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. T-RFLP 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.

Introduction

The goal of this project is to stimulate indigenous microbes that can immobilize uranium by reducing soluble U(VI) to an insoluble U(IV), without utilizing nitrate as an electron acceptor. While several bioremediation approaches require continual removal of nitrate to achieve bioreduction, our approach relies on low-pH anaerobic conditions and electron donors such as methanol and glycerol that favor the enrichment of acid-tolerant communities which do not have a large capacity for nitrate reduction. It is currently unknown whether the reduction of uranium in this system is intentional with a gain of energy for the microorganisms, or if U(VI) reduction is simply a consequence of the shunting of electrons from one place to another during a separate energy-acquiring metabolic process. Regardless, our work demonstrates the potential for nitrate-indifferent bioreduction of uranium after enrichment from FRC sediments.

Area 2 Enrichments

Enrichment of FRC sediments for uranium reduction in the presence of nitrate. The main goal for task 2 was to stimulate extant microbial populations and accelerate their activity toward metal/radionuclide reduction in order to immobilize metals/radionuclides under native conditions of high nitrate and low pH. MOPS/TRIS buffers with methanol and glycerol (donors which do not dissociate at lower pH) were used. Subsequent transfers contained yeast extract, trace minerals, and vitamins.

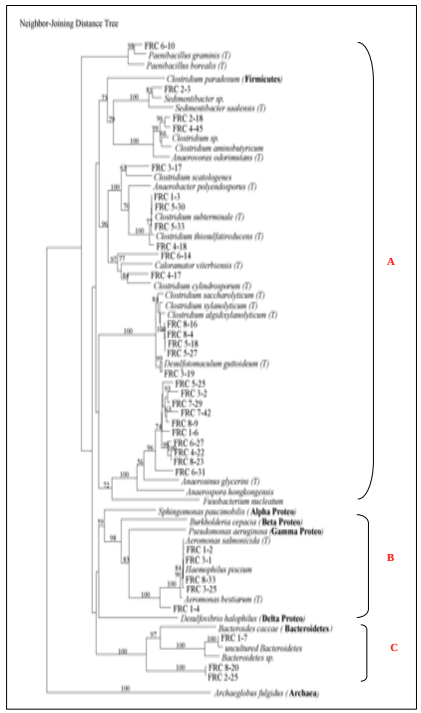
Label	donor	pH	%loss NO3-	%loss U
1 YMVGii	MeOH	6.2	13.7	99.0
2 YMVGi	MeOH	6.2	18.8	98.9
3 YMGGi	Glycerol	6.2	7.4	92.9
4 RMVGii	MeOH	5.9	4.6	90.1
5 RMVGi	MeOH	5.9	8.2	98.4
6 RMGGi	Glycerol	5.9	7.6	91.8
7 BMVGii	MeOH	5.7	22.1	98.2
8 BMVGi	MeOH	5.7	11.9	98.8
9 BMGGi	Glycerol	5.7	30.7	87.1
YMGGii	Glycerol	6.2	65.9	76.6

Label	donor	pH	%loss NO3-	%loss U
GRMVGii	MeOH	4.9	5.3	91.2
GBMVGi	MeOH	4.9	7.8	95.0
ORMVGii	MeOH	5.4	5.6	93.3
OBMVGi	MeOH	5.4	0	94.0

Several enrichments reduced uranium to near completion with little reduction of nitrate, even at lower pH.

16S rRNA community analysis

Clone sequences were present from three primary groups of bacteria:
A.) Low G+C gram positives (*Clostridia, Desulfotomaculum, Paenibacillus*)
B.) Bacteroidetes
C.) Gamma Proteobacteria



Phylogenetic analysis using 16S rRNA indicated that the large Cluster A is representative of that group of microorganisms. Although this group is composed primarily of low G+C gram-positive organisms, a small group of low G+C gram-negative bacteria were also found to be extremely important. Classifications within the *Clostridia* group have been, in the past, primarily based on phenotypic characteristics (i.e. Gram-positive staining, anaerobic growth, and endospore formation; The Prokaryotes). However, it is now known that some of these organisms possess a true gram-type negative cell wall, while others incorrectly stain as Gram-negative, leading to the misclassification of those organisms and the taxonomic disorder of the genus *Clostridia*. All of the true *Clostridia* (i.e. senso stricto) are endospore-formers, but several of the newly created genera do not contain spore-formers (i.e. *Anaerosinus*). All clones related to *Anaerosinus glycerini* had similarity ranks of 0.7 or less, indicating the likely presence of unknown organisms with variable physiological traits.

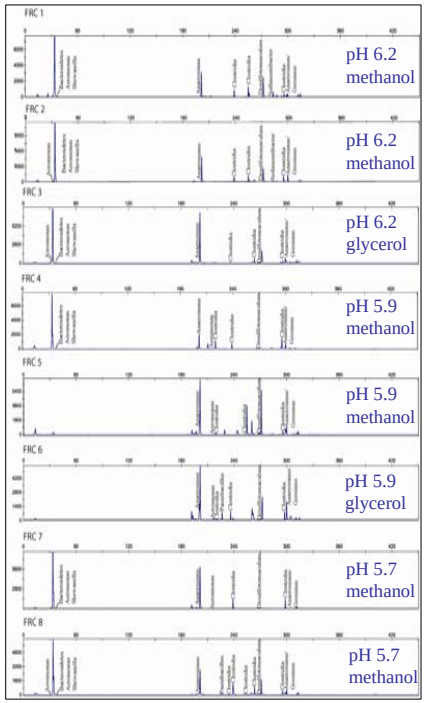
T-RFLP community analysis

According to T-RFLP profiles of the enrichments, clone sequences most closely related to *Desulfotmaculum guttoidem* and *Anaerosinus glycerini* were the dominant organisms in nearly all cases. Both organisms tree within the broadly defined Clostridiaceae family.

Desulfotomaculum guttoideum has been found to closely group with Clostridia-like species. *Anaerosinus glycerini* is the type strain for the *Anaerosinus* genus and is a low G+C (35 mol %), gram-negative bacterium.

Differences in T-RFLP profiles do not correlate well with changes in pH or electron donor.

The large band at ~33-35 has yet to be explained based on the observed clones.



Current and Future Work

Ongoing research includes: identification of nitrate reducers within the enrichments in order to identify conditions more partial to nitrate reducers, isolation of potential Clostridaceae which may be contributing to the uranium reduction, triethylphosphate utilization for free phosphate production and stable U-P precipitation, electron balance through monitoring of gas phase and aqueous species during growth, verification of the sustainability of U reduction through repeated transfers and new enrichments, and reoxidation experiments involving varying levels of oxygen stress in the presence of iron.

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