Determination of the Metabolically Active Microbial Groups in Contaminated ORFRC Subsurface Sediments Using Stable Isotope Probing (SIP)

D. M. Akob[1], H. J. Mills[1], L. Kerkhof[2], and J. E. Kostka[1*]

[1]Florida State University, Tallahassee, FL; [2]Rutgers University, New Brunswick, NJ

Abstract

The goal of this study was to employ stable isotope probing (SIP) techniques to provide a link between the composition and function of subsurface microbial communities with a high potential for uranium bioremediation. In subsurface sediments contaminated with mixed wastes, metal- and nitrate-reducing bacteria have been shown to mediate electron flow in subsurface sediments, thereby controlling the fate and transport of radionuclides. However, at the Oak Ridge Field Research Center (ORFRC), there is no consensus on the composition and distribution of active community members across changes in relevant environmental parameters likely to effect bioremediation potential. Ethanol has been shown in laboratory and field based bioremediation studies to promote microbial activity and the biological reduction of U(VI) in ORFRC sediments. In order to further characterize the metabolically active microbial community, we amended ORFRC Area 2 sediment microcosms with ^{13}C-labeled ethanol and monitored electron acceptor (NO_3^-, Fe(III) and U(VI)) and donor utilization. At selected time points corresponding to phases of terminal electron accepting processes, ^{13}C incorporation into community DNA was examined by density gradient centrifugation along with PCR amplification and terminal restriction fragment length polymorphism analysis (TRFLP). Incorporation of the ^{13}C-labeled substrate into microbial biomass was detected by day 6 of the incubation which corresponded to the onset of nitrate reduction. Following nitrate reduction, we observed a two-day lag phase before the onset of U(VI) and Fe(III) reduction and a shift in peak area for the predominant microbial groups, including members of the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes.* At all time points, we detected a TRFLP peak corresponding to the *Dechloromonas* group of the *Betaproteobacteria* which had incorporated the ^{13}C-ethanol suggesting that this group is active during both nitrate and metal reduction phases of the incubation. These results indicate that stable isotope probing methods can be used successfully to couple the composition and function of microbial communities in ORFRC subsurface sediments. Our data also suggest that members of the *Betaproteobacteria* may play an important role in nitrate removal and subsequent metal reduction, although further work is needed to elucidate the biogeochemical processes mediated by the microbial groups that incorporated ^{13}C-labeled ethanol.