Metabolically Active Microbial Communities in Acidic Uranium-Contaminated Subsurface Sediments

Denise M. Akob[1], Heath J. Mills[1], Lainie Petrie-Edwards[1], David L. Balkwill[2], Joel E. Kostka[1]

[1]Dept. Oceanography, [2]College of Medicine, Florida State University, Tallahassee, FL

The overall goal of this project is to determine the activity and community composition of microbial populations (such as metal- and nitrate-reducing bacteria, and other heterotrophs) that are likely to make strong contributions to the fate of uranium during in situ bioremediation. Sediments collected from the acidic (pH 3–4) and near-neutral pH (pH 6–7) zones of the DOE-NABIR Field Research Center (FRC), Oak Ridge, TN, were used in microcosm experiments and for total nucleic acid extraction. In these samples, we selectively targeted and amplified extracted DNA (16S rRNA gene sequence) to determine the composition of the in situ microbial communities and RNA (16S rRNA) to determine the metabolically active fraction of the microbial community.

We initially constructed clone libraries from amplified 16S rRNA gene products and from cDNA reverse transcribed from 16S rRNA. Clones were screened using restriction fragment length polymorphism analysis, followed by sequencing of cloned inserts. Clone libraries constructed from sediments prior to biostimulation contained representatives from the phyla Planctomycetes, Proteobacteria (α, β, δ, γ), Bacteroides, and Firmicutes. The diversity and numerical dominance of phylotypes varied between the DNA and RNA libraries. Phylotypes from the DNA clone libraries were more diverse than those found in the RNA-derived libraries. The most abundant phylotypes found in the DNA-derived libraries were members of the Alphaproteobacteria, while the Gammaproteobacteria were more frequently detected in the RNA-derived libraries.

Currently, we are examining the structure-function relationships of microbial communities in microcosms of biostimulated sediments by comparing community composition as determined by clone libraries to rates of electron donor/acceptor utilization (see companion abstract of Kostka, et al., p. 60). We plan to utilize cloning and sequencing data to develop more rapid and quantitative approaches such as tRFLP and real-time PCR. By targeting rRNA, we provide a novel assessment of the metabolically active fraction of the microbial populations that is likely to catalyze biogeochemical processes contributing to the bioremediation of uranium contamination in the acidic subsurface.