Changes in microbial community structure in response to changing oxygen stress in column tests of denitrification and selenium reduction

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Abstract Microbial community structure in nitrate and selenium-reducing biofilm from saturated waste rock backfill was described using 16S rRNA sequencing. Microaerophilic biofilm contained sequences highly similar to nitrate-reducing, iron-oxidizing *Thiobacillus* and iron-reducing *Albidiferax,* with most sequences similar to the hydrocarbon-degrading genus *Polaromonas*. Less abundant sulfate-reducing *Desulfosporosinus* was observed with the sulfur-oxidizing genera *Sulfuritalea, especially* in suboxic columns. *Methylotenera* were common in methanol-fed columns. Abundance of Se-reducing bacteria such as *Dechloromonas*, *Anaeromyxobacter*, and *Acidovorax* increased as oxygen decreased. The Se-reducing genera were less abundant than iron, nitrate and sulfur-cycling genera, which represent more of the energy and mass cycled in mined environments.

Key words reduction, selenium, microbial, column tests

Introduction

Metal-microbe interactions, such as microbial respiration of metals for metabolic purposes or metal detoxification mechanisms, *e.g.* metal sequestration to microbial biofilms, are increasingly recognized as a valid strategy to stabilize and/or remove metals in a sustainable and cost-effective manner. Reduction of selenium (Se), for instance, capitalizes on the capability of microbes to alter selenium speciation to less mobile forms such as using selenate (SeO_4^{2-}) as the terminal electron acceptor during respiratory processes. For biological Se-reduction to proceed efficiently, however, competing electron acceptors such as oxygen (O_2) or nitrate (NO_3^-) need to be removed first.

In this study, bench-scale column laboratory experiments were conducted to evaluate the influence of O_2 on biological NO_3^- and Se-reduction by indigenous microorganisms (*i.e.* those native in groundwater and waste rock) in saturated rock fills (SRF). Columns were fed with site groundwater from a saturated waste rock backfill at a coal mine. Columns were amended with either methanol or glycerol to stimulate microbial growth. Controls without carbon amendment were also carried out to evaluate intrinsic rates of NO_3^- and Se reduction. The performance of the column reactors was compared by monitoring physicochemical parameters during operation and microbial communities were analyzed using high throughput Illumina sequencing at the end of each experiment.

Methods

Column reactors. For each oxygen stress experiment, dissolved oxygen in the influent groundwater was monitored to achieve oxic or microaerophilic and anoxic conditions by sparging with nitrogen gas. Each experiment was set up with a total of six 3.7 L column reactors packed with waste rock material, which included a duplicate of control (no carbon amendment) and methanol or glycerol amended columns. Influent groundwater was added with 1.0 mg/L Se-SeO_4 (1.81 mg/L selenate). Experiments were conducted in darkness in a temperature controlled refrigerator set at 10°C (+/- 1 ˚C and influent flow rate was 0.85 mL/min to simulate conditions relevant to the mine site.

The reactors were periodically monitored for temperature (˚C), pH, oxidation reduction potential (ORP, mV), and concentrations of dissolved oxygen (DO), total organic carbon, non-particulate organic carbon, total inorganic carbon, total Se, selenate (SeO42-), selenite (SeO32-), NO3-, nitrite (NO2-), sulfate (SO42-), sulfide (S2-) and dissolved metals. Microbial cell counts were collected frequently. Ammonia and biochemical oxygen demand were also measured for selected samples. Each experimental phase lasted upon stability of NO_3^- and Se reduction was observed, which was approximately 10–12 weeks. These results are presented by Kirk et al. this volume.

Microbial community analysis. Samples collected from each section of the column (*e.g.* influent, middle, and effluent section) at the end of each experiment were analyzed to describe the microbial community, together with a sample of the composited waste rock material used to charge the columns. Microbial DNA was extracted from 10 g of waste rock using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) with minor adjustments to manufacturer's protocol. Microbial community composition and structure were characterized by high throughput Illumina sequencing of the 16S rRNA gene. Sequence reads were processed with the MiSeq SOP pipeline of the MOTHUR software package (Kozich et al. 2013). The unique contig sequences were classified with MOTHUR formatted version of the RDP training set using the Bayesian classifier at a 0.6 confidence score. Sequences were binned into phylotypes according to their taxonomic classification (*e.g.* genus-level) and a relative abundance plot for each library was obtained.

Results

Microbial community composition and structure. The microbial communities of the initial waste rock composite and of the samples collected from column reactors at the end of the experiment were analysed with high throughput Illumina sequencing. Overall, microbial communities in the columns responded to carbon amendments as well as varied dissolved oxygen concentration in the influent groundwater in each experiment (Figure 1).

Polaromonas (22–53%) was the most abundant bacterial genus in the initial composite waste rock material and remained predominant in the column reactors (22–38%). Bacterial genera that were also detected in the initial composite waste rock material and increased in relative abundance in the columns included *Thiobacillus* and *Albidiferax*. The relative abundance of *Albidiferax* was higher in control columns (13–26%) than in carbon amended columns (7–16%) regardless of varied dissolved oxygen conditions. In oxic columns, the highest relative abundance of *Thiobacillus* was observed in the control columns (10%) compared to the carbon amended columns (1–2%). In microaerophilic and anoxic columns, however, *Thiobacillus* was present at 5–11% and 6–9%, respectively, despite differing carbon amendments (Figure 1).

Desulfosporosinus was most abundant in all of the glycerol-amended columns (16–23%). A higher relative abundance of *Desulfosporosinus* was also observed in methanol-amended columns than in control columns; however, as microaerophilic and anoxic conditions were achieved in the columns, the relative abundance of *Desulfosporosinus* decreased (*i.e.* from 14% in oxic condition to 6% and 2% in microaerophilic and anoxic conditions, respectively). A similar trend was observed for methanol amended columns, where *Methylotenera* (3–9%) was always observed, but its relative abundance also decreased as oxygen levels decreased. The opposite was observed for *Sulfuritalea. Its relative abundance increased* as columns became more anoxic (*i.e.* from 0.5–1% in oxic conditions to 2–4% and 6–7% in microaerophilic and anoxic conditions, respectively) regardless of the different carbon amendments (Figure 1). Microbial genera that were not detectable under oxic conditions, but increased in microaerophilic and anoxic conditions included *Dechloromonas (*0.3–5%), *Anaeromyxobacter (0.2–0.6%)*, and *Acidovorax (0.5–1%). These organisms are known to influence Se speciation.*

Column reactor performance in response to O_2 stress and carbon amendment. Under oxic conditions, the slowest rate of Se reduction was observed in the control columns. However, Se reduction occurred under oxic conditions with carbon amendments and was most pronounced in the methanol-amended columns. Under microaerophilic and sub-oxic conditions, however, Se reduction occurred even without carbon amendment. Nonetheless, Se reduction was most rapid and efficient in glycerol-amended columns under these conditions. Specific rate data are presented by Kirk et al., this volume.

Table 1 *Assessment of selenium reduction efficiency under different dissolved oxygen concentrations and carbon amendments.*

	Oxic	**Microaerophilic**	**Sub-oxic**
Control	-	+	+
Glycerol	+	++	++
Methanol	++	+	+

* The minus sign indicates slowest rate of selenium reduction; plus sign, increased rate of selenium reduction; double plus signs, fastest rate of selenium reduction.

Discussion

Compared to the composite sample, all of the column experiments had an increase in relative abundance of *Albidiferax* spp., whose members are associated with Mn-oxidation and whose close relatives are known iron-reducing bacteria (Akob et al. 2014; Kaden et al. 2014). The reducing conditions in the columns potentially allowed this bacterial group to thrive. *Polaromonas* spp., found in many cold environments (Margesin et al. 2012) where it is known for its capacity to degrade hydrocarbon, remained predominant members in the column experiments. In addition to *Polaromonas* spp. and *Albideferax* spp., the other major bacterial population was *Thiobacillus* spp., whose related members are capable of nitrate-dependent anoxic iron sulfide oxidation (Haaijer et al. 2006). Compared to the control and methanol-amended columns, *Desulfosporosinus* spp., a group of sulfate-reducing bacteria and major contributor in the treatment of acid mine drainage systems (Koschorreck et al. 2010; Battaglia-Brunet et al. 2012; Sánchez-Andrea et al. 2012), was especially abundant in the glycerol-amended columns. Methanol amendment selected for the growth of *Methylotenera* spp., a bacterial group capable of mainly using C1-compounds as carbon and energy sources and whose members have been attributed to carrying out denitrification in freshwater lake sediments (Kalyuhznaya et al. 2009). *Sulfuritalea* spp. became more abundant in anoxic columns than oxic and microaerophilic columns. Members of *Sulfuritalea* are facultative anaerobes capable of chemolithoautrophy under anoxic conditions by the oxidation of reduced sulfur compounds and hydrogen. They are also capable of denitrification (Kojima et al. 2014). Although they were present at much lower relative abundance than other bacterial groups, bacteria capable of Se-reduction including *Dechloromonas* spp., *Anaeromyxobacter* spp., and *Acidovorax* spp. (Williams et al. 2013; He and Yao 2011; Panke et al. 2012) were detected in microaerophilic columns and with increasing abundance in anoxic columns.

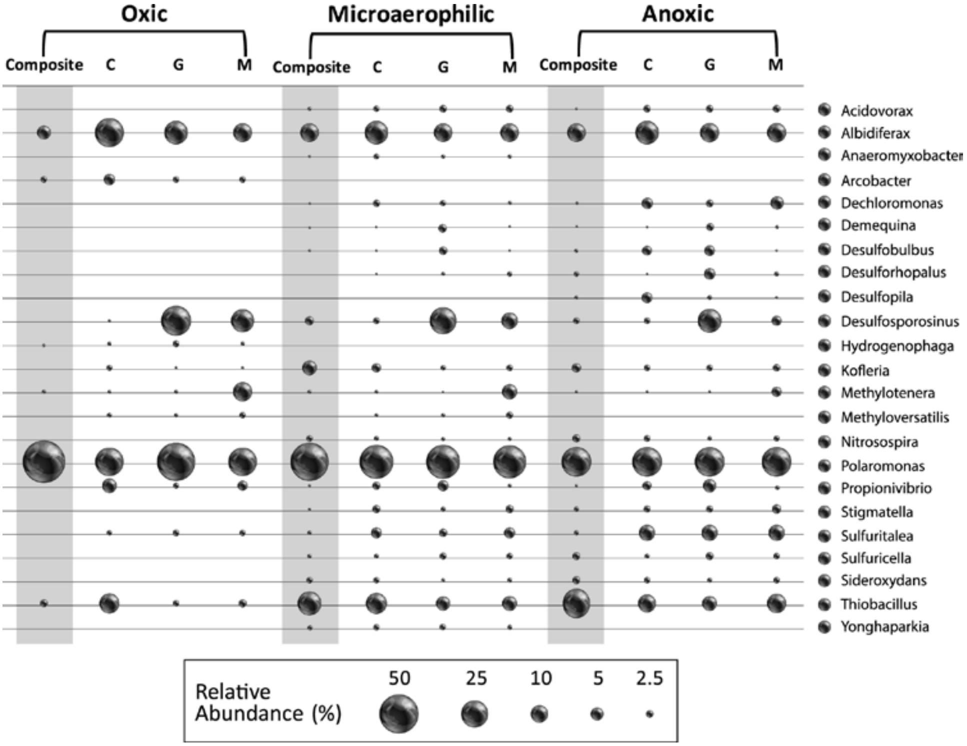

Figure 1 *Relative abundance of genera detected above 2.5% in oxic, microaerophilic, and anoxic column tests, for pre-test composite, no-carbon control (C), glycerol column (G) and methanol (M) treatments*

Conclusion

The study showed that Se attenuation can be promoted through carbon amendment in saturated rock fills. As the results indicated, methanol-amended columns performed the best under oxic condition, but glycerol-amended columns performed the best in microaerophilic and anoxic conditions. This was also a reflection of substrate requirements for growth of microbes in the deeper subsurface. Although reduction was observed in amended columns in spite of the presence of oxygen, and even without amendment under suboxic conditions, the choice of carbon substrate did influence the microbial community structure.

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