An Integrated Assessment of Geochemical and Community Structure Determinants of Metal Reduction Rates in Subsurface Sediments

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INTRODUCTION AND BACKGROUND

Fundamental questions still persist concerning the interactive effects of geochemistry and microbial community structure on rates of metal reduction in the subsurface. Some microorganisms can change the subsurface geochemical conditions (e.g., cause a drop in redox) so metal reduction becomes an energetically favored reaction. Other microbes can directly catalyze the necessary reactions so that metal reduction occurs at a more rapid rate than without microbial activity. Many microorganisms can accomplish the first role but many fewer can accomplish the second. Physical and geochemical factors such as mass transport, oxygen level, and nitrate concentration will likely dominate the rate of microbial change in the redox potential. Thus, it is possible that the importance of community structure at this stage of metal reduction may be minimal. However, the effect of community composition on the rate of metal reduction may be important. Factors influencing community structure could include both the specific electron donor and ratios of nutrients. Resource-ratio theory predicts that the structure of a biological community depends on the supply rates and ratios of potentially growth-limiting resources.

OBJECTIVES

The overall goal of our project is to provide an improved understanding of the relationships between microbial community structure, geochemistry, and metal reduction rates. The research seeks to address the following questions:

- Is the metabolic diversity of the in situ microbial community sufficiently large and redundant that bioimmobilization of uranium will occur regardless of the type of electron donor added to the system?
- Are there donor specific effects that lead to enrichment of specific community members that then impose limits on the functional capabilities of the system?
- Will addition of humics change rates of uranium reduction without changing community structure?
- Can resource-ratio theory be used to understand changes in uranium reduction rates and community structure as the C:P ratio changes?

APPROACH

We are using laboratory microcosms with sediments and groundwater from the NABIR Field Research Center (FRC). Three types of manipulations are planned with the goal of changing either the community or the geochemistry.

1) Change the community structure with minimal change in the geochemistry by employing different electron donors.
2) Change the geochemistry with minimal effect on the community structure by adding humics to serve as electron shuttles.
3) Change both the geochemistry and the community structure by adding phosphate to adjust the C:P ratio.

Figure 1 illustrates the planned manipulations and their interrelationships, and Table 1 lists the electron donors used in the various experiments..

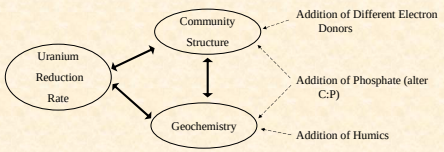

Figure 1. Relationships among planned manipulations and anticipated changes in community structure, geochemistry, and uranium reduction rate.

Table 1. Electron donors used in experiments. The number of electron equivalents (e^-) and the bacteria expected to most effectively utilize the substrate are also given.

Donor	Formula	e^-	Predominant Utilization	Exp
Acetate	$C_2H_3O_2$	8	FeRB/acetogenic methanogens	3
Lactate	$C_3H_6O_3$	12	SRB/FeRB	3
Pyruvate	C_3H_4O	10	SRB/FeRB	3
Methanol	CH_4O_3	6	Acetogens/methanogens	1,2,3,4
Ethanol	C_2H_6O	12	SRB/FeRB	1,2,3,4
Glycerol	$C_3H_8O_3$	14	Clostridia/gram positive anaerobes	3
Glucose	$C_6H_{12}O_6$	24	Clostridia/other heterotrophs	1,2,3,4

EXPERIMENTAL PROTOCOLS

Four experiments have been conducted to scope the effect of different electron donors on nitrate and uranium reduction rates. The results of these experiments will be used to narrow the range of conditions for which detailed community analysis will be done. The goal of these experiments is to define the lengths of experiments and sampling times and to choose specific substrates and appropriate substrate levels.

We are using laboratory microcosms containing sediment and groundwater to address the questions.

- Each microcosm used 20 g of sediment and 80 mL of groundwater from the FRC.
- Sediments samples were homogenized under anaerobic conditions prior to use in the microcosms.
- Carbon substrate concentrations were adjusted to give equivalent electron donor potential.
- Triplicate microcosms were run for each treatment.

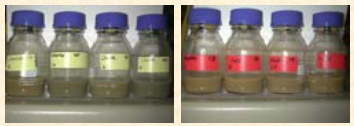

Figure 2. Photograph of microcosms.

EXPERIMENT 2 – SETUP

- Initial scoping experiment.
- Three electron donors and control:
  - Methanol (40 mM)
  - Ethanol (20 mM)
  - Glucose (10 mM)
  - Control
- Freshly collected sediments and groundwater.

EXPERIMENT 2 – RESULTS

- No lag times in nitrate reduction with fresh sediments.
- No uranium reduction with methanol.
- Glucose- and ethanol- (data not shown) amended microcosms exhibit both uranium and nitrate reduction.

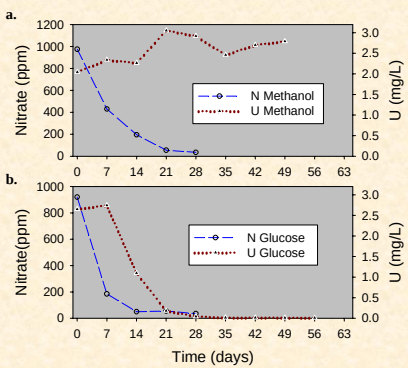

Figure 3. Loss of nitrate and uranium over time in (a) methanol- and (b) glucose-amended microcosms.

EXPERIMENT 2 – RESULTS

- Fastest rates of uranium reduction occurred with glucose.
- Substantial nitrate and uranium reduction with ethanol.
- Nitrate but no uranium reduction with methanol.

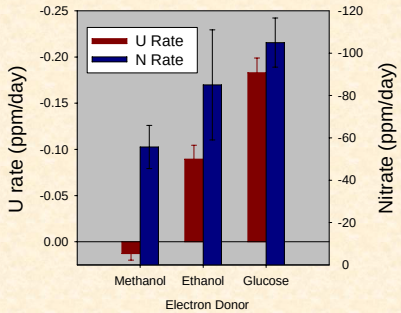

Figure 4. Comparison of nitrate and uranium reduction rates.

Hierarchical cluster analysis of phospholipid fatty acid profiles

Phospholipid fatty acid (PLFA) measurements were made on the final time point samples. The individual PLFA components were grouped into seven structural categories and relative percent composition of each sample was calculated. The resulting data were analyzed using average linkage hierarchical cluster analysis to identify sample groups.

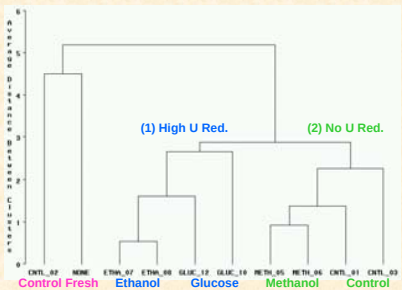

Figure 5. Cluster analysis dendrogram of PLFA analyses from final microcosm samples.

- Two major clusters: (1) high uranium reduction rate and (2) no uranium reduction.
- Control 2 and the fresh sediment are very different – lower biomass.
- PLFA biomarkers indicate nutritional stress in the methanol treatment and the control treatments was indicated by the cyclopropyl to monounsaturated fatty acid ratio.
- There also appears to be a potential toxicity stress in the methanol treatment indicated by the trans/cis ratio of monounsaturated fatty acids.

EXPERIMENT 4 – SETUP

- First attempt to incorporate humics (see Figure 1).
- Same experimental setup as Experiment 2 plus addition of ethanol and humics treatment:
  - Methanol (40 mM)
  - Ethanol (20 mM)
  - Glucose (10 mM)
  - Ethanol (20 mM) and humics (30 ppm)
  - Control
- Freshly collected sediments and groundwater.

EXPERIMENT 4 – RESULTS

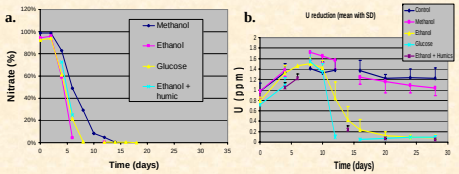

Figure 6. Loss of (a) nitrate and (b) uranium over time in microcosm.

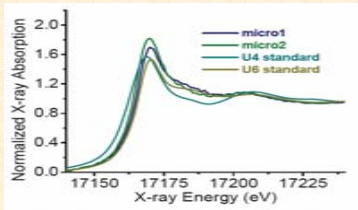

Figure 7. Uranium valence by X-ray spectroscopy (Kelly and Kemner).

Glucose end point	Ethanol end point
83 ±8 % U(VI)	96 ±4 % U(VI)
17 ±8 % U(IV)	4 ±4 % U(IV)

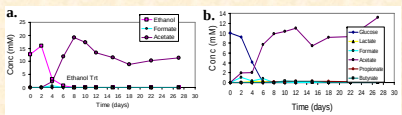

Figure 8. Donor composition and metabolite production in (a) ethanol- and (b) glucose-amended microcosms.

- Primary electron donor is consumed quickly.
- Acetate tends to accumulate over time and persist till end of experiment.

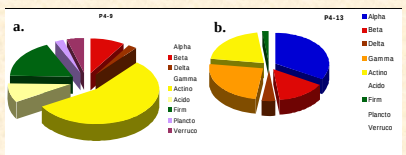

Figure 9. Community composition of final (a) ethanol- and (b) glucose-amended microcosm samples based on T-RFLP analysis.

SUMMARY

- Consistent results in the experiments indicating:
  - All substrates promoted nitrate reduction
  - Methanol did not promote uranium reduction but glucose and ethanol promoted rapid uranium reduction
  - PLFA indicated different communities with methanol
  - T-RFLP indicated distinct differences among communities even in treatments that promoted uranium reduction
- There appear to be limitations imposed on the community related to some substrates (e.g., methanol).
- Further data and analysis of the community structure is on going (e.g. functional gene arrays, T-RFLP, clone libraries).

ACKNOWLEDGEMENTS

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