U.S. Department of Energy, Office of Science
Environmental Remediation Sciences Division (ERSD)
FY08 Fourth Quarter Performance Measure

Introduction

This report is the fourth in a series of quarterly progress reports from the Rifle Integrated Field Research Challenge (IFRC) project on the ERSD general performance measure: **"Identify the critical redox reactions and metabolic pathways involved in the transformation/sequestration of at least one key DOE contaminant in a field environment."** The first quarter report documented the approach to identify critical redox reactions in the subsurface during stimulated uranium biotransformation. The second quarter report documented the approach to identify key microbial metabolic processes in the subsurface during stimulated uranium biotransformation. The third quarter report documented development of the conceptual process models from new knowledge of uranium biotransformation and the approach to coupling these models in numerical simulations of field scale flow and reactive transport. The topic of this report (Fourth Quarter Performance Measure) is ***the evaluation of collected field results on microbial metabolic processes with the detected changes in redox reactions in situ during uranium biotransformation.*** Previous performance measures are available at: http://www.lbl.gov/ERSP/generalinfo/milestones/ersd_data08.html.

Background

Over the last several years, a number of field-scale experiments have been conducted at contaminated sites in an effort to understand the stimulated immobilization of contaminant metals and radionuclides as a potential remediation strategy. Such approaches could lead to lower cost techniques for immobilizing low concentrations of metal and radionuclide contaminants found across large areas at DOE sites. The underlying processes of stimulated immobilization include a stimulation of reducing conditions within the subsurface that facilitates a change in redox status of the contaminant metals and/or radionuclides leading to an incorporation of contaminants into precipitated mineral phases, enhanced sorption on residual biomass or enhanced sorption onto mineral surfaces. These stimulated processes include both biotically and abiotically catalyzed reactions which, collectively, contribute to a decrease in groundwater concentrations of contaminants below applicable standards. In this report we summarize the results of a series of field-scale experiments performed at the Old Rifle uranium mill tailings remedial action (UMTRA) site located in Rifle, Colorado beginning in 2002 and continuing through 2008. In these experiments, acetate was injected into the subsurface of a uranium-contaminated aquifer in controlled test plots to stimulate the activity of anaerobic bacteria capable of enzymatically reducing metals and radionuclides to insoluble forms. Previous laboratory studies had indicated that the addition of acetate to natural sediments stimulates the activity of Fe(III)-reducing bacteria of the *Geobacter* family, a species known to reduce uranium to an insoluble form and indeed couple growth to this process (Gorby and Lovley, 1992; Lovley et al., 1991; Lovley et al., 1993). Experiments at the Old Rifle site determine the efficacy of stimulating metal- and radionuclide-reducing microorganisms in the field at a contaminated site as a test of an *in situ* remediation strategy. Initial experiments showed that metal-reducing microorganisms of the *Geobacter* family could be stimulated *in situ* and this stimulation

coincides with a loss of soluble uranium in the groundwater, consistent with the known metabolism of these organisms (Anderson et al., 2003; Ortiz-Bernad et al., 2004; Vrionis et al., 2005). Recent experiments have focused on the mechanistic details of stimulated metal and uranium reduction, including specific gene and protein expression profiles of the *in situ* microbial community that correlates with uranium reduction, geochemical changes in the subsurface associated with uranium reduction and the transition from metal-reducing conditions to sulfate-reducing conditions reduction in these test plots.

Field experiments conducted at the Rifle site to date

Field experiments conducted at the Rifle site are summarized in Table 1. Each experiment was conducted with specific goals and objectives to test scientific hypotheses. Sophistication of the experiments in terms of methods of amendment injection, use of cross-well mixing, etc. were improved from year to year and the extent and nature of sampling increased dramatically starting in 2007 when the first experiment was conducted at the site after it was established as an Integrated Field Research Challenge Site (IFRC) by the U.S. Department of Energy, Office of Science.

All of the experiments were conducted *in situ* under natural conditions of groundwater flow in structured porous media consisting of an alluvial aquifer deposited by the Colorado River. This material is mapped as Quaternary Flood Plain (Qfp) deposits (Shroba and Scott, 1997). These deposits rest on the Wasatch Formation that serves as an impermeable lower boundary to the alluvial aquifer. All of the experiments were performed by introducing moderate concentrations of acetate stock solution into a line of wells spaced ~1 m apart such that (Fig. 1) dilution in the well bore and in the surrounding aquifer resulted in a concentration of 3 to 15 mM in the formation as noted above. The target *in situ* concentration was selected depending on the particular experiment and its objectives. Up-gradient monitoring wells were used to track U(VI) concentrations coming into the experimental plot and wells down gradient from the injection wells were used to observe the impact of acetate introduction on U(VI) concentration and other geochemical and microbiological parameters of interest. It is important to note that in this configuration, up-gradient concentrations of U(VI) are continually introduced to the experimental plot by groundwater flow. Thus, decreases between up- and down-gradient concentrations of U(VI) must be maintained by a continuous reaction that precipitates U(VI) from solution, in this case direct enzymatic reduction of soluble U(VI) to an insoluble U(IV) mineral. This conclusion assumes that sorption of U(VI) remains nearly constant throughout the system, an assumption that will be directly tested as part of continued experiments at this test site.

Field Experiment	*Objectives*	*Key Observations/results*	*Conclusions/selected peer-reviewed papers*
2002	Determine if biostimulation removes U(VI) from flowing groundwater under *in situ* conditions by microbial reduction of U(VI) to U(IV).	U(VI) decrease in groundwater; rate of decrease goes down with sulfate reduction; *Geobacter* growth associated with U(VI) decrease in groundwater	Electron donor amendment works at the field scale, *Geobacter* apparently responsible for U(VI) decrease (Anderson et al., 2003)
2003	Extend Fe(III) reduction and U(VI) decrease in time and space by increasing acetate concentration.	Extensive sulfate reduction, Fe(III) reduction down gradient, U(VI) decrease; unexpected prolonged U(VI) reduction post-acetate addition.	Increasing electron donor works, but sulfate reduction may be both problematic during acetate amendment or may help limit reoxidation of U(IV) during post-amendment phase (Vrionis et al., 2005)
2004	Replicate U(VI) bioreduction, obtain genomic and mRNA samples, stable isotope probing, test geophysical monitoring.	U(VI) similar to 2002 experiment. *Geobacter* dominance, ^{13}C observed in PLFA, geophysics consistent with Fe reduction.	U(VI) decrease replicable at field scale, mRNA promising for site assessment and monitoring of remediation, SIP tracking of acetate utilizers possible. Geophysics may provide 3-D subsurface image of bioremediation progress (Chang et al., 2005; Holmes et al., 2005b)
2005	Replicate U(VI) bioreduction, obtain additional samples for new gene expression analyses, test function of new minigallery for 2006 experiment, conduct geophysical monitoring.	U(VI) removal similar to 2002 and 2004 experiment, with *Geobacter* dominance. Rebound in U(VI). Geophysics consistent with low amount of sulfide precipitation.	U(VI) decrease replicable at field scale. Reoxidation may be correlated with little sulfate reduction/low biomass. Geophysical monitoring continues to be promising.
2006	Direct comparison of post-acetate amendment U concentration changes after Fe-reducing and sulfate reducing conditions	U(VI) decrease in groundwater sustained after sulfate reduction, rapid U(VI) rebound after Fe-reduction.	Confirms apparent importance of sulfate reduction (sulfide) or biomass associated with sulfate reduction for sustained decrease in U(VI) after acetate amendment (N'Guessan et al., 2008).
2007* (Winchester)	Replicate Fe-reduction (*Geobacter* dominated) during acetate amendment while testing proteomic sampling. Obtain full suite of related samples and monitoring.	Proteomic and genomic samples successfully obtained. U(VI) decreased below applicable standards, rapid U(VI) rebound.	Four species of *Geobacter* dominate groundwater samples. Proteomic data analysis still underway. Several papers nearing submission to journals for peer review.
2008* (Big Rusty)	Obtain time-series samples for proteomics and genomics to track microbial population during acetate amendment. Fe-reduction and transition to sulfate reduction.	U(VI) decreased below applicable standards. Detailed time series proteomics samples obtained from 200 to 400 liter samples. Significant changes in permeability observed.	Experiment complete, samples still being analyzed.
2008* (Little Rusty)	Conduct field-scale de-sorption tracer test by adding bicarbonate and tracer to subsurface and monitoring U(VI) desorption under ambient Rifle IFC conditions.	Initial tracer test complete. Desorption tracer test started 18 Sept 2008.	Experiment still in progress. Future experiments will address desorption behavior under abiotic Fe-reducing conditions.

*Rifle IFRC field experiments.

Table 1. Summary of field experiments conducted at the Old Rifle site 2002-2008.

Overview of results from field experiments

Since 2002, field studies of *in situ* bioremediation of uranium-contaminated groundwater at the field study site in Rifle, Colorado have shown remarkable repeatability in both geochemical and microbiological response. Results from these field experiments have indicated that there are three distinct phases following the addition of acetate to the subsurface to stimulate microbial activity. In phase I, both Fe(III) minerals and soluble

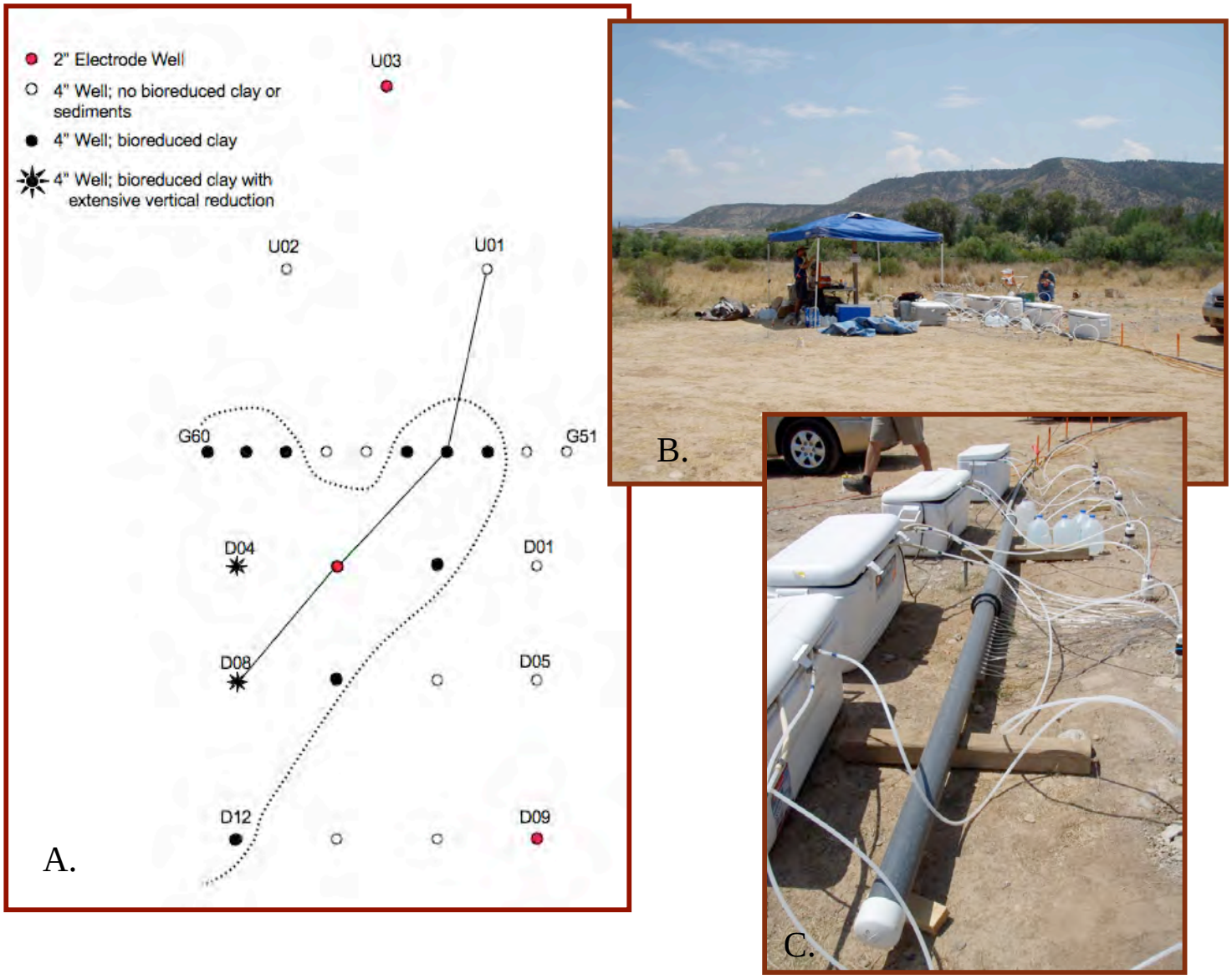

Figure 1. A) Well layout for the Big Rusty field experiment conducted during the 2008 field season. Groundwater flows from from well U02 toward D11. Acetate injection wells are G51-G60. Dashed line outlines zone of naturally bioreduced sediments in the subsurface. B) Photograph of well field. C) Photograph of acetate injection system.

U(VI) are reduced, reduced uranium precipitates and is removed from the groundwater coincident with the stimulation and prevalence of *Geobacteraceae* within the subsurface (Anderson et al., 2003). In phase II, the microbial reduction of sulfate begins as part of a normal transition of stimulated anaerobic processes from metal-reduction to sulfate reduction. Interestingly, the removal efficiency of U(VI) is diminished as sulfate-reducing bacteria (SRB) become the predominant microbial community (Vrionis et al., 2005). In phase III, U(VI) removal resumes and continues even after acetate additions are stopped (N'Guessan et al., 2008). Conditions required for this third phase include amendment with high acetate concentrations or amendment of the same plot for two successive years. N'Guessan and coauthors (2008) showed that phase III field behavior could be largely replicated in column experiments and concluded that sustained removal of U(VI) from groundwater was caused by sorption of U(VI). The study also suggested that biosorption plays an important role during this phase, with members of the *Firmicutes* contributing to the bulk of the microbial community sorbing U(VI). However, it is also true that sulfides precipitated during sulfate reduction are present in these sediments and their role in long-term removal of U(VI) is still being determined. What is clear is that if Fe-reduction is the dominant terminal electron-accepting process during a field experiment, cessation of acetate amendment results in a rapid rebound of U(VI) to concentrations equal to the up-gradient values. However, there is no strong evidence of a reoxidation pulse of U(VI) resulting in concentrations greater than up-gradient values, suggesting that U(IV) is stabilized or very slowly oxidized under field conditions at the Rifle Site. For longer experiments transitioning to sulfate reduction, rebound is limited and then may reverse or may not occur until later in time. In these cases there is thought to be both greater biomass and precipitated sulfide and calcite present in the subsurface than with just iron reduction.

In all four experimental plots that have been amended with acetate in a total seven different experiments, the common result has consistently been a decrease in soluble U(VI) concentration in groundwater that is directly correlated to *Geobacter* growth as determined using multiple techniques. These techniques have been performed mainly on groundwater samples, but also on sediment samples and include 16S-based rRNA microbial community analysis (Anderson et al., 2003; Vrionis et al., 2005), TRFLP (terminal restriction fragment length polymorphism), and phospholipid fatty acid (PLFA) analysis (Anderson et al., 2003; Chang et al., 2005). In addition, stable isotope probing (SIP) experiments have confirmed that *Geobacteraceae* are stimulated and become dominant in the groundwater following acetate amendment. Further studies quantifying mRNA transcripts have also shown that the predominant *Geobacteraceae* community becomes more active upon addition of acetate to the aquifer and this increased activity also correlated with uranium removal from the groundwater (Holmes et al., 2005a; Holmes et al., 2004; Holmes et al., 2005b).

Evaluation of microbial metabolic processes and changes in redox reactions

Microbial metabolic processes during Phase I-**Iron reduction**:

The availability of genome sequences for *Geobacter* spp. has enabled the study of specific metabolic characteristics of *Geobacteraceae* during *in situ* biostimulation. Given our ability to evaluate the metabolic state of bacteria by measuring the abundance of key

genes, several studies quantifying *Geobacter*-specific mRNA transcripts have been performed (e.g., (Holmes et al., 2005a; Holmes et al., 2004). Because *Geobacteraceae* have been determined to be the predominant organisms responsible for the bulk of uranium removal under iron-reducing conditions, studies have been undertaken with the goal of developing a more fundamental understanding of the metabolic state of these bacteria during both iron and uranium reduction. For example, studies evaluating our ability to monitor nutrient limitation in *Geobacteraceae* were performed during the 2007 Winchester field experiment. Investigations of key nutrient uptake genes for acetate, nitrogen and phosphate have suggested that it may now be possible to identify the environmental stresses that may affect the ability of *Geobacteraceae* to thrive and continuously and efficiently reduce uranium throughout the biostimulation period. Furthermore, genes that would allow the monitoring of the *in situ* growth rates of *Geobacter* were evaluated and could potentially enable us to determine under which metabolic state (or growth rate) the optimal uranium removal rates can be achieved. Such approaches could potentially enable a better optimization of the biostimulation method by finding ways to alleviate environmental stresses that inhibit the ability of microorganisms to reduce uranium. In addition to the quantification of mRNA transcripts, proteomics studies may also direct us to key microbial metabolic processes under the varying geochemical conditions that exist at the site during biostimulation.

Microbial metabolic processes during Phase II-**Transition phase**:
During the initial transition from iron to sulfate reduction - as determined by decreasing ferrous iron concentration in groundwater accompanying the accumulation of dissolved sulfide (Chapelle et al., 1995) - uranium concentrations continue to decrease, in some cases reaching levels at or below the EPA's maximum contaminant level (MCL; 0.13μM). At later stages into the transition, uranium removal becomes less effective, with uranium concentrations fluctuating. It has been hypothesized that this "loss" of uranium removal efficiency is a result of 1) the decline in abundance of *Geobacteraceae* responsible for uranium removal under iron-reducing conditions and 2) the rise of a microbial community dominated by acetate-oxidizing sulfate reducing bacteria incapable of reducing uranium. Further investigation of the metabolic processes during this transition phase could elucidate this behavior from the microbial standpoint. Proteomic, genomic, and transcriptomic analysis of the microbial community during the transition phase would provide some insight about the state of Geobacteraceae during this transition period. Some information about the sulfate-reducing bacteria community may also be inferred. However, the current availability of little genomic information for sulfate-reducing bacteria that predominate during biostimulation at the site, may limit the full interpretation of the data. Efforts are underway to isolate SRB from the site in order to obtain their genomic sequences and allow for the full analysis of the proteomic and genomic samples.

Microbial metabolic processes during Phase III-**Sulfate reduction and beyond**:
Once sulfate reduction becomes the dominant process, as determined by a significant (mM) removal of sulfate from the groundwater, uranium concentrations again begin to decrease, albeit to levels in excess of those achieved during the earliest phase of the transition period (Phase II). Uranium removal under these conditions may be promoted

by the strong reducing conditions that exist in the subsurface at this point during the biostimulation tests. It is not yet clear which sulfate-reducing organisms could be responsible for uranium reduction under these conditions. Onsite screening of sulfate-reducing bacteria during the Big Rusty experiment (Table 1) have suggested that *Desulfobacter sp*. may be the predominant acetate-oxidizing SRB during phase III of biostimulation. However, there are no known *Desulfobacter spp*. able to utilize uranium as an electron acceptor. On the other hand, *Desulfovibrio spp*. have been shown to be capable of reducing uranium. *Desulfovibrio spp*. prefers more complex sources of carbon such as lactate and are not able to oxidize acetate.

It is possible that, as we advance further in the biostimulation phases, more complex organic material may be produced in the subsurface as a result of microbial activity or as the stimulated microbial community begin to die. This complex mixture of carbon could become a substrate source for organisms such as *Clostridia, Desulfovibrio* or even *Mollicutes* or certain *Geobacter spp*. during prolonged addition of acetate as well as once acetate is stopped. Most field studies at the site have focused on the extensive characterization of the Phase I of biostimulation. The Big Rusty experiment will be the only experiment since 2002 and 2003 to study in more detail the microbial metabolic processes involved in phase III of biostimulation.

Outstanding science questions and uncertainties

Several outstanding science questions remain to be addressed through planned research activities at the Old Rifle Site. Each of these questions is related to the overall objective of the research conducted at the site, which is the development of a mechanistic understanding of the long-term behavior of uranium in the subsurface. From the standpoint of stimulated bioremediation, the feasibility of employing low infrastructure approaches for *sustained* uranium immobilization remains to be further developed. The aforementioned field experiments have demonstrated our ability to successfully remove uranium from groundwater to levels at or below the EPA's maximum contaminant level MCL following acetate injection and the stimulation of metal-reducing conditions in the subsurface (Fig. 2). What is unclear at this point, however, is whether this strategy is sustainable over extended periods of time. Microbial activity plays an important role in limiting groundwater concentrations of uranium. Sustaining this activity *in situ* will require continued, systematic evaluation of the functioning of microbial communities to develop methods to target and control desired microbial activities such as metal reduction. Planned proteomic and gene expression research activities are poised to address the key processes needed for optimized and sustained uranium removal. Our working hypothesis is that the onset of limited sulfate reduction creates environmental conditions favoring enhanced uranium removal, possibly through the alteration of geochemical conditions that induce metabolic changes in the microorganisms primarily responsible for uranium immobilization (Fig. 3). Identification of *Geobacter*-specific genes and proteins expressed during the period of optimized uranium removal is an ongoing research objective at the Rifle site that will greatly assist our understanding of the processes controlling efficient uranium bioremediation.

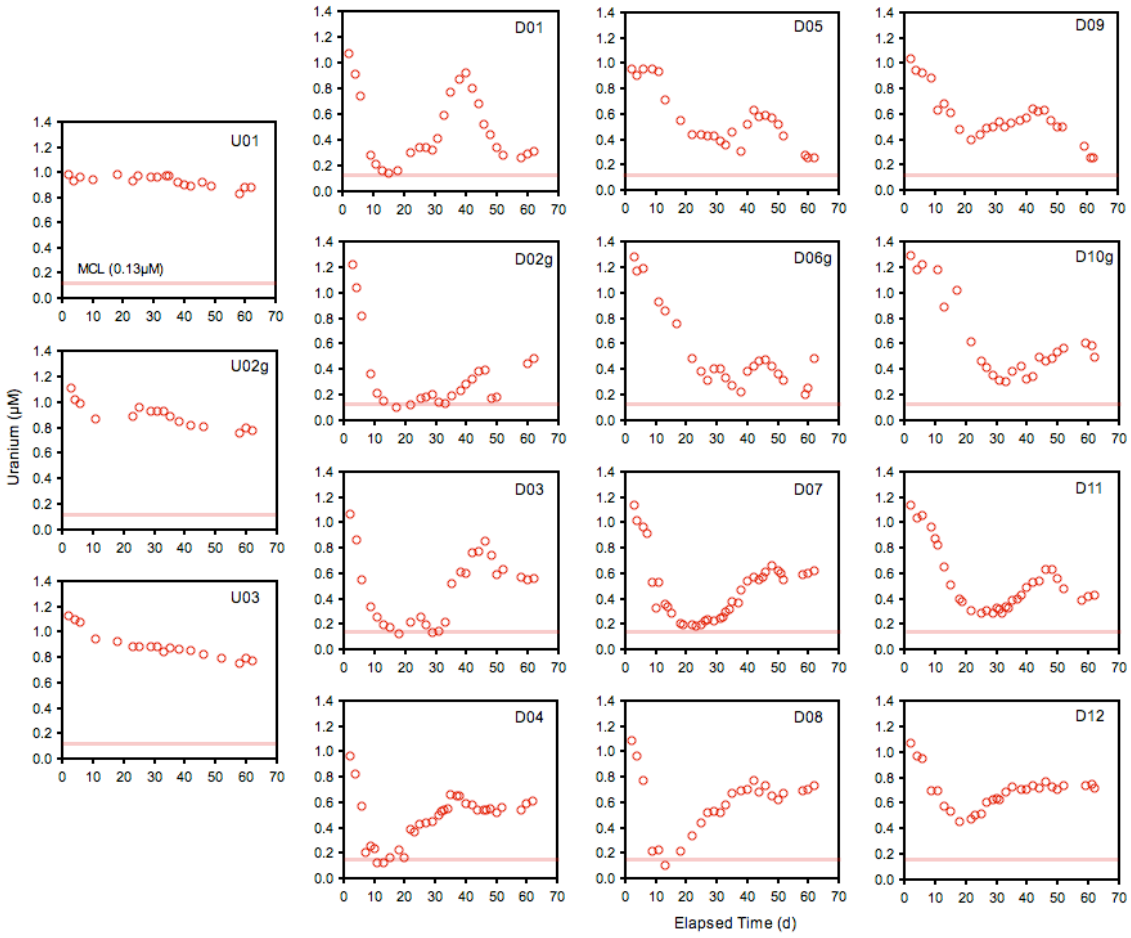

Figure 2. Spatiotemporal changes in groundwater uranium concentration accompanying the injection of acetate at the Old Rifle site during the 2008 "Big Rusty" field experiment (Table 1); the red bar indicates the EPA's MCL for uranium (0.13µM). Locations labeled "U" and "D" represent wells "up" and "down"-gradient from the region of acetate amendment, respectively; groundwater flow is from left to right. An acetate-free groundwater flush was conducted from days 16-27, after which time injection of acetate (5mM) was resumed. After confirming complete consumption of acetate between the region of injection and the first row of down-gradient wells (D01 to D04), the injected acetate concentration was increased from 5mM to 25mM on day 40.

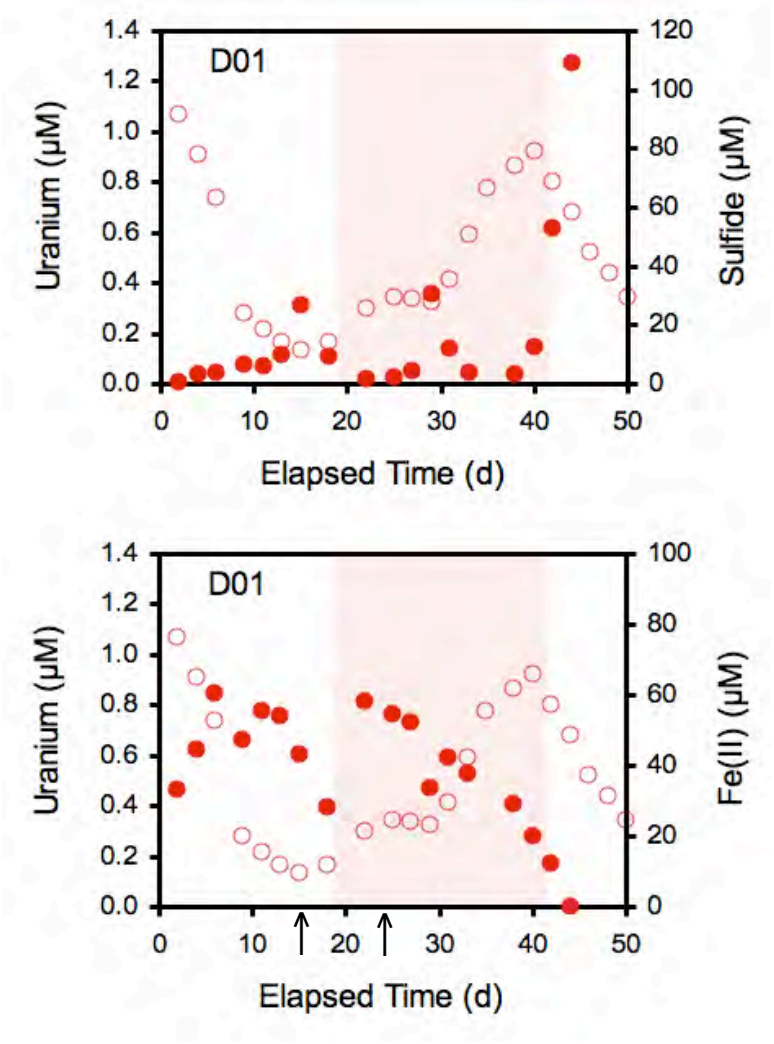

Figure 3. Temporal change in the concentration of uranium (open symbols), sulfide, and ferrous iron in well D01 during the 2008 "Big Rusty" field experiment. The initial increase in ferrous iron corresponds to the rapid removal of uranium from groundwater. The onset of limited sulfate reduction (ca. day 14) accompanies both the removal of ferrous iron from solution (likely as FeS) and removal of uranium to levels below the MCL (0.13µM). Following the acetate-free groundwater flush (arrows on lower panel mark start and end of the flush) and the complete consumption of acetate upgradient from well D01, acetate concentrations fell below 80µM over the 23-day time period highlighted in red. The injected acetate concentration was increased from 5 to 25mM on day 40; following the reappearance of acetate in the vicinity of D01, uranium concentrations again began to decrease dramatically before fluctuating at later times (Fig. 2). The relationship between acetate availability and uranium removal from groundwater cannot be overstated.

Furthermore, field research activities investigating the pool of sorbed uranium in the presence and absence of stimulated microbial activity are critical to assessing the extent to which uranium immobilization can be sustained. In addition to recharge from upgradient locations, the observed rebound in groundwater uranium concentrations accompanying the complete consumption of acetate is related to the pool of uranium sorbed to aquifer sediments and the rate at which it desorbs under different geochemical conditions. We need to identify the extent to which uranium desorption occurs during our current approach to stimulated bioremediation (e.g. acetate injection) and how it extends to background conditions (e.g. desorption from both naturally bioreduced and relatively oxidized sediments). Insofar as the long-term management of contaminated DOE legacy sites is concerned, a vastly improved understanding of the extent to which sorbed uranium is released to groundwater during stimulated and natural conditions may represent one of the more important scientific question to be addressed by our research team.

We are also actively investigating the extent to which regions of elevated subsurface microbial activity influence the fate and transport of uranium in the subsurface. We are studying such zones of natural bioreduction in terms of their role as net sources or sinks for uranium, an understanding of which will be critical in the decision making process regarding long-term management of legacy sites where such features are known to occur (e.g. the Hanford 300 area). Although naturally occurring, the biogeochemical conditions present in such zones are clearly related to those that develop during acetate-mediated biostimulation. As such, the research approaches presented above are ideally suited to addressing the outstanding scientific questions associated with these features.

References Cited

Anderson, R.T., Vrionis, H.A., Ortiz-Bernad, I., Resch, C.T., Long, P.E., Dayvault, R., Karp, K., Marutzky, S., Metzler, D.R., Peacock, A., White, D.C., Lowe, M., and Lovley, D.R., 2003, Stimulating the in situ activity of Geobacter species to remove uranium from the groundwater of a uranium-contaminated aquifer: Applied and Environmental Microbiology, v. 69, p. 5884-5891.

Chang, Y.J., Long, P.E., Geyer, R., Peacock, A.D., Resch, C.T., Sublette, K., Pfiffner, S., Smithgall, A., Anderson, R.T., Vrionis, H.A., Stephen, J.R., Dayvault, R., Ortiz-Bernad, I., Lovley, D.R., and White, D.C., 2005, Microbial incorporation of C-13-labeled acetate at the field scale: Detection of microbes responsible for reduction of U(VI): Environmental Science & Technology, v. 39, p. 9039-9048.

Chapelle, F.H., Mcmahon, P.B., Dubrovsky, N.M., Fujii, R.F., Oaksford, E.T., and Vroblesky, D.A., 1995, Deducing the Distribution of Terminal Electron-Accepting Processes in Hydrologically Diverse Groundwater Systems: Water Resources Research, v. 31, p. 359-371.

Gorby, Y.A., and Lovley, D.R., 1992, Enzymatic Uranium Precipitation: Environmental Science & Technology, v. 26, p. 205-207.

Holmes, D., O'Neil, R., Nevin, K., Ward, J., Adams, L., and Lovley, D.R., 2005a, Gene Expression Analysis of *in situ* Metabolism and Nutrient Status of Geobacteraceae in Subsurface Environments, The 105th General Meeting of the American Society for Microbiology: Atlanta, GA.

Holmes, D.E., Nevin, K.P., and Lovley, D.R., 2004, In situ expression of nifD in Geobacteraceae in subsurface sediments: Applied and Environmental Microbiology, v. 70, p. 7251-7259.

Holmes, D.E., Nevin, K.P., O'Neil, R.A., Ward, J.E., Adams, L.A., Woodard, T.L., Vrionis, H.A., and Lovley, D.R., 2005b, Potential for quantifying expression of the Geobacteraceae citrate synthase gene to assess the activity of Geobacteraceae in the subsurface and on current-harvesting electrodes: Applied and Environmental Microbiology, v. 71, p. 6870-6877.

Lovley, D.R., Phillips, E.J.P., Gorby, Y.A., and Landa, E.R., 1991, Microbial Reduction of Uranium: Nature, v. 350, p. 413-416.

Lovley, D.R., Roden, E.E., Phillips, E.J.P., and Woodward, J.C., 1993, Enzymatic Iron and Uranium Reduction by Sulfate-Reducing Bacteria: Marine Geology, v. 113, p. 41-53.

N'Guessan, A.L., Williams, K.H., Yabusaki, S., Long, P.E., and Lovley, D.R., 2008, Biogeochemical differences in pilot-scale bioremediation treatment plots undergoing iron reduction or sulfate reduction in a uranium-contaminated aquifer. : Geochemistry Division Program for the 236th American Chemical Society meeting, Philadelphia, PA, v. Section A.

N'Guessan, A.L., Vrionis, H.A., Resch, C.T., Long, P.E., and Lovley, D.R., 2008, Sustained Removal of Uranium From Contaminated Groundwater Following Stimulation of Dissimilatory Metal Reduction: Environ. Sci. Technol. , v. 42, p. 2999-3004.

Ortiz-Bernad, I., Anderson, R.T., Vrionis, H.A., and Lovley, D.R., 2004, Resistance of solid-phase U(VI) to microbial reduction during in situ bioremediation of uranium-contaminated groundwater: Appl. Environ. Microbiol. , v. 70, p. 7558-7560.

Shroba, R.R., and Scott, R.B., 1997, Geologic Map of the Rifle Quadrangle, Garfield County, Colorado: U.S. Geological Survey, Washington, DC, v. Open File Report 97-852.

Vrionis, H.A., Anderson, R.T., Ortiz-Bernad, I., O'Neill, K.R., Resch, C.T., Peacock, A.D., Dayvault, R., White, D.C., Long, P.E., and Lovley, D.R., 2005, Microbiological and geochemical heterogeneity in an in situ uranium bioremediation field site: Applied and Environmental Microbiology, v. 71, p. 6308-6318.