Probing for active metal-reducers at the FRC using stable isotope probing, new enrichment strategies and metagenomics

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Abstract
Stable-isotope probing (SIP) methods are being applied to identify bacteria that play a role in nitrate reduction in an ethanol-bioleached fluidized bed biolewisor for treatment of FRC groundwater, and eventually to identify bacteria important to metal reduction in FRC sediments. SIP methodology involves the addition of 13C-labeled substrates to a mixed microbial community extract, and the high-efficiency centrifugation to isolate 13C-labeled nucleic acids of microorganisms that utilized the substrate, and then sequencing of 16S rRNA and 16S rRNA for analysis. A biolewisor constructed from biomarker molecular biolewisors was previously used to biolewisr nitrate reduction in the reactor, and we monitored for 24 hours at 13C02 enrichment in the headspace and the data indicated that the substrate was utilized within several hours. Community analyses by DGGE and TES 16S rRNA sequencing indicate that the biomass that was comprised predominantly of nitrate-reducing beta-proteobacteria. Preliminary SIP analysis suggests that some 13C-enrichment of RNA may have occurred within 6 hrs, but further analyses in progress are needed to draw definitive conclusions regarding bacterial taxa that utilized ethanol.

The community composition of three bacterial enrichment cultures from contaminated subsurface sediment (FW109) was determined with SSU DNA cloning libraries. Microorganisms closely related to Anaeromicrobium dehalogenans and an uncultured Anaeromicrobium sp. species predominated the iron-reducing enrichment while microorganisms closely related to Desulfitobacterium sp. and Desulforospermum sp. predominated the nitrate-reducing enrichment. A nitrosative enrichment constructed from Pseudomonas sp. and Chlorobacterium sp. were isolated.