Biostimulation of Iron Reduction and Uranium Immobilization: Microbial and Mineralogical Controls

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The overall objective of our project is to understand the microbial and geochemical mechanisms controlling the reduction and immobilization of U(VI) during biostimulation in acidic subsurface sediments such as those present at the Field Research Center (FRC). The focus is on the activity and community composition of microbial populations (metal- and nitrate-reducing bacteria) and iron minerals which are likely to make strong contributions to the fate of uranium during in situ bioremediation.

We conducted microcosm experiments using near in situ conditions with FRC subsurface materials co-contaminated with high levels of U(VI) and nitrate. Rates of electron acceptor/ donor utilization were measured in acidic subsurface sediments across a range of environmental variables (pH, nitrate) relevant to bioremediation. Microbial activity was minimal at pH 5 or below, indicating that acidity is a master variable controlling microbial metabolism in FRC sediments. In concurrence with previous studies of neutrophilic uranium-contaminated subsurface environments, metal reduction in the acidic subsurface did not occur until after nitrate was depleted to low levels in response to pH neutralization and carbon substrate addition. Nitrate reduction, iron reduction, and electron donor utilization rates in neutralized acidic microcosms were among the most rapid reported for aquatic sedimentary environments. Acidity influenced not only the rates but also the pathways of microbial activity. The majority of C equivalents in neutralized glucose-amended microcosms were recovered as fermentation products, mainly as acetate. Although biostimulation leads to rapid nitrate and metal reduction in acidic subsurface sediments, environmental extremes appear to have selected for microbial communities with different metabolisms, and metal reduction may be substantially catalyzed by fermentative bacteria. By targeting rRNA, we sought to provide a novel assessment of the metabolically-active microbial groups as well as their response to changing environmental conditions in microcosm sediments. Clone libraries, constructed from the 16S rRNA gene and cDNA reverse transcribed from the 16S rRNA, contained representatives from the phyla Planctomycetes, Proteobacteria (α, β, δ, γ), Bacteroides, and Firmicutes. The most abundant phylotypes found in the DNA libraries were members of the Alphaproteobacteria, while Gammaproteobacteria-related clones dominated the RNA libraries. Currently, the response of metal-reducers and other heterotrophic communities is being investigated further in sediment microcosms in which metal reduction was stimulated by carbon substrate addition and pH neutralization.

Three novel iron(III)-reducing organisms were isolated from the contaminated FRC subsurface. Stable enrichment cultures were established and isolations were performed in soft gelrite dilution series using FeOOH and AQDS as the sole electron acceptor, respectively. Using the 16S rRNA gene as a molecular marker, pure cultures shared high sequence identity (96 to 99%) to *Geobacter bremensis*, *Clostridium* sp. CITR8, and *Desulfotomaculum ruminis*, respectively. Isolates were also closely affiliated with phylotypes detected in the FRC subsurface using cultivation-independent methods.