Nitrate-Independent Uranium Bioreduction

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Bioremediation strategies for the FRC are faced with the challenge of extreme levels of nitrate that have permeated the sediments. Field-scale bioreduction experiments at the FRC have demonstrated that nitrate concentrations recover soon after cessation of donor addition. This project investigates the possibility of stimulating nitrate-indifferent pH-tolerant organisms to achieve biologically mediated reduction of U(VI) despite nitrate persistence. Enrichments from Area 2 sediments were prepared using a variety of electron donors and MOPS/TRIS buffers at pHs ranging from 4.9 to 7. Successful enrichments from pHs 4.9-5.5 containing 10-20 mM methanol or glycerol have demonstrated ~90% reduction of uranium (~10 ppm) with less than 10% loss of nitrate (~850 ppm). Higher pH enrichments demonstrated similar U reduction potential with 5-30% nitrate loss.

Bacterial16S rRNA genes from uranium-reducing enrichments at pH 5.7-6.7 were PCR-amplified and sequenced. A majority of the clone sequences were most closely related to *Clostridia*, *Clostridia*-like organisms, and uncultured organisms related to *Anaerospora.* Other clone sequences were representative of aerobic Gram-negative Haemophilus and Aeromonas, along with members of the phylum Bacteroidetes. TRFLP analysis of bacterial 16S rRNA genes indicated that the two most dominant groups of organisms present in these enrichments were those most closely related to *Anaerosinus glycerini* and *Desulfotomaculum guttoideum*, strictly anaerobic members of the phylum Firmicutes. Aerobic Proteobacteria and metabolically versatile Bacteroidetes only comprised a very small portion of the bacterial community. There was a great deal of diversity found among the genera of *Clostridia*, and lower pH enrichments with methanol as the electron donor tended to yield overall lower diversity.

Additional experiments tested the stability of reduced uranium. Tubes were prepared with ~2 ppm U(IV) or 0.9 in tap, degassed, deoxygenated, and deoxygenated water plus 1000 ppm nitrate or 100 ppm nitrite solutions. Nitrite did induce oxidation of Fe(II) in similarly prepared tubes containing 50 ppm Fe(II); however, none of the treatments induced >0.1 ppm U oxidation after more than 200 days.