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

Introduction

This report documents the approach and methods in which key microbial metabolic processes are monitored and evaluated in the subsurface at the Rifle Integrated Field-Scale Challenge site, and how these data are being incorporated into fate and transport modeling efforts. In the second quarter of fiscal year 2008, the Rifle Integrated Field Challenge Site (IFC) team has continued sampling, and analysis of data collected from the Winchester field experiment (the first field experiment of the Rifle IFC) and other laboratory activities including column studies and method development.

Background

Microbial Metabolic Processes and Uranium Bioreduction. The bioreduction and subsequent precipitation of uranium from groundwater through the addition of an electron donor to stimulate uranium reducing microbial populations has shown promising potential to prevent uranium migration from contaminated sites (Lovley and Coates, 1997; Lovley and Phillips,1992; Abdelouas, et al., 1998). Biological (i.e., enzymatic) U(VI) reduction to U(IV) occurs simultaneously with Fe(III) reduction (Finneran, et al., 2002; Anderson, et al., 2003; Istok, et al. 2004) and has been shown to decrease during the transition from iron-reducing to sulfate-reducing conditions (Anderson et al., 2003). U(VI) reduction still may occur through different processes or microbes e.g. *Desulfovibrio spp* (Finneran, et al., 2002; Anderson, et al., 2003).

Current evidence from microcosms, column studies and field-scale studies such as those performed at the Rifle IFC, suggests that two major classes of microorganisms are involved in uranium reduction in the subsurface; organisms that utilize Fe(III) and organisms that use SO_4^{2-} as terminal electron acceptors. The SO_4^{2-}-reducers (SRBs) include a wide range of morphological and physiological types. Sulfate-reducing bacteria can however, be broadly separated into two physiological groups: (1) those that can completely oxidize multicarbon substrates to CO_2 and, (2) those that can incompletely oxidize these substrates to acetate. However, it is generally thought that acetate and H_2 represent the major electron donors for SO_4^{2-} reduction in anoxic sediments (Winfrey and Zeikus 1979; Lovley, Dwyer et al. 1982). Sulfate reducing bacteria can reduce U(VI) in cell suspensions and as bioflims. For example, the direct electron acceptor for the hydrogenase in *D. vulgaris* (cytochrome c3) is capable of reducing U(VI) (Lovley et al., 1993). Furthermore, *Desulfotomaculum reducens* has been shown to grow with U as its electron acceptor (Tebo and Obratsova 1998). A Desulfosporosinus sp. has also been shown to reduce U(VI) (Suzuki et al., 2003).

Bacteria able to utilize Fe(III) as electron acceptors can also be separated into two groups. *Geobacter metallireducens* is representative of the first group (Lonergan, Jenter et al. 1996), which consists of strict anaerobes that completely oxidize multicarbon electron donors to CO_2 concurrent with the reduction of Fe(III). The electron donors for these organisms include a variety of aromatics and the simple C2-C4 carboxylic acids that are generated as products from the fermentation of complex organic matter. Acetate is thought to be the most significant electron donor for these types of organisms. The second group of Fe(III) -reducing organisms is typified by the facultative anaerobe *Shewanella putrefaciens*. While this organism can readily oxidize H_2 and formate, it can only partially oxidize simple organic compounds such as pyruvate. Representative organisms from both groups utilize U(VI) as an electron acceptor (Gorby and

Lovley 1992) and methods have been described for the bioremediation of uranium using Fe(III)-reducing organisms for quite some time (Lovley and Phillips 1992).

Although both SO_4^{2-} and Fe(III) reducing microorganisms have the capability to reduce U(VI) in the subsurface, the rate of uranium reduction in many subsurface environments can be limited by the lack of availability of suitable electron donors and/or overabundance of certain electron acceptors that drive the underlying microbial respiratory processes. For example, SO_4^{2-} reduction is known to be inhibited by the presence of more electrochemically-positive electron acceptors such as NO_3^- (Sørensen 1982), while the rate of Fe(III) reduction is known to be limited by bioavailability and the low aqueous solubility of Fe(III). Likewise, the addition of Fe(III) is known to inhibit SO_4^{2-} reduction and methanogenesis (Lovley 1991). This latter effect is thought to occur due to the ability of Fe(III)-reducers to establish steady-state electron-donor concentrations that are too low for these less energetically favorable forms of respiration. As another example, when Fe(III) availability is not limiting, Fe(III)-reducers may outcompete SO_4^{2-} reducers for electron donors that are common to both (Chapelle and Lovley, 1992). It is therefore important to determine which group of organisms is reducing U in mixed microbial communities, and which electron donors are most effective in stimulating their activities.

<u>*Rifle, CO Field Site.*</u> The field study site at Rifle has proven to be ideal for conducting *in situ* biostimulation experiments for removal of U(VI) from groundwater. Shallow depth to water (3-4 m), thin saturated thickness (~2.5 m), and impermeable lower boundary create a readily accessible, well-defined groundwater flow system that makes it inexpensive to monitor microbial and geochemical processes and to assess the progress of *in situ* uranium bioremediation in real time. The site exhibits typical geochemical effects on uranium mobility (e.g. bicarbonate control on U(VI) sorption) and is also readily biostimulated, indicating that it offers a balance among competing biotic and abiotic processes, making it feasible to determine which processes dominate.

The results of repeated field experiments at the Rifle site have shown the predominance of *Geobacteaceae* initially (30 to 40 days) followed SRB during biostimulation. During the first field biostimulation experiment, Anderson et al. (2003), showed that after 17 days of biostimulation with acetate "*Geobacteaceae*" accounted for 89% of the microbial community. As time progressed the fraction of "*Geobacteaceae*" decreased, accounting for less than 7% by day 80, at which time the microbial community was dominated by the family "*Desulfobacteraceae*". The dominance of *Geobacter* during the Fe(III) reduction phase has been demonstrated in every subsequent field experiment at the Rifle site including the Winchester experiment in 2007. It has also been shown through repeated field-scale experiments that as the growth conditions became less favorable for the *Geobacter* species the overall U(VI) removal decreased and with continued donor addition SO_4^{2-} reducing organisms become dominant.

<u>*Current conceptual model for the site under biostimulated conditions.*</u> The field-scale biostimulation experiments at the Rifle site have shown that electron-donor (acetate) addition consistently promotes the growth of dissimilatory metal-reducing bacteria (DMRB) of the *Geobacter* family that are known to enzymatically reduce U(VI) to U(IV). With biostimulation, detectable biomass increases by more than 10-fold, and a significant percentage of that biomass is dominated by *Geobacter* and related *Desulfuromonas* and *Pelobacter* species. The reproducible, stimulated growth of DMRB correlating with the loss of U(VI) from the groundwater strongly suggests that these organisms are responsible for the *in situ* reduction of U(VI). However, maintaining metal-reducing conditions *in situ* depends on the amount of Fe(III) available. After consumption of about one-half of the bioavailable Fe(III), the anaerobic community shifts to a system dominated by sulfate-reducing bacteria (SRB), where removal of U(VI) from solution is significantly less than under Fe-reducing conditions. Bioavailable Fe(III) is typically represented by poorly crystalline iron hydroxide. However, recent column studies on Rifle sediments indicate

that phyllosilicate Fe(III) is actually reduced first and that there is little or no amorphous Fe(III) in Rifle sediments. The response of Rifle sediments contrasts with that observed at the Oak Ridge Field Research Center (Wu et al., 2006) where U(VI) loss increased during sulfate reduction. The marked differences in response suggest site-specific factors strongly influence biostimulation, confirming the need for a better mechanistic understanding.

Approach to Identifying Key Microbial Metabolic Processes in the Subsurface During Biostimulation

In the past, considerable laboratory-scale research has investigated the effects of microbial activity on radionuclides (including uranium). A variety of methods have been used to obtain this information including: analysis of geochemical data (Lovley and Goodwin 1988); batch, column, and microcosm reactor studies (Wilson, McNabb et al. 1983); direct observation and culture techniques (Harvey, Smith et al. 1984); biochemical marker techniques (Balkwill et al., 1988); molecular methods (Bowman et al. 1993); and ecological modeling (Kelly et al. 1988). The relative advantages and disadvantages of many of these methods are discussed in Chapelle 1993 and Ehrlich 1996. However, it is apparent that *in situ* testing and analysis methods are required to fully understand and characterize subsurface microbial metabolic activity, especially in environments that display steep biogeochemical gradients (Madsen 1991; Madsen 1998).

A universal theme that has emerged from 20 years of fundamental microbiology research within the U.S. Department of Energy's (DOE's) Subsurface Science, NABIR, EMSP and ERSP programs is the importance of metal-, and sulfate- reducing bacteria in subsurface geochemical processes. The results at the Rifle IFC have repeatedly shown the dominance of Fe(III) reducing bacteria (*Geobacter*) and SO_4^{2-} reducing bacteria during sequential phases of biostimulation. As a consequence of this reality, the Rifle microbiology team has focused on these two specific microbial groups. The project is therefore designed to better understand the roles played by the microorganisms responsible for uranium reduction at Rifle using SIP, RNA probing/fingerprinting, PLFA assay, and proteomics technologies. Initial characterization of the microbial community has involved bi-monthly sampling at the Rifle site to assess temporal/spatial variability in the field. Similar methods have been employed to monitor the biostimulation experiments (Winchester) and are being used in the laboratory column studies in order to elucidate the active members during and after experimental manipulation.

Analysis of Microbial Community Dynamics – Proteomics. Systems-level biological analysis, a concept in existence for more than 50 years (Konopka, 2004), requires insight into fundamental properties such as structure and dynamics (Kitano, 2002a; Kitano, 2002b). Recent advances in genome sequencing and other geochemical and biological measurements have stimulated interest in this approach because they enable description of the architecture of microbial communities and identification of the roles, activity levels, and environmental impacts of each of its members. The ability to comprehensively and accurately measure biological molecules in microbial communities in a high throughput mode has been a pivotal advantage. This has opened the way for monitoring over time and under a variety of conditions to reveal the workings of the biological networks. Developing a system-level understanding of microbes requires global, genome-wide, analysis that can be accomplished by monitoring changes in RNA expression (transcriptomics) or protein abundance (proteomics). The value of each of these analyses is enhanced through their integration (Hedge, 2003), especially if they are combined with information about the physical and geochemical context.

Many challenges are associated with the transition from pure culture-based studies to studies of natural microbial consortia. However in the Rifle project, the focus is primarily on the development of proteomic methods because it has been shown they can be applied to at least simple microbial communities (Ram et al., 2003). Results from the Winchester biostimulation

experiment have shown that sequenced genomic information is essential for understanding meta-proteomic datasets. In the absence of meta-genomic information for a sample, genomic information for individual species is being used to identify detected peptides. As protein samples are usually digested by trypsin prior to being analyzed by LC-MS/MS, all possible protein fragments can be predicted for any sequenced microbial genome. These predicted peptides are then matched against peptide spectra from the MS. At the Rifle site, a number of complementary techniques (stable isotope probing, 16S rRNA clone libraries, PLFA) indicated that following the addition of acetate to the subsurface, *Geobacter* species dominated the subsurface microbial community during periods of Fe(III) reduction. An initial proteomic search database was constructed using predicted peptides from all sequenced microorganisms available at the Department of Energy Joint Genome Institute website (www.jgi.doe.gov). When the proteomic data was searched against these predicted peptides, nearly all (> 95%) matches were from species within the *Geobacteraceae*. This further confirmed that the samples were dominated by *Geobacter* species, as had been observed in previous studies. It was subsequently decided that the meta-proteomic datasets would be searched against predicted peptides from all seven sequenced *Geobacter* strains: *G. bemidjiensis*, *G. metallireducens*, *G. sulfurreducens*, *G. uraniireducens*, *G. lovleyi*, *G.* FRC-32 and *G.* M-21. Two of these species, *G. uraniireducens* and *G.* M-21 were isolated from the Rifle subsurface under biostimulated conditions during prior experiments. This data analysis technique allowed about 3000 proteins per LC-MS/MS run to be detected, and identified. Further analysis of this data has indicated that multiple *Geobacter* strains are present in the Rifle subsurface during biostimulation, with the most abundant being closely related to *G. bemidjiensis*/*G.* M-21.

<u>*Nucleic acid techniques*</u>. Quantitative PCR targets being used to track Fe(III) and SRB reducing microbes at the Rifle IFC..

- Metal (iron) Reducing Bacteria. Functional gene: No conserved functional genes are currently defined for Fe(III) reducing bacteria. However, because *Geobacter* sp. has been shown to make up more than 90% of the population of iron-reducing bacteria, gene for citrate synthase (*gltA*) and nitrogen fixation (*nifD*) have been used to track these iron reducing bacteria at the site (Holmes et al., 2004 and 2005). In addition, specific 16S rDNA of the low G+C Gram-positive Fe(III) reducer (Anaeromyxobacter sp.) that predominate in most environments (Petrie et al., 2003) has been used.
- Sulfate-Reducing Bacteria. Functional gene: The sulfate-reducing bacteria (SRB) have been shown to be enormously versatile metabolically with activities far beyond the classical utilization of lactate or acetate as electron donors (Singleton, 1992). The functional gene *dsr* for dissimilatory sulfite reductase is a reference gene for sulfate reducing activity/microorganisms in many settings. This gene has been used to define SRB in UMTRA uranium-contaminated aquifers effectively (Chang et al., 2001). We understand that some dissimilatory iron reducers are also SRB's (Coleman et al., 1993), and do take that into account when interpreting results.
- Fermenters. Functional Genes: Fermenters are a widely divergent group and two putative functional genes are assessed. SpoOA represents phosphorylation-activated transcription factor of *Bacillus subtilis* that is widespread amongst Gram-positive bacteria. (Brown et al., 1994). A glucanotransferase (*malQ*) is also being examined as it represents a common fermenter activity (Goda et al., 1997).
- Methanogens. Functional Genes: The Archaea which generate methane from acetate or carbon dioxide/hydrogen are characterized universally by the functional gene *mcrA* for methyl coenzyme m reductase (Hallam et al., 2003).

Although none of these gene targets are a perfect representation of a given activity they can be used in a weight of evidence approach along with the other data such as geochemistry generated during the biostimulation experiments.

Stable Isotope Probing (SIP); Stable isotope probing identifies the active bacteria in the subsurface by following the uptake of the ^{13}C carbon added as acetate. SIP/tRFLP experiments are ongoing in conjunction with columns studies at Princeton University. A microcosm-uptake experiment was performed during the Winchester experiment using either groundwater from monitoring wells or a 10% (v/v) soil/sediment slurry prepared using water and sediment at the beginning and end of the experimental effort. The microcosms were sparged with a 70%–30% mixture of N_2-CO_2 and amended with 10-100 μM of uniformly ^{13}C-labeled acetate or no additional carbon source. Bacterial biomass was collected after a 3-h incubation. The time points for the final soil amendment were 1h, 6 h, 24 h, and 2-, 5-, 7-, and 21-day (three active tubes with labeled substrate or unlabeled substrate). Extraction and purification used a carrier technology that significantly reduced the time needed to detect the SIP signal as in Gallagher et al. (Gallagher et al., 2005). Results from the Winchester experiment showed that most of the bacterial activity (acetate uptake) was with the bacterial population in close association with the sediment fines suspended in the groundwater. Detailed sequencing of the resulting tRFLP profiles is ongoing.

Microarray Profiling of Microbial Community Composition and Activity. The temporal and spatial changes in microbial community composition and activity associated with biostimulation as a treatment technology require a field compatible, near real-time monitoring tool. Nucleic acid microarrays provide an unparalleled opportunity for the multiplexed detection of not only active members of the microbial community (16S rRNA microarray) but also active microbial processes (mRNA microarray). Sediment and groundwater samples from laboratory and field experiments have been obtained for DNA/RNA extraction and subsequent community profiling by 16S rRNA and mRNA microarrays. The probes for the 16S rRNA microarray were chosen based on relevance to DOE legacy sites and include probes specific for known DMRB and SRB. The use of the 16S rRNA microarray permits the direct detection of specific rRNA targets in mixed microbial communities without the biases associated with differential PCR amplification. The mRNA microarray is being used to determine critical microbial activities including terminal electron accepting processes, response to nutrient limitation, metals resistance mechanisms, and response to oxidative stress. The current 16S rRNA and mRNA microarrays are 3-D gel element arrays, which represent an improvement over the tunable bead arrays that were successfully, but retrospectively, used to track microbial community composition at the Rifle site previously (Chandler et al., 2006). Moreover, the gel element microarrays are readily expandable to include additional target sequences, as needed, based on ongoing and planned research activities. The microarray data will be integrated with the proteomic data and the mRNA arrays will be analyzed in the light of the population genomic analysis.

Lipid Analyses and Biomass decay: Variations in the microbial community biomass, composition, metabolic status and viability are being assessed using various lipid analyses. PLFA profiles for both the background and stimulated sediments have already been assembled as part of past experiments for Rifle, and they have provided a framework for characterizing spatiotemporal changes in column and field microbial communities. The determination of the total PLFA provides a quantitative measure of the viable microbial biomass because the phosphate group of PFLA present in intact membranes of viable microbes is enzymatically hydrolyzed from the polar lipid shortly after cell death (White et al., 1979). The amount of resulting bacterial diglyceride in a sample reflects the amount of non-viable biomass and is being used to asses biomass decay after following the cessation of donor additions in the Winchester experiment and associated column studies. We have also published the dominant PLFA and quinone patterns for *Geobacter* at the Rifle IFC site and have quantitatively followed its biochemical signal *in situ*. PLFA patterns of

the total microbial community have also been assayed at Rifle and are being used to quantitatively define differences between stimulated and unstimulated areas. Formation of different respiratory quinones (part of isoprenoid lipoquinone class) in response to different TEAPs indicate *in-situ* terminal electron-accepting conditions and we are investigating the possibility of using quinones from *Geobacter* as an activity indicator. Additionally UQ/MK ratios are being used to demonstrate, e.g., the shift from aerobic to anaerobic conditions with depth in Rifle sediments (White and Long, 2006).

Coupling of Biology and Geochemistry (Modeling)

Mechanistically unraveling the impact of biostimulation under the dynamic and spatially variable hydrologic and geochemical conditions at the Rifle site has been a significant scientific challenge. Past and ongoing investigations at Rifle have made considerable progress identifying complex linkages between community structure/function, biological reactions, and geochemical reactions. The current series of investigations is building on this knowledge to address the goal of developing a quantitatively predictive understanding of field-scale uranium behavior at the Rifle site. One challenge is how to incorporate microbiological information into the modeling efforts. The general formulation of reaction solvers that are being used for Rifle is a system of differential (ODEs, i.e., kinetic reactions) and algebraic (nonlinear equilibrium reactions, e.g., mass action; and arithmetic mass balance, e.g., total Ca as a sum of all Ca species) equations that are solved. Since microbially-mediated reactions include chemical components from other geochemical reactions, it is important to incorporate them using the same specification for the general reaction solver in order to simultaneously solve for the complete biogeochemical system of reactions. To adapt microbial behaviors to this approach, they need to be described in the form of reactions and rate laws. The reactions require the specification of a stoichiometric transformation of reactants (e.g., acetate, TEAs, nutrients) to products (e.g., bicarbonate, reduced components, biomass). The rate laws describe the rate (including onset and termination) of the reactions as a function of input and modeled quantities, for example, the concentration and bioavailability of solid-phase Fe(III).

For the case of biostimulation at the Rifle IFC there are several questions that are being addressed.

1. Defining what microorganisms are the principal acetate consumers.
2. Describing what microbially-mediated reactions (e.g., TEAPs) need to be included in the reaction models and stoichiometries for these reactions, e.g., conversion of acetate, terminal electron acceptors, and nutrients to biomass, and reduced phases.
    a. The reactions for the iron, sulfate, and uranium TEAPs are based on an assumption of a particular energy transfer efficiency and are available, for example:
        i. $0.125\ CH_3COO^- + 0.6\ FeOOH(s) + 1.155\ H^+ + 0.02\ NH_4^+ = 0.02$ Biomass_iron_reducer $+ 0.6\ Fe^{++} + 0.96\ H_2O + 0.15\ HCO_3^-$
        ii. $0.125\ CH_3COO^- + 0.3875\ UO_2^{++} + 0.3538\ H_2O + 0.0113\ NH_4^+ =$ 0.0113 Biomass_iron_reducer $+ 0.3875\ UO_2(s) + 0.855\ H^+ +$ $0.1938\ HCO_3^-$
        iii. $0.125\ CH_3COO^- + 0.1155\ SO_4^{--} + 0.0057\ H^+ + 0.0038\ NH_4^+ =$ 0.0038 Biomass_sulfate_reducer $+ 0.1155\ HS^- + 0.114\ H_2O +$ $0.231\ HCO_3^-$

3. For standard geochemical reactions, stoichiometries are not only fixed but are thermodynamically constrained (e.g., stability constants in ΔG or logK). Microbially-mediated reactions therefore have the potential to change stoichiometry depending on the amount of energy being used for cell synthesis and maintenance, exogenous solution chemistry, and metabolic status. In order to solve for these constituents our team is defining;

    a. The principal TEAP reactions.

    b. What controls the onset of these reactions, whether it be thermodynamics, chemistry or the sequential consumption of TEAs.

    c. What factors affect/control the stoichiometry of these reactions (e.g., nutrients, biomass/cell synthesis, cell maintenance, geochemistry) and how they are quantified. Additionally the thermodynamic constraints on these reactions.

4. Defining the principal mechanisms controlling the rates of microbially- mediated reactions and a rate law for each microbially-mediated reaction is needed. A variety of rate laws have been developed that include first-order Monod terms, thermodynamic terms, inhibition constants, etc. For example, a rate law for microbially-mediated iron reduction:

$$R = k \frac{[U(VI)aq]}{K_U + [U(VI)aq]} \frac{[\mathrm{Ac}]}{\mathrm{K_{Ac}} + [\mathrm{Ac}]} \left(1 - \exp\left(\Delta G_r^0 + 2.3RT \log\left(\frac{\{H^+\}^9 \{HCO_3^-\}^2}{\{UO_2^{2+}\}^4 \{CH_3COO^-\}} \right) - \Delta G_{\min} \right) / RT \right)$$

    a. The Rifle group is investigating whether this Monod formulation (1st two terms above) are a reasonable rate law approximation for field-scale biogeochemical reactive transport simulations.

    b. The role of bioavailable TEAs (e.g., Fe(III) mineral, aqueous U(VI), sulfate) in the reaction rates is also being investigated In the Monod terms, declining acetate and TEA concentrations result in decreasing rates. TEA concentrations have also been used in signaling the termination of a TEAP reaction and/or the onset of succeeding TEAP reactions. Discovering if these reactions are valid is key to the effort.

    c. Answering the question "Is a thermodynamic impetus for reaction rate such as transition state theory (i.e., distance from thermodynamic equilibrium) appropriate for microbially-mediated reactions?" is also being investigated and how to quantify and specify the output in the rate law.

    d. Determining if threshold levels of certain chemical components (e.g., nutrients, reaction products) will inhibit or enhance reaction rates.

    e. Understanding the significance of biomass in reaction rates. If significant, then defining what controls the biomass abundance (which could relate back to the yield in the stoichiometric reaction).

    f. Exploring if metabolic lag is important for microbially-mediated reactions at Rifle and how this should be quantified and modeled.

    g. Investigating if there are inherent limitations on the overlapping presence of bacterial reducers for different redox couples (e.g., iron and sulfate reducers).

5. Determining what microbial behaviors require incorporation into existing process models.
    a. Understanding the need to differentiate between attached and planktonic populations/biomass and under what conditions.
    b. Exploring the process and role of biosorption.
    c. Investigating if biomass production affects the reactivity of mineral surfaces.

A mechanistic and quantitative understanding of microbial processes in the environment has been an elusive goal, but is now possible with advances in genomic-enabled techniques. By employing a range of these techniques including SIP, RNA/DNA probing/fingerprinting, PLFA assay, and proteomics to the detection of Fe(III) reducing and SO_4^{2-} reducing bacteria at the Rifle site, our team is providing critical information that is now being integrated into the described modeling efforts. A major goal of this project is to couple advances in genomics and proteomics technology with an integrative multi-disciplinary approach to describe the functioning of subsurface microbial communities under stimulated metal-reducing conditions. The Rifle approach specifically targets new knowledge that can be translated into scientifically defensible flow and reactive transport process models of microbially mediated and abiotic reactions, taking a major step toward ERSP's long-term goal to "…incorporate coupled biological, chemical and physical processes into decision making for environmental remediation." The integration of geochemical, geophysical, microbial, and hydrological data by the research team is resulting in significant progress toward this goal.

References:

Abdelouas, A., et al. 1998. Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water. Journal of Contaminant Hydrology. 35(1-3): p. 217-233.

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

Balkwill, D. L., F. R. Leach, et al. (1988). "Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate." Microbial Ecology 16: 73-84.

Bowman, J. P., L. Jimenez, et al. (1993). "Characterization of the methanotrophic bacterial community present in a trichloroethylene-contaminated subsurface groundwater site." Applied and Environmental Microbiology 59: 2380-2387.

Brown, D.P., L. Ganova-Raeva, B.D. Green, S.R. Wilkinson, M. Young, P. Youngman. 1994. Characterization of spoOA homologues in diverse Bacillus and Clostridium species identifies a probable DNA-binding domain. Mol Microbiol.114(3):411-426.

Chandler, D.P., A. E. Jarrell, E.R. Roden, J. Golova, B. Chernov, A.D. Peacock, and P.E. Long. (2006) Suspension Array Analysis of 16S rRNA from Fe- and SO42- Reducing Bacteria in Uranium Contaminated Sediments Undergoing Bioremediation. Applied and Environmental Microbiology, Vol. 72, No. 7, p. 4672-4687.

Chang, Y.-J., A. D. Peacock, et al. (2001). "Diversity and Characterization of Sulfate-Reducing Bacteria in Groundwater at a Uranium Mill Tailings Site." Appl. Envir. Microbiol 67: 3149-3160.

Chapelle, F.H., and D.R. Lovley. 1992. Competitive exclusion of sulfatereduction by Fe(III)-reducing bacteria: a mechanism for producing discrete zones of high iron groundwater. Gound Water. 30:29-36.

Chapelle, F.H. 1993. Ground-water microbiology and geochemistry by F. H. Chapelle. John Wiley & Sons, Inc., 1993, 424p., (ISBN 0-471-52951-6)

Coleman, M. L., D. B. Hedrick, et al. (1993). "Reduction of Fe(III) in sediments by sulfate-reducing bacteria." Nature 361: 436-438.

Ehrlich, H. L. (1996). Geomicrobiology. New York, Marcel Dekker, Inc.
Elshahed, M., J. M. Senko, F.Z. Najar, S. M. Kenton, B.A. Roe, T.A. Dewers, J.R. Spear, and L. R. Krumholz. 2003. Bacterial Diversity and Sulfur Cycling in a Mesophilic Sulfide-Rich Spring. Applied and Environmental Microbiology. 2003 69: 5609-5621.

Finneran, K.T., et al. 2002. Potential for Bioremediation of uranium-contaminated aquifers with microbial U(VI) reduction. Soil & Sediment Contamination. 11(3): p. 339-357.

Gallagher, E., L. McGuinness, C. Phelps, L. Y. Young, and L. J. Kerkhof. 2005. 13C-carrier DNA shortens the incubation time needed to detect benzoate-utilizing denitrifying bacteria by stable-isotope probing. Applied and Environmental Microbiology 71:5192-5196

Goda, S.K., O. Eissa, M. Akhtar, N.P. Minton. 1997. Molecular analysis of Clostridium butyricum NCIMB 7423 gene encoding 4-alpha-glucanotransferase and acharacterization of the recombinant enzyme produced in Escherichia coli. Microbiology 143 (part 10):3287-94.

Gorby, Y. A. and D. R. Lovley (1992). "Enzymatic uranium reduction." Environmental Science and Technology 26: 205-207.

Harvey, R. W., R. L. Smith, et al. (1984). "Effect of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod, Mass., aquifer." Applied and Environmental Microbiology 53: 2992-2996.

Hallam, S.J., P.R. Girguis, C.M. Preston, P.M. Richardson, and E.F. DeLong. 2003. Identification of Methyl Coenzyme M Reductase A (mcrA) Genes associated with Methane Oxidizing Archaea. Applied and Environmental Microbiology 69 (9): 5483-5491.

Hegde, P. S., I. R. White, and C. Debouck. 2003. Interplay of transcriptomics and proteomics. Current Opinion in Biotechnology 14:647-651.

Holmes, D.E., K.T. Finneran, R.A. O'Neil, and D.R. Lovley. 2002. Enrichment of the members of the family Geobacteracea associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. Applied and Environmental Microbiology 68:2300-6.

Holmes DE, Nevin KP, Lovley DR. 2004. In situ expression of nifD in Geobacteraceae in subsurface sediments. Appl Environ Microbiol. 2004 Dec;70(12):7251-9.

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. 2005. Potential for quantifying expression of the Geobacteraceae citrate synthase gene to assess the activity of Geobacteraceae in the subsurface and on current-harvesting electrodes. Appl Environ Microbiol. 2005 Nov;71(11):6870-7.

Istok, J.D., et al. 2004. In situ bioreduction of technetium and uranium in a nitrate-contaminated aquifer. Environmental Science & Technology. 38(2): p. 468-475.

Kelly, F. X., K. J. Dapsis, et al. (1988). "Effect of bacterial chemotaxis on dynamics of microbial competition." Microbial Ecology 16: 116-131.

Kitano, H. 2002a. Computational systems biology. Nature 420:206-210.

Kitano, H. 2002b. Systems biology: A brief overview. Science 295:1662-1664.

Konopka, A. 2004. Systems biology - not brand new, but different. ASM News 70:163-168.

Lonergan, D. J., H. L. Jenter, et al. (1996). "Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria." Journal of Bacteriology 178: 2402-2408.

Lovley, D. R. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol. Rev. 55:259–287.

Lovley, D.R. and J.D. Coates. 1997. Bioremediation of metal contamination. Current Opinion in Biotechnology. 8(3): p. 285-289.

Lovley, D. R.; Goodwin, S. 1988. Geochimica et Cosmochimica Acta, 52, 2993-3003.

Lovley DR, Widman PK, Woodward JC, Phillips EJP. 1993. Appl Environ Microbiol. 59(11):3572-6.

Lovley, D.R. and E.J.P. Phillips. 1992. Bioremediation of Uranium Contamination with Enzymatic Uranium Reduction. Environmental Science & Technology. 26(11): p. 2228-2234.

Lovley, D. R., D. F. Dwyer, et al. (1982). "Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments." Applied and Environmental Microbiology 43: 1373-1379.

Madsen, E. L. (1991). "Determining in situ biodegradation - facts and challenges." Environmental Science and Technology 25: 1663-1673.

Madsen, E. L. (1998). "Epistemology of environmental microbiology." Environmental Science & Technology 32: 429-438.

Petrie, L., N. N. North, S. L. Dollhopf, D. L. Balkwill, and J. E. Kostka. 2003. Enumeration and characterization of iron(III)-reducing microbial communities from acidic subsurface sediments contaminated with uranium(VI). Applied and Environmental Microbiology 69:7467-7479.

Ram, R. J., N. C. VerBerkmoes, M. P. Thelen, G. W. Tyson, B. J. Baker, R. C. Blake, M. Shah, R. L. Hettich, and J. F. Banfield. 2005. Community proteomics of a natural microbial biofilm. Science 308:1915-1920.

Singleton, R. (1992). The sulfate-reducing bacteria: an overview. The sulfate-reducing bacteria:Contemporary perspectives. J. M. Odom and R. Singleton. New York, Springer-Verlag: 1-20.

Sørensen, J. (1982). "Reduction of ferric iron in anaerobic, marine sediment and interaction with reduction of nitrate and sulfate." Applied and Environmental Microbiology 43: 319-324.

Suzuki, Y., S.D. Kelly., K.M. Kemner, and J.F. Banfield. 2003. Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. Appl Environ Microbiol. 2003 Mar;69(3):1337-46.

Tebo, B. M. and A. Y. Obratsova (1998). "Sulfate reducing bacterium grows with Cr(VI), U(VI), Mn(IV) and Fe(III) as electron acceptors." FEMS Microbiol. Lett. 162: 193-198.

White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie. 1979. Determination of

the Sedimentary Microbial Biomass by Extractable Lipid Phosphate. Oecologia 40:51-62.

White, D. C., and P. E. Long. 2006. Lipid analysis of microbial processes and communities at a Uranium Bio-immobilization site Using 13C-labled Acetate Amendment. Department of Energy- ERSD PI Meeting. Warrenton, VA.

Wilson, J. T., J. F. McNabb, et al. (1983). "Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer." Ground Water 21: 134-142.

Winfrey, M. R. and J. G. Zeikus (1979). "Effect of sulfate on carbon and electron flow during microbial methonogenesis in freshwater sediments." Applied and Environmental Microbiology 33: 275-281.

Wu, W.M., Carley, J., Gentry, T., Ginder-Vogel, M.A., Fienen, M., Mehlhorn, T., Yan, H., Caroll, S., Pace, M.N., Nyman, J., Luo, J., Gentile, M.E., Fields, M.W., Hickey, R.F., Gu, B.H., Watson, D., Cirpka, O.A., Zhou, J.Z., Fendorf, S., Kitanidis, P.K., Jardine, P.M., and Criddle, C.S., 2006, Pilot-scale in situ bioremedation of uranium in a highly contaminated aquifer. 2. Reduction of U(VI) and geochemical control of U(VI) bioavailability: Environmental Science & Technology, v. 40, p. 3986-3995.