Influence of Protozoa on Anaerobic Groundwater Bioremediation

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The importance of bacteria in the anaerobic bioremediation of groundwater polluted with organic and/or metal contaminants is well recognized and in some instances so well understood that modeling of the *in situ* metabolic activity of the relevant subsurface microorganisms in response to changes in subsurface geochemistry is feasible. However, a potentially significant impact on bacterial growth and activity in the subsurface that has not been adequately addressed is protozoan predation of the microorganisms responsible for bioremediation. In order to evaluate the potential role of protozoa, anaerobic bioremediation studies were conducted at a uranium-contaminated aquifer located in Rifle, CO in which microbial reductive precipitation of uranium was stimulated with the addition of acetate to the groundwater. As observed in previous field experiments, acetate amendments initially promoted the growth of metal-reducing *Geobacter* species followed by the growth of sulfate-reducers. Analysis of 18S rRNA gene sequences revealed a broad diversity of sequences closely related to known bacteriovorous protozoa in the groundwater prior to the addition of acetate. In 2010, 41 different protozoan species were detected in the untreated groundwater and 37 species were detected in 2011. The bloom of *Geobacter* species associated with added acetate was accompanied by a specific enrichment of protozoa most closely related to the amoeboid flagellate, *Breviata anathema*, which accounted for over 80% of the recovered protozoan sequences. The abundance of *Geobacter* species declined following the rapid emergence of *B. ananthema*. The subsequent growth of sulfate reducers in the acetate-amended groundwater was accompanied by another specific enrichment of protozoa, but with sequences most similar to the diplomonad flagellate, *Hexamita inflata*, which accounted for over 80 % of the protozoan sequences recovered during this phase of the bioremediation. These results suggest a prey-predator response during uranium bioremediation with specific protozoa responding to increased availability of preferred prey bacteria. Thus, quantifying the influence of protozoan predation on the growth, activity, and composition of the subsurface bacterial community is essential for predictive modeling of bioremediation strategies. It is expected that protozoa may play an important role in controlling the activity of microorganisms in a diversity of anaerobic soils and sediments.