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

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Abstract

Our overall objective is to understand the microbial and geochemical mechanisms controlling the reduction and immobilization of U(VI) during biostimulation in shallow subsurface sediments contaminated with uranium and nitrate. The focus is on the activity and community composition of microbial populations (metallureducing bacteria and iron minerals) that likely make strong contributions to the fate of U during in situ bioremediation. Our initial efforts focused on the isolation and characterization of alphaproteobacteria from FRC materials from Area 2 of the Field Research Center (FRC) at Oak Ridge, TN. Slightly enriched iron solutions were obtained from the sediments of Area 2, and samples were stored in anaerobic bottles at room temperature until used.

Cultivation-Dependent Community Analysis

- Incubated at 30°C and sampled for geochemical analysis of iron and uranium.
- Treatments (3 replicates each):
  1. Area 2 sediment (FB094) was combined with microbial populations from other Geochemical analyses (Fe(III)-reducing bacteria in the ethanol treatments were numerically abundance compared to glucose-amended treatments in parallel with enhanced U(VI) removal in ethanol treatments. Cultivable Fe(III)-reducing bacteria in the ethanol treatments were numerically dominated by Geobacter sp., while those cultured on glucose were dominated for ferromanganese). Efforts are underway to associate in situ activity with microbial processes at the FRC.

Microcosms

- Novel iron(III)- and sulfate-reducing organisms were isolated from the FRC with the bacteria from the microcosms by fingerprinting.
- Isolates were identified by 16S rRNA sequencing and analysis of microcosm samples.
- Cultivation-independent Community Analysis
  1. DNA was extracted from FRC materials and MPCA cultures.
  2. PCR-DGGE: fingerprinting of PCR-amplified 16S rRNA genes.
  3. T-RFLP: analysis of PCR-amplified 16S rRNA genes with a fluoro-labeled 27F primer.
  4. PCR products were digested with restriction enzyme Mnl

Iron Mineralogy

- The following are estimated for goethite: a surface area of 30 m²/g, a crystallite size of 100 Å.

Microbial Community Analysis

- In collaboration with Dave Thomas and Frank Löffler (Georgia Tech), several isolates were obtained from highly contaminated sediments of Area 1

Approach

- Isolates were selected, cultured, and flushed with N₂.
- Treatments (3 replicates each):
  1. 20 mM Ethanol, 10mM Glucose; Unamended control.
  2. 20 mM Ethanol, 10mM Glucose, 15mM NO₃⁻; Unamended control.

Isolates

- Geobacter strain FRC-32
  1. Microbial community analysis
  3. Growth was not detected with nitrate and sulfate as electron acceptors.

- Anaeromyxobacter sp.
  1. The optimal pH was ~ 6 with no growth detected at pH 5.
  2. Growth was not detected with nitrate and sulfate as electron acceptors.

- Anaeromyxobacter strain FRC-32
  1. Microbial community composition of FRC-32 was determined using clone libraries.
  2. Isolates were identified by 16S rRNA sequencing and analysis of microcosm samples.

Conclusions

- The mechanism of U(VI) reduction is electron donor dependent, with substantial reduction occurring prior to Fe(III)-reduction in microcosms.
- Carbon utilization defined by fermentative metabolism and incomplete oxidation of ethanol.
- Geobacter sp. numerically dominates cultivable Fe(III)-reducing bacteria in ethanol microcosms.
- However, only Anaeromyxobacter is detected in the metabolically active profiles of highly contaminated groundwater columns near FRC in situ sites.
- Highly impacted, upgradient groundwater samples had roughly half the T-RFLP pairs of degradant samples suggesting lower diversity in contaminated groundwater columns.

Isolates

- > 1400 16S rRNA genes sequences retrieved from FRC materials in new available ~400 have been examined with silicic digestion for T-RFLP profiling.
- Please see Denise Akob’s poster for microbial community analysis of microcosm samples!

Iron Mineralogy

- Pure cultures have been obtained from FRC surface sediments for at least four groups of Fe(III)-reducing bacteria (Geobacter, Desulfotomaculum, Anaeromyxobacter). 
- Geobacter and Desulfotomaculum strains are physiologically distinct from close relatives. The Desulfotomaculum isolate is capable of growth using both Fe(III) and sulfate.

- The genome sequence of Geobacter strain FRC-32 is now available from JGI.
- In collaboration with the Löffler lab (Georgia Tech), several Anaeromyxobacter strains were isolated, these have yet to be characterized.

Iron Mineralogy

- Milobarium is the sequence of PCR-amplified 16S rRNA genes.
- PCR products were digested with restriction enzyme Mnl.

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References