Microbial Activity and Community Composition in Microcosms of FRC Area 2 Uranium Contaminated Subsurface Sediment and Groundwater

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Abstract

Uranium contamination is widespread at subsurface sites across the U.S and this contamination tends to be acidic because acids were used in the processing of uranium ore for nuclear weapons production. Metal-reducing microbial communities in subsurface sediments have been shown to effectively immobilize uranium contamination. In order to design appropriate strategies for *in situ* bioremediation of uranium contaminates it is important to understand the activity and community composition of microbial populations (metal- and nitrate-reducing bacteria) and iron minerals in subsurface materials present at the Field Research Center (FRC). The goal of this study was to evaluate the differences in activity (electron acceptor/ donor utilization) and microbial community composition present during nitrate and iron reduction phases in biostimulated FRC Area 2 subsurface materials cocontaminated with high levels of U(VI) and nitrate. FRC Area 2 subsurface sediments and groundwater were combined under anoxic conditions and neutralized with bicarbonate. Electron donor utilization was evaluated with three treatment conditions: addition of 10mM glucose, 20 mM ethanol and no carbon controls. Electron acceptor utilization was evaluated by measuring the rate of nitrate-, iron-, and uranium-reduction and electron donor utilization will be evaluated using high performance liquid chromatography (HPLC) analysis. Nitrate concentrations at the start of the experiment were approximately 15 mM in all microcosm treatments and iron (II) concentrations were below detection. Nitrate reduction occurred rapidly in all carbon amended treatments, disappearing completely in ethanol and glucose treatments on days 4 and 6, respectively. Iron (II) was detected in ethanol and glucose treatments on day 10 and 6 indicating the beginning of the iron reduction phase. Nitrate concentrations remained constant in all no carbon control treatments. The presence of nitrate- and iron-reducing bacteria in the three microcosm treatments during nitrate-reduction (day 7) and iron-reduction (day 21) was evaluated by pooling samples from three replicates and inoculating a most probable number dilution series. MPN tubes inoculated during nitrate reduction showed that the number of nitrate reducing bacteria in ethanol treatments were 1-2 orders of magnitude higher than in the glucose treatment. Nitrate reducing bacteria were 2-3 orders of magnitude higher than iron-reducing bacteria in the glucose treatment and detected in equal abundance for the ethanol treatment. MPN tubes inoculated during iron reduction are currently being evaluated to determine differences in the number of nitrate and iron reducing bacteria present. Samples from the three treatments and intact core material will also be used to investigate the microbial communities present with cultivation-independent techniques, including fluorescence *in situ* hybridization (FISH), terminal restriction fragment length polymorphism analysis (t-RFLP) and SSU rRNA cloning and sequencing. The work from this study will provide important information of the metabolic potential of microbial communities present in the Area 2 subsurface available for bioremediation strategies.

Goals

•To evaluate the differences in electron acceptor/ donor utilization in biostimulated Area 2 subsurface materials.

• To evaluate the shift in microbial community composition present during nitrate- and metal-reduction conditions in FRC Area 2 subsurface materials.

Methods

Microcosm Design

•Area 2 sediment (FB094) was diluted 1:4 with Area 2 groundwater (FW209).
•Microcosms were neutralized and flushed with N_2 to create anoxic conditions.
•Treatments (3 replicates each):
  •20 mM Ethanol
  •10 mM Glucose
  •No carbon control
•Incubated at 30°C and sampled for geochemical analysis every 1-5 days.

Geochemical Analysis

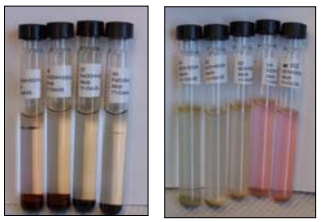
•Electron acceptor usage:
  •NO_3 reduction
  •Fe-reduction (accumulation of Fe(II))
  •Uranium reduction (*in progress*)
•High performance liquid chromatography (HPLC) for electron donor usage (*in progress*)
•Fe-mineral analysis (*in progress*)

Cultivation-Dependent Community Analysis

•Most probable number dilution series were prepared for nitrate- and iron-reducing bacteria.
•Pooled samples from replicate treatments were used to inoculate MPN tubes during nitrate-and iron-reducing conditions.
•Highest dilutions were sampled for NO_3-reduction, Fe(II)-accumulation, HPLC and molecular analysis at 2 weeks of growth.

Table 1: Experimental design for most probable number (MPN) dilution series. Day 7 and 21 represent nitrate- and iron-reducing conditions, respectively.

		MPN Media	
	Microcosm Treatment	Carbon Source	Electron Donor
Day 7	Glucose	Glucose	Nitrate
	Glucose	Glucose	Iron
	Ethanol	Ethanol	Nitrate
	Ethanol	Ethanol	Iron
Day 21	Glucose	Glucose	Nitrate
	Glucose	Glucose	Iron
	Ethanol	Ethanol	Nitrate
	Ethanol	Ethanol	Iron
	Control	Glucose	Nitrate
	Control	Glucose	Iron
	Control	Ethanol	Nitrate
	Control	Ethanol	Iron



Results

Microcosm Experiment

Figure 1: Electron acceptor usage in microcosm treatments over time. (A) nitrate-reduction and (B) iron-reduction measured by Fe(II) accumulation. Error bars indicate standard deviation. Arrows indicate MPN sampling times during nitrate- or iron-reducing conditions

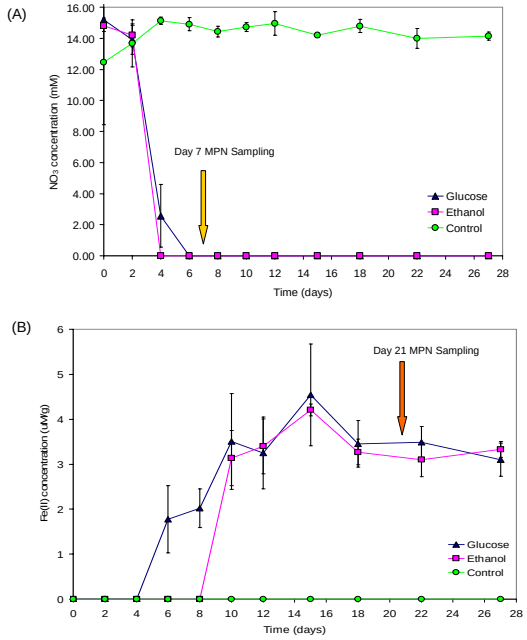


Figure 2. Photographs of Control, Glucose and Ethanol (left to right) microcosm experimental treatments at (A) day 7 and (B) day 15. Note significant color change in carbon treatments compared to the control.

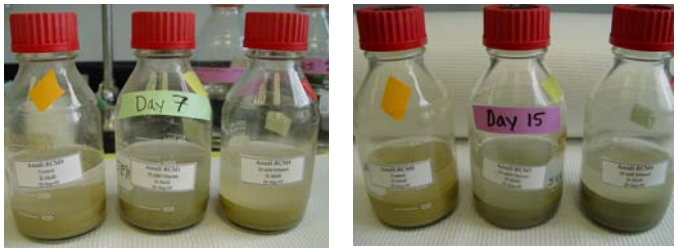
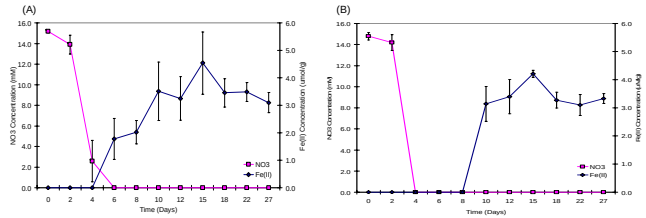


Figure 3: Shift from nitrate-reducing to metal-reducing conditions in replicate (A) glucose and (B) ethanol microcosms . Error bars indicate standard deviation.



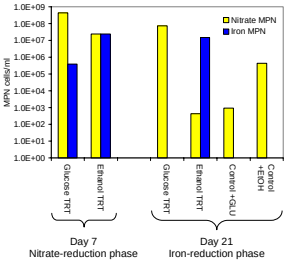
Results

MPN experiments

•Day 7 MPNs were recorded after 3 weeks of growth.

•Preliminary data collected after only 1 week of growth is presented for Day 21 MPNs.

•No iron reduction has been detected in day 21 MPNs for glucose and control treatments.



Conclusions

•The shift between nitrate- and iron-reduction phases in the microcosms occurred at different times in the two electron donor treatments. In the glucose amended microcosms there appears to be an overlap in nitrate- and iron-reduction phases while in ethanol amended treatments a lag phase was observed between the end of nitrate-reduction and the beginning of iron-reduction.

•The overlap between nitrate- and iron-reduction in glucose amended treatments indicates that there may be little shift in abundance and composition of metal-reducing bacteria. Further analysis of MPN results from inoculations during the iron-reduction phase of the microcosm will provide evidence for this hypothesis.

•The four-day lag between nitrate- and iron-reducing conditions in the ethanol treatments suggests that there is a change in the abundance of metal-reducing bacteria. Preliminary comparisons of MPN counts indicates that with the change in reducing conditions there is a significant decrease in the number of nitrate-reducing bacteria while the number of iron-reducing bacteria significantly increases.

•Nitrate reduction was observed in MPN tubes inoculated with material from the control microcosm which indicates that the abundance of nitrate-reducing bacteria is most likely high under *in situ* conditions.

Future Work

•Analyze data for additional geochemical parameters (i.e. uranium, HPLC, and iron mineral analysis)
•Inoculate MPN dilution series for FB094 sediments to determine the *in situ* nitrate- and iron-reducing microbial communities.
•Cultivation-independent Community Analysis:
  •SSU rRNA analysis (cloning, sequencing, t-RFLP)
    - Homogenized sediment (*in situ* community) from day 0
    - Pooled microcosm material from three replicates of each treatment from days 4 and 21
    - Material from each microcosm from day 27
    - Samples from MPN highest dilution tubes
  •Fluorescence *in situ* hybridization (FISH) on microcosms from days 8 and 21.
  ▪Isolate nitrate- and metal-reducing bacteria from MPN dilution series.

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