

**Summary of Istok Group Breakout Session.
FRC meeting at Oak ridge National Lab. Sept. 24, 2003.**

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Krumholz:

Both laboratory and field experiments have demonstrated that as denitrification is stimulated in FRC sediments containing nitrate, U(IV) is oxidized resulting in increased levels of dissolved U(VI). Our original hypothesis was that oxidized N intermediates from denitrification were directly oxidizing the U(IV) to U(VI). Recent experiments have verified that U oxidation occurs in FRC groundwater with abundant nitrate (FW021) but does not occur in groundwater with low levels of nitrate (GW835). Experiments were carried out in a model system (FW021 groundwater and washed cells of a denitrifying bacterium isolated from the site). Results showed that electron donor addition, which results in formation of nitrite dramatically stimulates U oxidation. In this model system dissolved Fe(II) is rapidly oxidized presumably forming Fe(III). Further lab experiments showed that Fe(III) oxidizes U but the extent of oxidation is limited unless nitrite is present. Nitrite does not oxidize U but maintains Fe in the oxidized form allowing Fe(III) to continue to oxidize a large fraction of U.

Experiments showed that Tc(VII) is biologically reduced in subsurface sediments and nitrate addition does not result in reoxidation.

Most of the locations in area 1 have a low pH (3-6) and biostimulation is the ultimate goal of our experiment. However, microbial activity in FRC sediments appears to be inhibited in sediments with a pH below 4.5. In order to study this process, we have isolated microorganisms capable of denitrification from biostimulated wells and tested their ability to grow under low pH conditions. Two of the strains will grow in media down to a pH of 4.5. These strains will allow us to test the effect of Al(III) on growth at low pH values. Humic compounds appear to aid in survival of these strains at low pH and further studies are being carried out in this area.

2003 publications related to this work:

1. Chang, I.S., J. L. Steger, M. M. Ramsey, J.D. Ballard and L.R. Krumholz. 2004. Differential Expression of *Desulfovibrio vulgaris* in Response to Metal Toxicity. **Appl. Environ. Microbiol.** (Submitted 8/03).
2. Elias, D.A., J.M. Suflita, M.J. McInerney and L.R. Krumholz. 2004. The periplasmic cytochrome C_3 of *Desulfovibrio vulgaris* is directly involved in H_2 mediated metal-, but not sulfate-reduction. **Appl. Environ. Microbiol.** (In press).
3. Istok, J.D., J.M. Senko, L. R. Krumholz, D. Watson, M.-A. Bogle, A. Peacock, Y-J. Chang, and D.C. White. In Situ Bio-Reduction of Technetium and Uranium in a Nitrate-Contaminated Aquifer. **Env. Sci. Technol.** (In press).

4. Peacock, A.D. Y-J. Chang, J. D. Istok, L. R. Krumholz, R. Geyer, and D.C. White. 2003. Utilization of Microbial Biofilms as Monitors of Bioremediation. **Microbial Ecology**. (In Press)
5. Wong, D., J.M. Suflita, J.P. McKinley and L.R. Krumholz. 2003. Impact of Clay Minerals on Sulfate-Reducing Activity in Aquifers. **Microbial Ecology**. (In press, Online First).
6. Elias, D.A., D. Wong, L.R. Krumholz and J.M. Suflita. 2003. Characterization of microbial activities and uranium reduction in a shallow aquifer contaminated by an UMTRA disposal cell. **Microbial Ecol.** 46:83-91.
7. Chang, I., J.D. Ballard and L.R. Krumholz. 2003. Evidence for Chimeric sequences during random arbitrarily primed PCR. **J. Microbiol Meth.** 54:427-431.
8. Elias, D.A., J.M. Senko and L.R. Krumholz. 2003. A procedure for quantitation of total oxidized uranium for bioremediation studies. **J. Microbiol Meth.** 53/3:343 – 353.
9. Krumholz, L.R., D.A. Elias and J. M. Suflita. 2003. Immobilization of cobalt by sulfate-reducing bacteria in subsurface sediments. **Geomicrobiol. J.** 20(1):61-72.

Kostka:

Our laboratory group discussed results from push-pull experiments conducted in Area 1. We analyzed the solid phase of sediment cores sampled immediately adjacent to the Istok wells in Area 1 before and after *in situ* addition of electron donors, glucose and ethanol. Microbial communities were analyzed by generating clone libraries for the 16S rRNA genes and using a quantitative PCR approach to determine the gene copy number for known metal-reducing bacteria detected in previous cultivation-based experiments by our group. Quantitative PCR results indicated that *Geobacter* sequences increased by 1 to 2 orders of magnitude upon addition of electron donors, indicating that this group was important to electron acceptor utilization in contaminated FRC sediments. Results from clone libraries corroborated those from quantitative PCR to show that sequences from the δ -Proteobacteria increased from 7 % to 40 % of the sequence retrieved from sediments after addition of electron donors. Geochemical results from our group showed that sediment pH increased and the majority of nitrate was depleted in the same FRC sediments that were studied for microbial communities.

Joseph W. Stucki

IRON MINERALOGY OF FRC SOILS AND SEDIMENTS

The objectives of this study are to determine the basic Fe mineralogy of the soils at the FRC site, then to assess changes in Fe mineralogy that occur as a result of

biostimulation. Soil samples from the background area and Area 1 were studied with respect to their Fe mineralogy using Mössbauer spectroscopy and wet chemical methods of analysis. Samples from biostimulated cores were also investigated by these methods and correlations made, to the extent possible, with nearby wells that received no biostimulation. Samples were protected as much as possible from the atmosphere and were kept frozen until analysis.

Analyses of the background sample (FWB-302) revealed that more than half of the particles are in the gravel (> 2 mm) range, whereas only about 6.5 % is in the clay-size fraction. Chemical data revealed an overall pH of 5.4, no carbonate, total Fe content of 1.83% by weight, Fe(II) content of 3.6% of total Fe, and organic C content (loss on ignition) of 2.8% by weight. The total Fe content of the clay-size fraction of this sample was 7.9% by weight, which could be separated into coarse clay and fine clay, with total Fe contents of 7.6 and 8.2%, respectively. Iron was distributed as approximately 40% to 70% in the layer silicates, depending on the specific well location, and the balance in one or more Fe oxide phases. The Fe oxide phases present are most likely goethite, with the possibility of a poorly crystallized superparamagnetic phase. No definitive conclusions can be made on that question until Mössbauer spectra at 4.2 K can be acquired (coming soon).

Biostimulation of soil profiles adjacent to the contaminated wells were performed by injecting an electron donor (C source) in the form of either ethanol or glucose. Sediments were then sampled under Ar gas, frozen, and transported to the lab where they were kept in the freezer until analysis. The Mössbauer and chemical analyses for oxidation state revealed similar trends in both unstimulated and biostimulated samples. Biostimulated sample 45-01-42 (paired with unstimulated sample FWB-032) had the highest level of Fe(II) at about 11% of the total Fe. Sample 49-01-8 (paired with unstimulated sample FWB-034) had the least amount at about 3.6%. Comparisons with the original sediments showed that biostimulation increased Fe(II) content. In FWB-032 it increased from 6.87 to 11.07% if ethanol was the electron donor or to 8.31% if glucose was the electron donor. Similar trends were noted for the other samples.

Evidently the choice of electron donor (ethanol or glucose) not only caused some difference in the extent of reduction, but also changed the amount of Fe oxide removed from the sample. For example, in sample FWB-032 biostimulation with ethanol as the electron donor (sample 45-01-42) decreased the Fe oxide content from 56.3% of total Fe to only 35.1%. If glucose was the electron donor (sample 46-01-07), virtually no Fe oxide was removed. The particular Fe oxide removed with ethanol as the electron donor appears to be the least crystalline phases. No surprise there, but it is interesting that we can verify it. This is clearly visible in the Mossbauer spectra (Figure 5). The peak area for the Fe(II) doublet in the silicate phase increased from about 17% of total Fe to about 23% of total Fe (Table 8); or in terms of Fe(II):Fe(III) ratio, the changes were 0.21 to 0.31.

Biostimulation of sediment FWB-033 with glucose (sample 47-01-39 [or 36]) created a more complex magnetically ordered system (Figure 5) than the original sample and than in any of the other biostimulated samples. A new sextet at high field appeared, and the Fe(II) content increased. We need to complete analysis of the oxalate and CBD extractions before drawing further conclusions about this, except the amount of oxide dissolution appears to be rather small, similar to the glucose treatment of FWB-032.

In contrast with that observation, biostimulation of sediment from well FWB-034,

with glucose (sample 49-01-48) as the electron donor, removed almost all of the silicate phase, and sextet patterns dominate the Mössbauer spectra; but, the total Fe content is greater (6.56%) than in the other biostimulated samples (4.7 to 4.99%), and even greater than in the unstimulated sample (4.50%).

From these observations we conclude that biostimulation increases the Fe(II) content of the sediment, and ethanol more effectively promotes Fe oxide dissolution than does glucose. Glucose, however, promotes the dissolution of the silicate phase in favor of greater amounts of Fe oxide phases.

Inspection of the data indicates that some difficulties exist in these analyses, because treatments sometime increase the Fe content rather than decreasing it and some other inconsistencies are apparent. Further work is in progress to check three variables that could influence conclusions regarding these samples. The first is a preferential distribution of Fe minerals among different aggregate sizes. We did not dry, crush, and homogenize the sample, but rather selected the finer particle size fraction for Mössbauer analysis. The larger grains need to be checked also. Second, we are conducting oxalate and CBD extractions on these samples, which will give us even better quantitative results regarding the dissolution of the Fe oxide phases. And third, acquisition of Mössbauer spectra at 4.2 K are in progress in order to confirm the presence or absence of ferrihydrite and/or other superparamagnetic phases.

Baohua Gu, Oak Ridge National Laboratory

The FRC area 1 groundwater is not only characterized by a low pH but also a high toxic metal content (e.g., Al and Ni), which greatly limit the microbial activity and the success of bioremediation at the site. In this study, **the complexation of humic substances with Ni and Al are being investigated** to evaluate the effect of humics in reducing metal toxicity to anaerobic microorganisms. We first studied the ability of humics in complexing metals in laboratory. Results to date indicate that humic substances have a relatively high capacity in complexing Ni and Al; even in the presence of a high concentration of Ca, the FRC humic acid was able to retain ~0.8 mmol/g FRC humics. The FRC humic acid could be characterized by two adsorption sites: a low pH site and a high pH site which correspond to carboxylic and phenolic functional groups. Potentiometric titrations were also used to characterize the intrinsic acidity constants of the two sites and the concentrations of the different surface functional groups. Results of these studies are being summarized in a technical manuscript entitled “the complexation of humic substances with heavy metals in a contaminated groundwater.”

A number of field push-pull tests were recently performed in collaboration with J. Istok at OSU to evaluate the effects of humic substances in enhancing the bioreduction of U(VI) in situ at the NABIR FRC area 1 and 2 sites. Preliminary results appear to be encouraging; we found that the addition of FRC humic acid increased the rate of U(VI) removal (as high as 100%) as compared with the control (without addition of humics in the same injection well). Although the exact mechanisms of the enhanced U(VI) removal by humics are yet to be determined, these observations could be attributed to (1) an increased bioreduction rate of U(VI) due to a reduced toxicity of metals to microorganisms, (2) an increased electron shuttling effect of humics, and (3) an increased

precipitation or co-precipitation of U(VI) with humics (or metal-humic complexes). Field samples and data are still being analyzed, and results will be summarized in the coming NABIR workshop in March 2004. Additionally, laboratory batch studies are being performed to determine the effect of FRC humics on the bioreduction rate of U(VI) in the presence or absence of toxic metals such as Ni and Al.

An important finding of our recent studies suggests that the reduced U(IV) species appear to form complexes with humics and thus stay in the solution phase so that U(IV) could **not** be filtered out by using 0.2- μm filters. This technique (filtration by 0.2- μm filters) has been widely used to separate the reduced forms of U(IV) (as precipitates) from the oxidized forms of U(VI). Apparently, this assumption is false in the presence of humics or other organic ligands which may prevent the precipitation of the reduced U(IV) species in solution. In collaboration with B. Burgos at PSU, who initially reported that humics completely inhibit the U(VI) bioreduction (NABIR workshop, March 2003), we found that the reduced U(IV) (passed through the 0.2- μm filters with humics) could be rapidly re-oxidized so that subsequent KPA analysis significantly underestimated the reduced forms of U(IV). Further studies are still in progress to evaluate if reduced forms of U(IV) are truly complexed with humic molecules or stay in the solution phase as precipitated U(IV) nanocolloids (which also pass through the 0.2- μm filter), as some researchers suggested. A manuscript is in preparation (led by Burgos), which summarizes our recent findings and discrepancies on the bioreduction of U(VI) in the presence or absence of humic substances.

Publications:

Gu, B., S. C. Brooks, Y. Roh, and P. M. Jardine. 2003. Geochemical reactions and dynamics during titration of a contaminated groundwater with high uranium, aluminum, and calcium. *Geochim. Cosmochim. Acta.* 67,2749-2761.

Gu, B. and J. Chen. 2003. Enhanced microbial reduction of Cr(VI) and U(VI) by different natural organic matter fractions. *Geochim. Cosmochim. Acta* 67:3575-3582.

Chen, J., B. Gu, R. A. Royer, and W. D. Burgos. 2003. The roles of natural organic matter fractions in chemical and microbial reduction of ferric iron. *Sci. Total Environ.* 307:167-178.

W. Wu, B. Gu, M. W. Fields, M. Gentile, Y. Ku, S. Tiquias, J. Nyman, J. Zhou, P. M. Jardine, C. S. Criddle. 2004. Characterization of uranium(VI) reduction by microbial biomass from a denitrifying fluidized bed reactor. *Environ. Sci. Technol.* (Manuscript completed).

Gu, B., Y. K. Ku, and P. M. Jardine. 2004. Sorption and binary exchange of nitrate, sulfate, and uranium on an anion-exchange resin. *Environ. Sci. Technol.* (in review).

McKinley

Reaction path modeling may aid in determining the chemical reactions responsible for the solute concentration observed during push-pull testing. We completed three tests to

evaluate this methodology, in which a complete suite of relevant analytes are being analyzed. These include dissolved anions, cations, inorganic carbon, pH, along with dissolved U, Tc. Time point results will be used to calculate reactant products for plausible hypothetical reactions through the withdrawal phase of the tests. Also, the impact of fine-scale vertical heterogeneity will be evaluated using three newly installed boreholes located in close proximity to one another. Multilevel samplers installed in these boreholes are in place to determine pre-test compositional variation. Subsequent testing will determine whether the zone of injection includes only a portion of the screened interval. The results of these investigations will be used in designing future tests.