Biostimulation of Iron Reduction and Uranium Immobilization: Microbial and Mineralogical Controls

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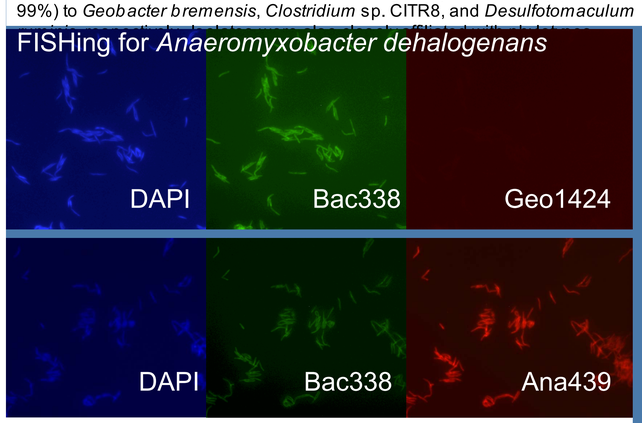
Abstract

The overall objective of our project is to understand the microbial and geochemical mechanisms controlling the reduction and immobilization of U(VI) during biostimulation in acidic subsurface sediments such as those present at the Field Research Center (FRC). The focus is on the activity and community composition of microbial populations (metal- and nitrate-reducing bacteria) and iron minerals which are likely to make strong contributions to the fate of uranium during *in situ* bioremediation.

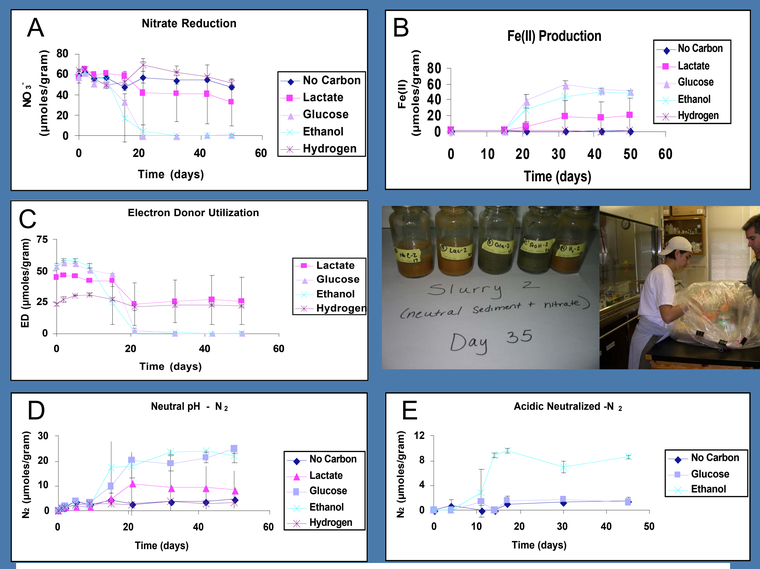
We conducted microcosm experiments using near *in situ* conditions with FRC subsurface materials cocontaminated with high levels of U(VI) and nitrate. Rates of electron acceptor/ donor utilization were measured in acidic subsurface sediments across a range of environmental variables (pH, nitrate) relevant to bioremediation. Microbial activity was minimal at pH 5 or below, indicating that acidity is a master variable controlling microbial metabolism in FRC sediments. In concurrence with previous studies of neutrophilic uranium-contaminated subsurface environments, metal reduction in the acidic subsurface did not occur until after nitrate was depleted to low levels in response to pH neutralization and carbon substrate addition. Nitrate reduction, iron reduction, and electron donor utilization rates in neutralized acidic microcosms were among the most rapid reported for aquatic sedimentary environments. Acidity influenced not only the rates but also the pathways of microbial activity. The majority of C equivalents in neutralized glucose-amended microcosms were recovered as fermentation products, mainly as acetate. Though biostimulation leads to rapid nitrate and metal reduction in acidic subsurface sediments, environmental extremes appear to have selected for microbial communities with different metabolisms, and metal reduction may be substantially catalyzed by fermentative bacteria. By targeting rRNA, we sought to provide a novel assessment of the metabolically-active microbial groups as well as their response to changing environmental conditions in microcosm sediments. Clone libraries, constructed from the 16S rRNA gene and cDNA reverse transcribed from the 16S rRNA, contained representatives from the phyla *Planctomycetes*, *Proteobacteria* (a, b, d, g), *Bacteroides*, and *Firmicutes*. The most abundant phylotypes found in the DNA libraries of neutral pH sediments were members of the *Alphaproteobacteria*, while *Gammaproteobacteria*-related clones dominated the RNA libraries. Currently, the response of metal-reducers and other heterotrophic communities is being further investigated in sediment microcosms in which metal reduction was stimulated by carbon substrate addition and pH neutralization.

Three groups of novel iron(III)-reducing organisms were isolated from the contaminated FRC subsurface. Stable enrichment cultures were established and isolations were performed in soft gelrite dilution series using FeOOH and AQDS as the sole electron acceptor, respectively. Using the 16S rRNA gene as a molecular marker, pure cultures shared high sequence identity (96 to 99%) to *Geobacter bremensis*, *Clostridium* sp. CITR8, and *Desulfotomaculum*



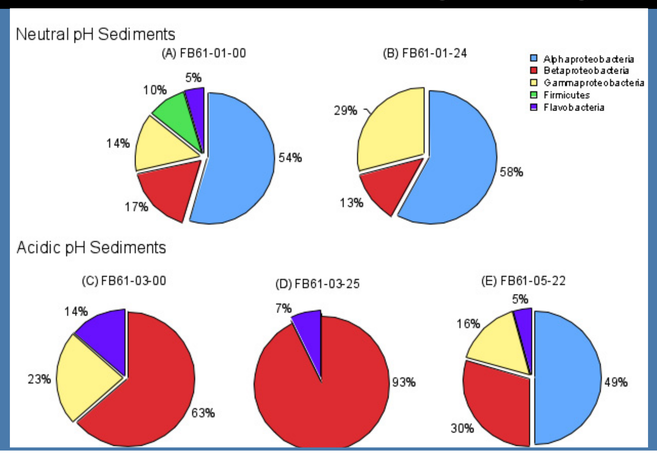
Fluorescent *in situ* hybridization (FISH) probes were developed for metal-reducing bacteria detected/ cultivated from FRC materials. Probes were tested successfully in pure cultures (see above) and in microcosm

Microcosm Experiments



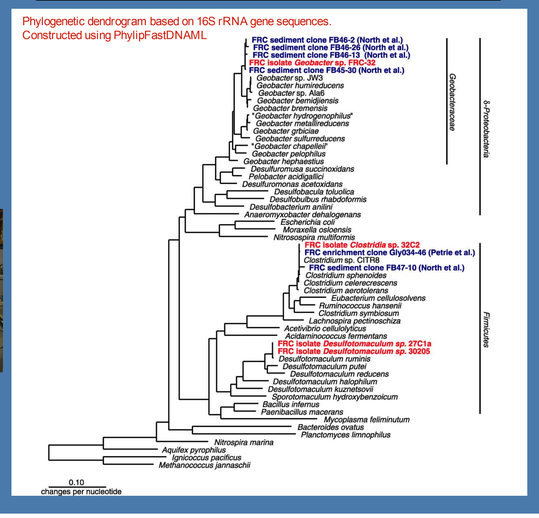
Greater than 300 microcosms were studied over a range of treatments and site materials. Representative plots are shown for electron acceptor (A, B) and electron donor utilization (C) in incubations of neutral pH sediments from Area 1. N_2 accumulation is compared for incubations of neutral pH (D) and acidic, neutralized sediments (E) from Area 1. In the neutral pH incubations, complete denitrification was followed by Fe(III) reduction. Carbon equivalents balanced fairly well with the amount of electrons accepted. 4-5x more N_2 was produced in ethanol supplemented microcosms than in glucose microcosms, and ammonium accumulated in the glucose treatments only, indicative of different nitrate-reduction pathways.

Microbial Community Analysis



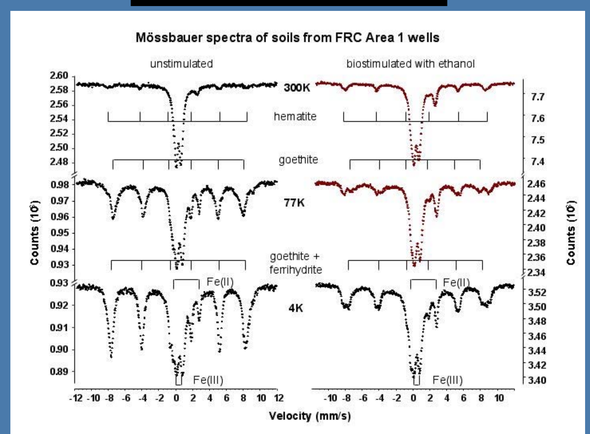
Community composition was determined for acidic and neutral pH sediment depth intervals (A-E) of borehole FB61, Area 1, that served as source materials for the microcosms. Shown above is the frequency of bacterial phylogenetic lineages in clone libraries of 16S rRNA gene sequences retrieved from sediments. Calculations were based on the total number of clones associated with phylotypes from which a representative clone had been sequenced.

Culture Work



13 strains of Fe(III)-reducing bacteria were isolated from background and Area 1 FRC subsurface sediments in gelrite dilutions on a variety of carbon substrates with AQDS as the electron acceptor. Many of the strains show high 16S rRNA gene sequence identity with phylotypes retrieved from FRC subsurface sediments (see above tree). Six strains each of **30205** and **27C** (*Desulfotomaculum* spp.) grow on FeOOH and lactate in the presence of AQDS as an electron shuttle. Six strains of **32C2** (*Clostridium* sp.) were isolated on acetate and are now growing with glucose, AQDS, and FeOOH. Strain **FRC-32** (*Geobacter* sp.) is a dissimilatory Fe(III)-reducing organism capable of growth on FeOOH and acetate in the absence of electron shuttle. The following electron donors were utilized by FRC-32 with FeOOH: acetate, ethanol, formate.

Iron Mineralogy



Mössbauer spectra were obtained using a Webb Research, Inc. spectrometer equipped with a Janis Model SHI-850-5 Closed Cycle Cryostat, operating at a sample temperature of 4 K.

Conclusions

Microcosms/ Community Analysis

- Rates of substrate utilization were among the most rapid reported for aquatic environments
- Acidity controlled not only the rates but also the pathways of microbial metabolism
- Electron donors were determined to stimulate metal reduction in the following order: glucose > ethanol > lactate > hydrogen
- Metal reduction may be catalyzed by fermentative bacteria in neutralized acidic sediments
- Microcosms provide more accurate rates than pure cultures of electron-accepting processes and carbon utilization for use in reactive transport models
- Microbial communities in microcosm source sediments were dominated by *Alpha-*, *Beta-*, and *Gammaproteobacteria*.
- Please see Denise Akob's poster for more data on microbial communities, including rRNA targets

Culture Work

- 13 strains of Fe(III)-reducing bacteria were isolated from the FRC subsurface
- Many of these strains are closely related to phylotypes detected in direct nucleic acid extractions of FRC sediments
- *Geobacter* sp. strain FRC-32 is the best characterized, grows on acetate and FeOOH, and its genome is currently being sequenced by JGI in the 2005 cohort.

Iron Mineralogy

- Biostimulation dissolves a significant fraction of original Fe oxide (goethite + ferrihydrite) in FRC subsurface sediments, producing hematite
- Mössbauer spectroscopy is reliable for identifying Fe oxide minerals only if measurements are taken at 4 K
- Some discrepancies exist between Mössbauer and wet chemical analysis for Fe(II)

Acknowledgements

This research was funded by the Natural and Accelerated Bioremediation Research (NABIR) program, Biological and Environmental Research (BER),and the U.S. Department of Energy (grant DE-FG02-00ER62986). We thank David Watson, Jack Istok, Lee Krumholz, and Barry Kinsall for sediment sampling and collaboration.