Probing for Active Metal-Reducers at the FRC Using Stable Isotope Probing, New Enrichment Strategies, and Metagenomics

James M. Tiedje[1] (PI), Jizhong Zhou[2], Terry Marsh[1], Weimin Gao[2], Mary Beth Leigh[1], Nathaniel Ostrom[1], Sarah Miller[1], Zamin Yang[2], Erik Cardenas[1] and Sang-Hoon Kim[1]

[1]Michigan State University, East Lansing, MI; and [2]Oak Ridge National Laboratory, Oak Ridge, TN

Stable isotope probing (SIP) methods are being developed to identify metabolically active bacteria that may play a role in metal and radionuclide reduction in FRC sediments. SIP methodology involves the addition of ^{13}C-labeled substrates to a mixed microbial community, extraction and density-gradient centrifugation to isolate ^{13}C-labeled nucleic acids of microorganisms that utilized the substrate, and then sequencing of 16S rRNA and rDNA to obtain phylogenetic information. Microcosms of FRC sediments and groundwater are being probed with the same substrates used for field biostimulation of metal reduction (ethanol, acetate). Optimization of SIP methodology, including RNA and DNA extraction methods, density-gradient centrifugation, and isotope-ratio mass spectrometry of nucleic acids, is underway. Highest recovery and quality of RNA and DNA were obtained by bead-beating/grinding sediments in guanidine thiocyanate denaturing solution, extraction at 65C with CTAB and SDS buffer and Sephadex gel filtration, followed by separation of RNA and DNA with Qiagen Tip 100 columns and further purification. RNA and DNA were banded isopycnically, in separate density gradients constructed with cesium trifluoroacetate, and were successfully recovered from fractions.

The community composition of three bacterial enrichment cultures from contaminated subsurface sediment (FW109) was determined with SSU rDNA clonal libraries. Microorganisms closely related to *Anaeromyxobacter dehalogenans* and an uncultured *Anaeromyxobacter* sp. predominated the iron-reducing enrichment, while microorganisms closely related to *Desulfotomaculum* sp. and *Clostridium* sp. predominated the sulfate-reducing enrichment. The nitrate-reducing enrichment consisted of species closely related to *Pseudomonas* sp. C05E and *Citrobacter amalonaticus*. Fosmid libraries have been constructed for the nitrate- and sulfate-reducing enrichments. Over 7,000 clones were obtained from nitrate-reducing enrichment and over 760 clones have been obtained for the sulfate-reducing enrichment. Additionally, fosmid libraries have been constructed from the DNA extracted from the U and acidic well FW005. We have also initiated an analysis of the colonization of surfaces by *Desulfitobacterium hafniense* under iron-reducing conditions. Using a chemostat capable of long-term operation under anaerobic conditions, we have identified several surfaces that support biofilm formation. *D. hafniense* is capable of reaching relatively high cell densities in the chemostat. Based on the genome sequence of *D. hafniense,* we have constructed a full ORF chip and have used this chip to evaluate gene expression under different electron-accepting conditions and under N_2-fixing conditions, and we will use it to study expression under biofilm conditions. Besides metal reduction, *Desulfitobacteria* dechlorinate some chlorinated co-contaminants. We found seven putative dehalogenase genes in the *D. hafniense* genome, and we are evaluating their expression and have cloned and expressed several in *E. coli. Desulfitobacteria* have several properties important to successful remediation at DOE sites.