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 subsurface sediments of the Field Research Center (FRC) which are cocontaminated with uranium and nitrate. The focus will be on activity 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. The project will: 1) quantify the relationships between active members of the microbial communities, iron mineralogy, and nitrogen transformations in the field and in laboratory incubations under a variety of biostimulation conditions, 2) purify and physiologically characterize new model metal-reducing bacteria isolated from moderately acidophilic FRC subsurface sediments, and 3) elucidate the biotic and abiotic mechanisms by which FRC aluminosilicate clay minerals are reduced and dissolved under environmental conditions resembling those during biostimulation. Active microbial communities will be assessed using quantitative molecular techniques along with geochemical measurements to determine the different terminal-electron-accepting pathways. Iron minerals will be characterized using a suite of physical, spectroscopic, and wet chemical methods. Monitoring the activity and composition of the denitrifier community in parallel with denitrification intermediates during nitrate removal will provide a better understanding of the indirect effects of nitrate reduction on uranium speciation. Through quantification of the activity of specific microbial populations and an in-depth characterization of Fe minerals likely to catalyze U sorption/ precipitation, we will provide important inputs for reaction-based biogeochemical models which will provide the basis for development of *in situ* U bioremediation strategies.

In collaboration with Jack Istok and Lee Krumholz, we have begun to study the change in microbial community composition of FRC sediments during *in situ* biostimulation in single well push-pull tests. Microbial communities were stimulated in the acidic subsurface via pH neutralization and addition of electron donor to wells. Examination of sediment chemistry in cores sampled immediately adjacent to treated wells revealed that sediment pH increased substantially (by 1-2 pH units), while nitrate was largely depleted. Following the *in situ* biostimulation, previously cultured metal-reducing elta-*Proteobacteria* 16S rRNA gene sequences substantially increased from 5% to nearly 40% of clone libraries. Quantitative PCR revealed that *Geobacter*-type 16S rRNA gene sequences increased in biostimulated sediments by one to two orders of magnitude at two of the four sites tested, thereby corroborating information obtained from clone libraries, and indicating that members of the elta-*Proteobacteria* (including *Anaeromyxobacter dehalogenans*-related and *Geobacter*-related organisms) are important metal-reducing bacteria in FRC

Results - In situ Biostimulation of Acidic FRC Subsurface Sediments

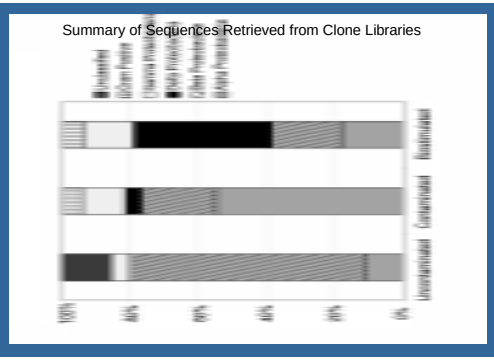
Cultivation-independent, Microbial Community Analysis

16S rRNA gene sequences retrieved from biostimulated FRC sediment.

FRC Contaminants and Potential Bioremediating Organisms

FRC Contaminant	Physiological potential	Potential bioremediating organisms	Clone library: % Before	% After
Uranium	Reduction and immobilization by FeRB	Geobacter sp. (33) *Anaeromyxobacter dehalogenans* (47) *Desulfitobacterium metallireducens* (13)	4.5%	37.8%
	Reduction and immobilization by fe-transition FeRB	*Clostridium beijerinckii* (56) *Serratia proteamaculans* (39)	5.7%	16.5%
Nitrate	Reduction	*Pseudomonas stutzeri* (40) *Alcaligenes defragans* (18) *Ralstonia pickettii* (41) *Anaeromyxobacter dehalogenans* (51) denitrifying Fe-oxidizing clone (98) *Paenibacillus sp.* (93)	22.8%	27.1%
Chlorinated hydrocarbons	Dechlorination	*Methylobacterium dichloromethanicum* (22) *Anaeromyxobacter dehalogenans* (91) Clone from TCE-contaminated site (8) *Dechloromonas sp.* (35)	42.5%	34.4%
Polychlorinated biphenyls	Dechlorination	*Alcaligenes xylosoxidans* (43) *Caulobacter leidyi* (42)	14.8%	2.2%
Fuel hydrocarbons	Degradation	*Burkholderia sp.* ADP1 (39) *Sphingomonas paucimobilis* (28)	5.7%	14.8%

Summary of Sequences Retrieved from Clone Libraries



Conclusions

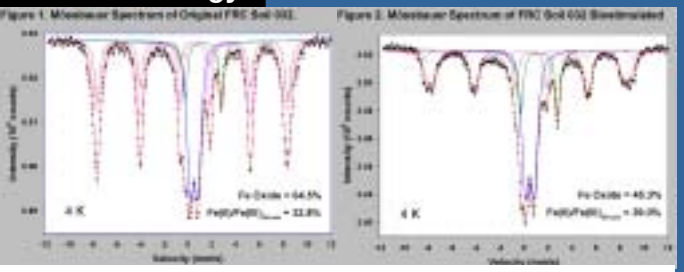
Iron Mineralogy

- Iron in the FRC subsurface is distributed 64.5 % in iron oxide and 35.5 % in silicate phases (Fig. 1)
- Silicate phases were partially reduced and iron oxides were dissolved during biostimulation (Fig. 2)
- Reliable Mossbauer results for iron phase distribution can be obtained only at 4 K

Microbial Community Analysis

- 16S rRNA gene sequences affiliated with the delta Proteobacteria increased from 5 % to 40 % of clone libraries during biostimulation
- Quantitative PCR revealed that *Geobacter*-type sequences increased by one to two orders of magnitude after biostimulation
- Many of the metal-reducers detected were closely affiliated with cultured organisms (*Geobacter*, *Anaeromyxobacter*, *Desulfitobacterium*) capable of coupling the reduction of nitrate, iron, or halogenated compounds to growth

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.

Borehole set	Organism Probed	Copies 16S gene/ gram sediment (95% Confidence limits) Before biostimulation	After biostimulation
A	*Geobacter*	$5.38*10^2$ ($4.23*10^2$; $2.63*10^3$)	$2.63*10^3$ ($2.19*10^3$; $9.50*10^3$)
B	*Geobacter*	$5.38*10^2$ ($4.23*10^2$; $2.63*10^3$)	$1.50*10^3$ ($1.06*10^3$; $7.38*10^3$)
C	*Geobacter*	$2.25*10^3$ ($1.60*10^3$; $8.65*10^3$)	$1.50*10^3$ ($1.06*10^3$; $7.38*10^3$)
D	*Geobacter*	$6.50*10^2$ ($4.25*10^2$; $1.30*10^3$)	$2.88*10^3$ ($2.38*10^3$; $1.50*10^4$)
A	*Anaeromyxobacter*	$2.63*10^3$ ($2.10*10^3$; $1.03*10^4$)	$1.16*10^3$ ($9.75*10^2$; $5.91*10^3$)
B	*Anaeromyxobacter*	$2.63*10^3$ ($2.10*10^3$; $1.03*10^4$)	$1.13*10^3$ ($1.03*10^3$; $5.63*10^3$)
C	*Anaeromyxobacter*	$3.45*10^3$ ($2.25*10^3$; $1.48*10^4$)	$5.38*10^3$ ($4.50*10^3$; $1.79*10^4$)
D	*Anaeromyxobacter*	$4.25*10^3$ ($3.18*10^3$; $1.65*10^4$)	$1.16*10^3$ ($9.75*10^2$; $5.91*10^3$)
A	*Paenibacillus/ Brevibacillus*	$1.16*10^3$ ($9.75*10^2$; $5.91*10^3$)	$1.43*10^3$ ($9.3*10^2$; $6.07*10^3$)
B	*Paenibacillus/ Brevibacillus*	$1.16*10^3$ ($9.75*10^2$; $5.91*10^3$)	$1.00*10^3$
C	*Paenibacillus/ Brevibacillus*	$1.88*10^3$	$6.90*10^3$ ($5.85*10^3$; $3.55*10^4$)
D	*Paenibacillus/ Brevibacillus*	$2.38*10^3$ ($1.65*10^3$; $7.85*10^3$)	$1.88*10^3$ ($1.5*10^3$; $7.38*10^3$)

Quantitative PCR before and after biostimulation of contaminated sediment with glucose: FB32 to nearby FB45 (borehole set A), FB32 to nearby FB46 (borehole set B), FB33 to nearby FB47 (borehole set C), and ethanol FB34 to nearby FB48 (borehole set D).

Acknowledgements

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