* of *Acetovibrio* decreases.

Conclusions

In passive treatment systems containing mixtures of complex organic materials, a consortium of microorganisms exist that work in synergy with each other to make the organic material available to sulphate-reducing bacteria and other competing microbial groups. Some groups of microorganisms survive under different environmental conditions. For example, *Methanobacterium* species grow better when the ORP is less than -340 mV as opposed to *Clostridia* that live at ORPs higher than -340 mV or *Desulfovibrio* that prefer ORPs higher than -280 mV. Dissolved oxygen is another important factor that affects microbial growth. Some species such as *Methanobacterium* and *Treponema* need an environment devoid of oxygen. On the other hand, some other bacteria such as *Syntrophomonadaceae* can tolerate some oxygen. Interestingly, SRB (e.g *Desulfovibrio*) can tolerate some oxygen (as was seen in this system where some albeit low concentrations of oxygen were measured (Figs.3 and 4)). This is an especially important point for on-site passive treatment systems, where the mine water cannot be de-aerated before

entering the treatment system. As the mine water flows into the treatment system, the aerobic bacteria and more oxygen tolerant bacteria will consume the oxygen and make it anaerobic. No selenium-removing bacteria were observed in the sequences. Selenium was measured in the effluent of Column 2 and it was removed successfully (to less than 2 µg/L). Absence of selenium-removing bacteria in this column shows that this element was likely removed by sulphate-reducing bacteria. In addition, results show that SRB and methanogens coexist in these systems and are competing for organic carbon. SRB are more active when sulphate concentration is higher.

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References

Calabrese, E.J., Kosteck, P.T., Dragun, J. (2005) Contaminated Soils, Sediments and Water: Successes and Challenges, Springer, New York, USA

Caporaso, G.J., Kuczynski, J., Stombaugh, J.,Bittinger, K.,Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez Peña, A.,Goodrich, J.K.,Gordon, J.I., Huttley, J.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R. (2010) QIIME allows analysis of high-throughput community sequencing data, Nature Methods, Vol 7, PP. 335-336

Cocos, I.A., Zagury, G.J., Clement, B., Samson, R. (2002) Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment, Water Research, Vol. 32, PP. 167-177.

Eaton, A.D. , Clesceri, L.S. , Rice, E.W. , Greenberg, A.E. (2005) Standard Methods For The Examination Of Water And Wastewater, American Public Health Association, Washington, DC, USA

Fujita, M., Ike, M., Kashiwa, M., Hashimoto, R., Soda, S. (2002) Laboratory-scale continuous reactor for soluble selenium removal using selenate-reducing bacterium, bacillus sp. Sf-1, Biotechnology and Bioengineering, Vol 80 (7), PP. 755-761

Holmer, M., Kristensen, E. (1994) Coexistence of sulfate reduction and methane production in an organic rich sediment, Marie Ecology Progress Series, Vol 107, PP. 177-184

MacLean, D., Jones, J.D.G. , Studholme, D.J. (2009) Application of "next generation" sequencing technologies to microbial genetics, Nature Reviews, Vol. 7, PP. 287-296

Metzeker, M. (2010) Sequencing technologies-the next generation, Nature Reviews, Vol. 11, PP. 31-46

Mirjafari, P.,Moger, L., Martel, R., Baldwin, S.A. (2011) Factors affecting the start-up, operation and decline of a laboratory-based passive treatment system for selenium and sulphate removal, In, proceedings of the Conference of Metallurgists, Oct 2-5, Montreal, Canada

Neculita, C.M., Zagury G.J., Bussièr, B. (2007) Passive treatment of acid mine drainage in bioreactors using sulphate-reducing bacteria: critical review and research needs, Journal of Environmental Quality, Vol. 36, PP. 1-16.

Postgate, J.R. (1984) The Sulphate-Reducing Bacteria, Cambridge University Press, Cambridge, UK.

Schmidtova, J., Baldwin, S.A. (2011) Correlation of bacterial communities supported with different organic materials with sulphate reduction in metal-rich landfill leachate, Water Research, Vol 45(3), PP. 1115-1128

Siddique, T., Arocena, J.M., Thring, R.W. (2007) Bacterial reduction of selenium in coal mine tailings pond sediment, Journal of Environmental Quality, Vol 36(3), PP. 621-627

Zagury, G.J., Kulnieks, V.I., Neculita, C.M. (2006) Characterization and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment, Chemosphere, Vol. 64, PP. 944-954.