Determination of the Metabolically Active Microbial Groups in Contaminated ORFRC Subsurface Sediments Using Stable Isotope Probing (SIP)

Denise M. Akob[1*], Heath J. Mills[1], Lee Kerkhof[2], and Joel E. Kostka[1]

[1]Florida State University, Department of Oceanography, Tallahassee, FL; [2]Rutgers University, Institute of Coastal and Marine Sciences, New Brunswick, NJ

*contact: akob@ocean.fsu.edu

Abstract

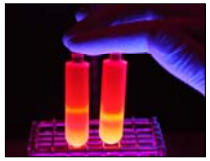
The goal of this study was to employ stable isotope probing (SIP) techniques to provide a link between the composition and function of subsurface microbial communities with a high potential for uranium bioremediation. In subsurface sediments contaminated with mixed wastes, metal- and nitrate-reducing bacteria have been shown to mediate electron flow in subsurface sediments, thereby controlling the fate and transport of radionuclides. However, at the Oak Ridge Field Research Center (ORFRC), there is no consensus on the composition and distribution of active community members across changes in relevant environmental parameters likely to effect bioremediation potential. Ethanol has been shown in laboratory and field based bioremediation studies to promote microbial activity and the biological reduction of U(VI) in ORFRC sediments. In order to further characterize the metabolically active microbial community, we amended ORFRC Area 2 sediment microcosms with ^{13}C-labeled ethanol and monitored electron acceptor (NO_3^-, Fe(III) and U(VI)) and donor utilization. At selected time points corresponding to phases of terminal electron accepting processes, ^{13}C incorporation into community DNA was examined by density gradient centrifugation along with PCR amplification and terminal restriction fragment length polymorphism analysis (TRFLP). Incorporation of the ^{13}C-labeled substrate into microbial biomass was detected by day 6 of the incubation which corresponded to the onset of nitrate reduction. Following nitrate reduction, we observed a two-day lag phase before the onset of U(VI) and Fe(III) reduction and a shift in peak area for the predominant microbial groups, including members of the *Proteobacteria, Actinobacteria, Firmicutes* and *Bacteroidetes.* At all time points, we detected a TRFLP peak corresponding to the *Dechloromonas* group of the *Betaproteobacteria* which had incorporated the ^{13}C-ethanol suggesting that this group is active during both nitrate and metal reduction phases of the incubation. These results indicate that stable isotope probing methods can be used successfully to couple the composition and function of microbial communities in ORFRC subsurface sediments. Our data also suggest that members of the *Betaproteobacteria* may play an important role in nitrate removal and subsequent metal reduction, although further work is needed to elucidate the biogeochemical processes mediated by the microbial groups that incorporated ^{13}C-labeled ethanol.

Background

- **Bioremediation strategies at the Oak Ridge Field Research Center (ORFRC) are focused towards stimulating *in situ* microbial communities to reduce U(VI) to insoluble U(IV).**
- **U(VI) reduction is limited by the availability of carbon, pH, and competing terminal electron acceptors.**
- **Nitric acid is a cocontaminant at the ORFRC**
  - **Nitrate serves as a competing electron acceptor for metal-reducing bacteria**
  - **Nitrate must be depleted prior to metal reduction (Finneran *et al*., 2002; Senko *et al*., 2002).**
  - **Both nitrate- and metal-reducing bacteria may be important for bioremediation strategies.**
- **Area 2 in the ORFRC**
  - **Located west of the S-3 pond site adjacent to Bear Creek**
  - **Contamination is likely due to transport through the groundwater**
  - **Sulfate present; neutral pH**

Objective: To employ stable isotope probing (SIP) to directly link biogeochemical processes with metabolically active microbial groups with a high potential for uranium bioremediation.

Experimental Approach



Microcosm Design

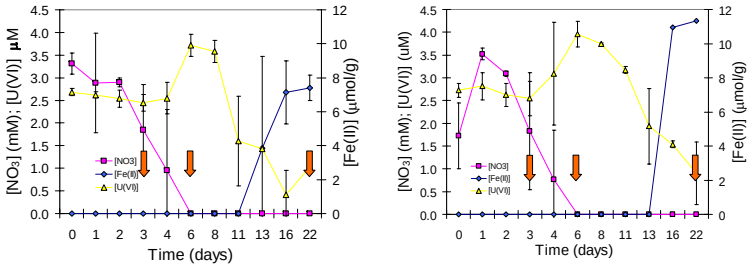
1. **ORFRC Area 2 sediment (FB097) was homogenized**
2. **Diluted with artificial groundwater under anoxic conditions**
3. **Treatments (in duplicate):**
   1. **5mM ^{13}C-EtOH (labeled) + 2.5 mM NO_3**
   2. **5mM ^{12}C-EtOH (unlabeled) + 2.5 mM NO_3**
4. **Incubated at 30°C for 22 days**
5. **Sampled to monitor:**
   - **Terminal electron accepting processes (TEAPs)**
   - **Carbon utilization (HPLC, *in progress*)**

Community Characterization

1. **Samples collected on days 0, 3, 6 & 22**
2. **DNA extracted and the ^{13}C/^{12}C fraction separated using density gradient centrifugation**
3. ***Bacteria*-domain specific primers (27F & 1392R) were used to PCR amplify community SSU rRNA genes**
4. **Community profiles generated using terminal restriction length polymorphism (TRFLP) analysis**

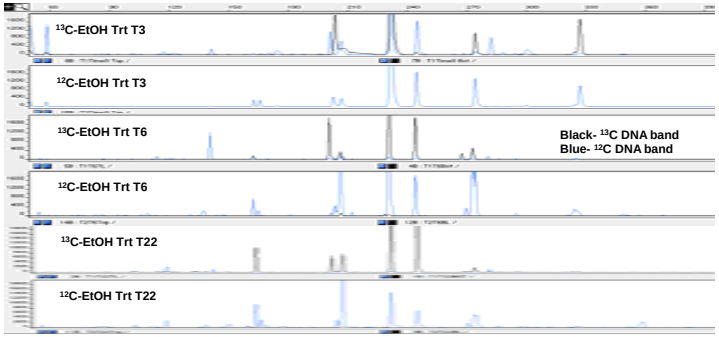
Results

Figure 1: Electron acceptor usage in (A)^{13}C- and (B)^{12}C-ethanol amended microcosms. Arrows indicate sampling points for TRFLP community fingerprinting. Values are averages of duplicate microcosms.



- **Ethanol addition stimulated microbial activity**
- **TEAPs in both the ^{13}C- and ^{12}C-ethanol treatments were temporally equivalent**
- **U(VI) reduction occurred only after nitrate depletion and prior to Fe(III)-reduction**
- **Rates of U(VI)-reduction:**
  - **^{13}C-EtOH = 0.9433 µM day^{-1}**
  - **^{12}C-EtOH = 0.7046 µM day^{-1}**

Figure 2: SSU rRNA gene TRFLP profiles in the ^{13}C- and ^{12}C-ethanol amended microcosm treatments during days 3, 6, and 22. The ^{13}C-DNA fraction is in black and the ^{12}C-DNA band is in blue.



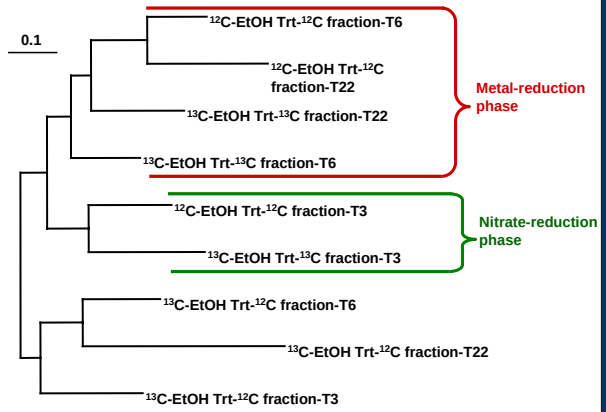
Peak Identification

- **SSU rRNA gene sequences derived from studies at the ORFRC site were compiled & digested *in silico* with *MnlI*.**
- **Peaks from TRFLP profiles were compared to results from *in silico* digests.**

Peaks of Interest:

- **232 bp = Betaproteobacteria (Dechloromonas)**
  - **Detected at all time points in ^{13}C-EtOH treatment**
- **245 bp = related to *Arthrobacter* spp. member of the *Actinobacteria* (*Micrococcaceae*)**
  - **Detected in both treatments and at all time points**

Figure 3. Sorensen's index derived from distance matrices of SSU rRNA gene TRFLP profiles.



Conclusions

- **The majority of the detectable microbial community in microcosms assimilated ^{13}C from the addition of ^{13}C-EtOH**
  - **Incorporation of ^{13}C-EtOH was detectable at day 3 of the incubation**
- **Peaks detected in the ^{12}C fraction of ^{13}C-EtOH treatment represent those groups not assimilating ^{13}C from labeled ethanol, such as dead, senescent, or sporeforming bacteria**
- **TRFLP profiles revealed changes in community structure with incubation time**
  - **Communities at T6 and T22 were most closely related.**
    - **Both sampled during metal-reduction**
    - **Communities are likely composed of microbial groups active in metal-reduction**
  - **Profiles at T3 are likely composed of active nitrate reducers**
- **The majority of the peak area identified to the *Betaproteobacteria***
  - **Present throughout the incubation**
  - **Genera identified: *Dechloromonas, Azoarcus, Alcaligenes, Ralstonia,* and *Diaphorobacter***
  - **Many families metal-resistant and capable of nitrate-reduction**
- **Detected members of the *Actinobacteria* and *Firmicutes***
  - ***Arthrobacter* –nitrate- and chromate-reducer**
  - ***Clostridium* –fermentative metal-reducer**

- **Microbial community structure changed with time and geochemistry of the microcosm**
- **Microbial groups are present in Area 2 sediments that have a high potential for bioremediation.**

Acknowledgments

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