

Microbiological Comparison of Two Field-Scale Sulfate-Reducing Permeable Reactive Zones Treating Mine Drainage

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Abstract. With an estimated 30,000 abandoned mines in Colorado alone, mine drainage poses a significant problem. Mine drainage is typically high in sulfates and heavy metals, and low in pH. Biological treatment through the implementation of a sulfate-reducing permeable reactive zone (SR-PRZ) that is installed transverse to flow is an attractive treatment option because of the low cost and maintenance requirements. This study focuses on two field-scale SR-PRZs, Luttrell and Peerless Jenny King, both located in the Ten Mile Creek area near Helena, MT. A suite of molecular biological tools was used to characterize the microbial communities of these two different field bioreactors with the goal of understanding the relationships between the microbial community, operating conditions, and performance. Compost-based substrate was used to promote the growth of a complex microbial community, including sulfate-reducing bacteria (SRB), in each bioreactor. The two bioreactors were sampled at multiple locations and depths in June, 2005, and Peerless Jenny King was sampled again in August, 2005. The overall bacterial composition of the microbial community was characterized through cloning of polymerase chain reaction (PCR)-amplified 16S rRNA genes followed by restriction digest screening and DNA sequencing. The SRB populations were directly examined using two approaches: cloning of the *apsA* gene and quantitative real-time PCR (Q-PCR). The *apsA* gene is specific to SRB and was PCR-amplified, cloned, and sequenced. This method revealed that two main SRB groups are prevalent in both bioreactors. The two SRB groups are both *Desulfovibrio* spp., and one is known to be particularly aerotolerant. The analysis also uncovered the presence of *Thiobacillus denitrificans*, a bacterium with the highly undesirable ability to oxidize sulfides in the presence of nitrate or oxygen. The second approach, Q-PCR, was used to quantify two specific groups of SRB, *Desulfovibrio* and *Desulfobacter*. The population sizes of these two SRB groups were found to vary with depth in both bioreactors. The results of this study demonstrate that the two different sulfate-reducing bioreactors harbor significantly different bacterial communities. The ultimate goal of this research is to develop an improved understanding of the microbiology of SR-PRZs so that the reliability of design and operation may be improved.

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