

PNNL ERSP SFA Annual Report

ROLE OF MICROENVIRONMENTS AND TRANSITION ZONES IN SUBSURFACE REACTIVE CONTAMINANT TRANSPORT

1. Program Overview

The PNNL Scientific Focus Area (SFA) is resolving critical Hanford basic subsurface science issues through integrated, multi-disciplinary, science-theme focused research on the role of microenvironments and transition zones in the reactive transport of technetium (Tc), uranium (U), and plutonium (Pu). Microenvironments are small domains within larger ones that exert a disproportionate influence on subsurface contaminant migration. They may be internal fractures or microbiologic niches within porous media lithic fragments; grain coatings, biofilms, or micro-colonies on larger mineral particles; or compact silt/clay stringers in gravel-dominated subsurface sediments. Transition zones are field scale features where chemical, physical, or microbiologic properties change dramatically over relatively short meter-scale distances. They exhibit steep, transport-controlled gradients of system-controlling chemical species such as O_2 , H^+ , or organic carbon. Microenvironments and transition zones frequently dominate subsurface contaminant reactivity, with strong effects resulting from the coupling of chemical reaction, physical transport (advection, diffusion), and microbiologic processes. Past DOE-BER funded research has documented the importance of these zones at the Hanford Site.

The overall ten-year goals of the SFA are to develop: i.) an integrated conceptual model for microbial ecology in the Hanford subsurface and its influence on contaminant migration, ii.) a fundamental understanding of chemical reaction, biotransformation, and physical transport processes in microenvironments and transition zones, and iii.) quantitative biogeochemical reactive transport models for Tc, U, and Pu that integrate multi-process coupling at different spatial scales for field-scale application. Targeted contaminant chemical reaction and biotransformation processes include heterogeneous/biologic electron transfer, precipitation and dissolution, and surface complexation. The SFA is emphasizing lab-based, coupled computational and experimental research using relevant physical/biologic models, and sediments and microbial consortia and isolates from various Hanford settings to explore molecular, microscopic, and macroscopic processes underlying field-scale contaminant migration. It will also pursue the refinement of geophysical techniques to define, characterize, and map spatial structures and reactive transport properties of microenvironments and transition zones in the field.

The SFA is partnering with the PNNL Environmental Molecular Sciences Laboratory (EMSL) to develop molecular understandings of key processes, and the Hanford Integrated Field Research Challenge (IFRC) for access to, and samples from subsurface environments where these zones exist and are important. The research program builds upon established areas of PNNL expertise in geochemistry, microbiology, and multi-scale modeling. Individual, but highly collaborative research projects are focused on different scales, coupled processes, and/or contaminants; each is managed by a PNNL-ERSP investigator of significant accomplishment and appropriate expertise. The projects fall in the following general science categories: i.) microbial ecology, ii.) molecular scale mechanisms, iii.) pore-scale coupled processes, iv.) reactive transport science, v.) multi-scale reactive transport models, and vi.) in-situ geophysical structures and reactive transport properties. An additional project will also be established to mentor a promising new or young investigator, or group of investigators. The PNNL team is supplemented with 12 external, PNNL-supported investigators (lab, university) with needed complementary expertise. Cohesiveness and integration are achieved by focus on SFA hypotheses and impactful science topics formulated to enhance information connectivity, upscaling, resolution of broader science issues, and Hanford impact.

The overall SFA science program is managed by John Zachara and Jim Fredrickson, two PNNL Chief Scientists who collectively have more than 40 years experience as BER PI's in subsurface biogeochemistry and contaminant fate and transport.

2. Scientific Hypotheses

The following global hypotheses will guide research in the first 5 years of the SFA:

1. *Hanford Microbial Ecology*: Microorganism species richness and equitability will increase in groundwaters near the Columbia River. The functional properties and redundancy of microbial communities will be determined by local microscale geological, chemical, and hydrological conditions that are subject to seasonal variation. Resident microbial communities will coincidentally catalyze biogeochemical transformations of U, Tc, or Pu and/or modify aqueous, mineralogical, or surface chemical properties to affect contaminant migration.
2. *Hanford Redox Microenvironment*: Hanford subsurface microenvironments and transition zones will be dominant regions of contaminant reaction. Reductive valence transformations (heterogeneous and biologic) of Pu, Tc, and U will occur in microenvironments where: oxygen is consumed by microbiologic respiration; microorganisms populate their cell envelope with redox-active biomolecules; or ubiquitous ferrous containing mineral solids are exposed for dissolution and reaction. Oxidation reactions [e.g., of U(IV) and Tc(IV)] will be retarded in these zones of higher intrinsic oxygen consumption as compared to advectively dominated ones where oxygen is more rapidly replenished. Contaminant precipitation reactions will also be promoted in these domains as a result of heterogeneous nucleation and local pore water compositions that differ from bulk.
3. *Diffusion-Controlled Mass Transfer*: The establishment and maintenance of microenvironments and transition zones in Hanford subsurface sediments will be strongly influenced by diffusion processes. Restricted solute mass transfer will occur at multiple scales and will cause disequilibrium between advectively transported waters in primary flow-paths and diffusion controlled fluids in nano- to micro-domain pores; and the interiors of grains, grain aggregates, microbial cell aggregates, and fine-grained sediment bodies. Mass transfer will regulate solute, nutrient, reactant, and contaminant fluxes to and from sites of biologic and geochemical reaction, and consequently the rates and extent of these important processes.
4. *Process Scale-Dependency*: The dominant processes within microenvironments and transition zones will vary with scale from microns to meters. At the smallest scale, heterogeneities in mineral and biological structures, and chemical composition/functionality will create nano-sites of disproportionate molecular reactivity that dominate the (bio)geochemical behavior of larger microscopic reaction domains. At the largest scale, geologically structured heterogeneities in grain size distribution, permeability/porosity/ tortuosity, and mineralogy and natural organic matter, will control linkages between abiotic (transport, geochemistry) and biotic processes at the macroscopic scale to create localized zones of reaction that dominate field scale contaminant migration.

3. National Laboratory Program Structure

The PNNL SFA includes a Laboratory Research Manager (Dr. Harvey Bolton, Jr.), two chief scientists [Drs. John Zachara (PI) and Jim Fredrickson (PI)], and a core research team of technical leads. Dr. Bolton in consultation with the Chief Scientists has overall management responsibility of the SFA, and

interfaces with OBER and PNNL management who have staff in the SFA. Dr. Zachara and Fredrickson will lead and manage the science. Listed below are the key staff members for the SFA, their role, and the task or tasks they are responsible for.

Harvey Bolton, Jr., Laboratory Research Manager. Dr. Harvey Bolton serves as the management point of contact between PNNL/SFA and BER/ERSP. Dr. Bolton also ensures that the significant accomplishments of the SFA are relayed to PNNL management, and that BER guidance and scientific direction is properly relayed to SFA principal investigators.

John M. Zachara, Principal Investigator. Drs. Zachara and Fredrickson jointly manage the science portfolio of the SFA and are responsible for its scientific outcome. Dr. Zachara coordinates SFA activities with the Hanford Site and is responsible for assuring and managing site impact. He also facilitates all coordination with PNNL's Hanford IFRC, working toward partnership and synergy on field experiments proposed by both projects. Dr. Zachara is the technical lead for the SFA project titled "Reactive Transport of U and Tc in Sediment Systems Containing Microenvironments and Transition Zones". He also participates in research activities related to reactive transport experimentation, biogeochemical transformations of U and Tc, spectroscopic measurements of speciation, and microscopic studies targeting mineralogic residence and association.

Jim K. Fredrickson, Principal Investigator. Dr. Fredrickson is the technical lead for the SFA project titled "Pore-Scale Biogeochemical Processes Controlling Contaminant Fate and Transport" and it's interfacing with SFA companion projects and key external collaborators. Dr. Fredrickson coordinates microbial biomolecular and ecology activities to ensure integration and linkage with contaminant biogeochemistry research. He also serves as point-of-contact for ERSP researchers desiring Hanford-relevant microbes and sediments for their research.

Donald R. Baer. Dr. Baer is the technical lead for the SFA project titled "Impact of the Local Environment and Electron-transfer Initiated Transformations of the Structure and Chemical Properties of Mineral and Contaminant Nanoparticles". Dr. Baer is the Environmental Molecular Sciences Laboratory Lead Scientist for Interfacial Chemistry and has extensive experience in nanoparticle characterization, oxide surface analysis, scanning probe microscopy, and interfacial studies of different type.

Alexander S. Beliaev. Dr. Beliaev is the technical lead for the SFA project titled "Biomolecular Studies of Microbiological Processes Controlling Contaminant Fate and Transport", which includes the isolation as well as the genetic and physiological characterization of both model and Hanford-specific metal reducing bacteria. He directs the experimental activities and is responsible for interfacing with collaborating SFA projects, data analysis and interpretation, and manuscript writing.

Andrew R. Felmy. Dr. Felmy is the technical lead for the SFA project titled "The Chemical Form and Transformation Reactions of Plutonium in Hanford Soils and Sediments". He has long experience in actinide chemistry research and thermodynamic modeling. He is responsible for conceptualizing and managing Pu research in a rigorous and safe manner within our 331 Building Radiochemistry Laboratory, interfacing with internal SFA collaborators, and guiding externally performed X-ray absorption spectroscopy studies on Pu-containing Hanford sediments.

Allan E. Konopka. Dr. Konopka is the technical lead for the SFA project titled "Microbial Ecology". He is responsible for the experimental design and interpretation of multiple types of analyses needed to define the complex microbial ecology of the Hanford vadose zone and saturated zone. Dr. Konopka guides collaborators focused on the molecular-based analysis of microbial communities and their

relationship to subsurface properties. He actively collaborates with other SFA activities investigating the fundamental biogeochemical behavior of Hanford microorganisms.

Chongxuan Liu. Dr. Liu is the technical lead for the SFA project titled “Microscopic Mass Transfer of U and Tc in Subsurface Sediments”. Dr. Liu has a unique combination of skills involving biogeochemistry, applied mathematics, and coupled reaction transport modeling at different scales. He directs and coordinates microscopic transport experimental design and measurements, microscopic and spectroscopic characterization of grain and sediment transport properties, microscopic transport modeling, and associated publications. He is responsible for the development of new microscopic reactive mass transfer models for the Reactive Transport Modeling Project, and coordinating external microscopic reaction and transport studies.

Kevin M. Rosso. Dr. Rosso is the technical lead for the SFA project titled “Molecular Scale Mechanisms of Biogeochemical Electron Transfer Underlying Subsurface U/Tc Valence Transformation and Stability”. He is responsible for scientific direction and coordination of all abiotic molecular scale studies, and for close collaboration with molecular scale, biochemical research performed by Dr. Shi and external collaborator Dr. Richardson. He directs the experimental activities of a full-time post-doc to perform surface and chemical characterization of heterogeneous electron transfer systems, and guides the electron transfer research and biomolecular modeling activities of Dr. Kerisit.

Timothy D. Scheibe. Dr. Scheibe is the technical lead for the SFA project titled “Multi-Scale Reactive Transport Modeling”. In this role, Dr. Scheibe is closely coordinating with multiple SFA laboratory projects investigating pore scale reaction and transport processes, and larger scale reactive transport experiments to develop robust coupled process models of porous media systems containing microenvironments and/or transition zones. His research team provides needed state-of-the-art modeling capabilities for experimental interpretation of these tasks, and in collaboration with Drs. Ward and Wood (Oregon State University), is developing theoretical and measurement-based concepts for upscaling of knowledge and process-based models to the field. Dr. Scheibe and his team are applying these robust models to SFA and IFRC field experiments.

Liang Shi. Dr. Shi is the technical lead for the SFA project titled “Functional Characterization of Microbial Macromolecules”. As a microbial biochemist, Dr. Shi is developing model macromolecular experimental systems and approaches to study fundamental electron transfer and other bio-directed reaction processes responsible for the biogeochemical transformations of polyvalent metal contaminants by Hanford subsurface microorganisms. He closely collaborates with other SFA microbiologists (Konopka and Fredrickson) to identify key, high impact microorganism and macromolecular systems for research. Dr. Shi will purify and characterize the target macromolecules, determine the bio-mechanisms that regulate their distribution and cellular localization, and, in collaboration with the project of Dr. Rosso, will perform fundamental research on the mechanisms of their interactions with soluble contaminants and various Hanford mineral surfaces.

Andy L. Ward. Dr. Ward is the technical lead for the SFA project titled “Facies-based Characterization of Hydrogeologic Structures and Reactive Transport Properties”. Dr. Ward is a soil physicist and geophysicist who deals with properties measurements, geostatistical relationships, and scaling laws for field-scale hydrogeologic and transport models. His project is obtaining independent measurements of pore distribution, surface-area-to-pore-volume ratios, and diffusion-limited surface trapping length using geophysical methods (including complex resistivity). Correlations are being established between geophysical measurements and sediment physical properties that support development of three-dimensional field scale hydrogeologic models of subsurface domains exhibiting complex stratigraphy, microenvironments of different scale, and transition zones.

At the beginning of FY10 (late October, 2009), the SFA leads have scheduled a one-day meeting for the PNNL investigators. The morning will consist of a poster session that will allow the investigators and leads to assess the technical progress and direction of each subproject. An afternoon session will be used to facilitate scientific integration by identifying hypothesis-specific coordinating science themes based on findings and progress from year one (FY09). We anticipate some-redirection of FY10 funding, relative to FY09, based on the outcome of this meeting.

4. Performance Milestones and Metrics

Performance against milestones and metrics is provided as progress towards addressing each of the global scientific hypotheses listed in Section 2 as communicated to Dr Todd Anderson in our letter of July 10, 2008. It is noted that the information provided below is a synopsis that includes a single science highlight. A more comprehensive description of progress towards all milestones, provided by each Technical Lead, may be found in the Appendix (Section 7). It should be emphasized that during FY09, the first year of the PNNL SFA, considerable effort was placed on obtaining and characterizing relevant Hanford materials to facilitate the transition to Hanford-inspired research themes. Such characterization and analysis efforts are critical for rigorously testing our stated scientific hypotheses.

Hypothesis 1. Hanford Microbial Ecology

Description of Scientific Progress. PNNL is developing a conceptual model for microbial ecology in Hanford's unconfined aquifer with specific emphasis on implications for long-term contaminant fate and transport. The first essential element in developing such a model is a characterization of microbial biomass, phylogenetic diversity, and biogeochemically-relevant activities. In coordination with the Hanford IFRC, core samples were collected during drilling of the 52 m "deep characterization borehole" (DCB, well C6209) at the 300A IFRC site in July 2008. Detailed microbiological analyses were made on 17 core subsamples from multiple geological formations including: the saturated zone of the Hanford formation (9-10 m), the oxic fine-grained upper Ringold formation (17.4-18.2 m), the reducing upper Ringold sediments immediately below the oxic interface, and reduced sediments within the lower Ringold formation extending to the surface of the Columbia River flood basalts.

Biomass and Functional Gene Analysis. Viable microbial biomass, as determined by direct microscopic counts, ranged from $1\text{-}29 \times 10^7$ cells per g, with the highest counts in the Hanford formation sediments. Both lipid biomarker analysis and real time quantitative PCR (qPCR) assays showed a 15-fold higher biomass concentration in the Hanford when compared to the Ringold formation, with less than 10-fold variation among samples collected from the Ringold. The most probable number (MPN) of cultivatable aerobes were 1.3 ± 2.2 % of the total lipid-based cell estimates, whereas the MPN of fermenters, denitrifiers, metal and sulfate reducers were estimated to comprise less than 0.5% of total lipid-based microbial biomass. Archaea were less than 8% of total microbial community, normalized to the absolute copy numbers of total Bacterial plus Archaeal 16S rRNA genes, with elevated relative abundances found near the upper Ringold oxic-reducing interface. The *nosZ* gene, associated with denitrification (nitrous oxide reductase), had a relative abundance of 5-17% of total 16S rRNA genes below 18.3 m and <5% in samples above 18.1 m. Most of *nosZ* gene sequences were affiliated most closely with *Ochrobactrum anthropi* (97% sequence similarity) or *Achromobacter xylosoxidans* (90% similarity). The potential for microbial dissimilatory sulfate reduction was assayed by analysis of *dsrA* (dissimilatory sulfite reductase) gene copies, which ranged from below detection in Hanford formation to as high as 7% in the oxic Ringold sediments. The majority of *dsrA* genes were related to *Desulfotomaculum thermosapovorae* in clone libraries constructed from samples near the Ringold oxic-reducing interface. The abundance of metal-reducing *Geobacteraceae*, *Anaeromyxobacter* or *Shewanella* employing phylogenetic primers was

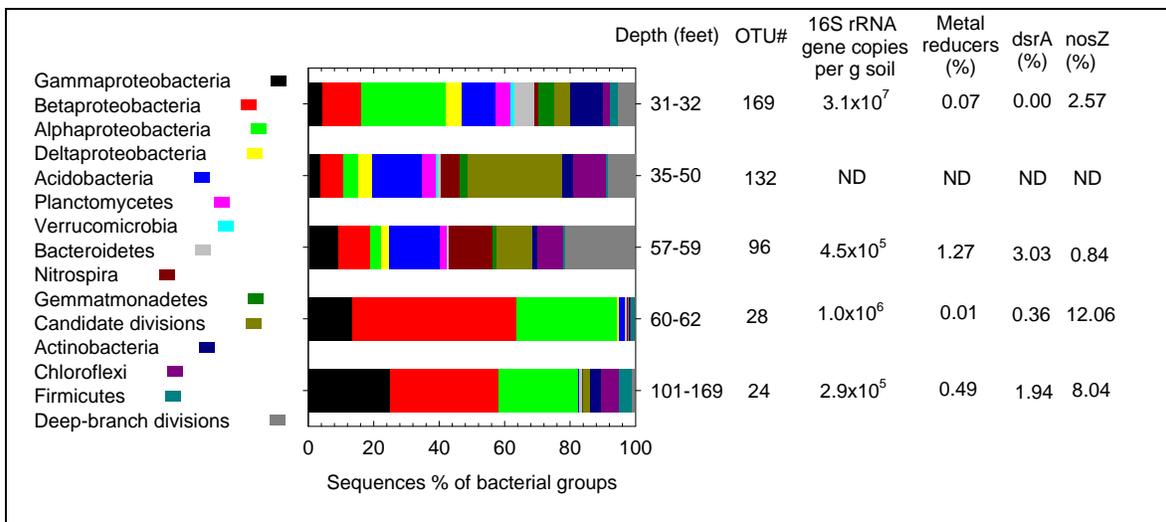


Figure 1. Abundance and diversity of the Hanford Site subsurface Bacteria assayed using 16S rRNA gene sequencing and real time quantitative PCR. Data shown are mean values averaged from a range of sampling depths in the Deep Characterization Borehole (DCB): Hanford 31-32', Hanford 35-50' (non-DCB samples), Ringold oxic 57-59', Ringold anoxic interface 60-62', and deeper Ringold 101-169'. Operational taxonomic unit (OTU) is defined using a cutoff value of 97% sequence similarity. The percentage of each physiological group is relative to the total 16S rRNA gene copy numbers. Metal reducers are the sum of Geobacteraceae, Anaeromyxobacter, and Shewanella. The *dsrA* and *nosZ* were used as biomarkers for detecting sulfate reducers and denitrifiers, respectively.

generally less than 0.5% of total 16S rRNA genes. These results suggest that the saturated zone within the Hanford formation is region of substantial microbial abundance, diversity and, potentially, high metabolic activities that could impact local biogeochemical reactions.

Microbial community composition was investigated by generating clone libraries after amplification of 16S rRNA genes from Bacteria (21 samples) or Archaea (12 samples). The samples were obtained by isolating high-quality DNA from subsurface sediment samples. Three hundred and eighty four clones were sequenced from each library at the Joint Genome Institute. The results, in general, demonstrate that the Hanford unconfined aquifer microbial community is phylogenetically very diverse, with 1,233 unique bacterial operational taxonomic units (OTUs) and 99 archaeal OTUs determined at 97% sequence similarity of 16S rRNA gene. Similar to microbial biomass distribution, bacterial diversity was significantly higher in the Hanford formation relative to the underlying Ringold Formation. In contrast, Archaea were most diverse in the oxic portion of the upper Ringold Formation. Some are affiliated with ammonia-oxidizing *Nitrososphaera sp.* and *Nitrosopumilus sp.* Bacterial OTUs were distributed among 42 divisions, with the most abundant ten divisions (from most abundant to least: *Proteobacteria*, *Acidobacteria*, GAL15, *Nitrospira*, *Chloroflexi*, NC10, *Actinobacteria*, SPAM, *Planctomycetes*, and *Gemmatimonadetes*) representing 88% of the bacterial clones in the Hanford formation and Ringold oxic sediments. In the reduced region of the upper Ringold Formation, the microbial community was dominated by proteobacteria, which accounted for 88.8% of the total bacterial clones. The Hanford subsurface was comparatively rich in candidate divisions (e.g., GAL15, NC10, and SPAM) and other novel clades at various taxonomic cutoffs. This novelty and uniqueness may reflect unique local geochemical and hydrological environments.

The collaboration with Dr. Rob Knight (University of Colorado, Boulder) is focused on utilizing their pyrosequencing and metadata analysis expertise for understanding vertical and horizontal changes in the subsurface microbial community similarity and composition, and relating these changes to subsurface environmental properties (geological/geochemical/geophysical properties). Ninety-six samples from the deep borehole as well as sediments from across the IFRC well field array have been sent to UCB and the pyrosequencing of 16S rRNA gene is completed. Data analysis and interpretation is pending; the capacity to integrate other environmental datasets that have been collected on material from sediment boreholes will be a significant factor in the quality of product from this activity.

Near-Term Science Objectives. The primary goals for FY10 include completion of the analysis of molecular data (16S rRNA gene clone libraries, 16S rRNA gene pyrosequencing data, and quantitative PCR data on functional genes), and performance of an integrated analysis using available geological, geochemical, and hydrological data from Hanford IFRC to generate hypotheses regarding environmental drivers of community structure. We will also utilize assays of microbial activity (incorporation of ¹H-leucine into biomass) to test hypotheses regarding environmental factors that drive changes in community composition and activity. These drivers include both geochemical nutrient resources and hydrological regimes. The experimental analyses include both microcosm experiments with sediment materials and in-well incubations of sediment surrogates.

Evolving data on the hydrological and physical properties, and their variation across the Hanford 300A IFRC site, create a unique opportunity to determine the effects of local environmental regimes upon microbial community composition and function. Although still in the early stages of characterization, it appears that there is considerably more vertical and horizontal variation in the hydrological properties within the Hanford formation than expected. Moreover, there is evidence suggesting that Columbia River water may reach the upper regions of the IFRC wells during high water stages in the spring and early summer. The implications of these findings to microbial community structure and function in the Hanford formation are unclear but could be extremely significant.

Hypothesis 2. Hanford Redox Microenvironment

Description of Scientific Progress. A major objective for FY09 was to define the nature and distribution of biogeochemical processes in Hanford and upper Ringold formation subsurface sediments collected from the 300A IFRC site. This included determining the potential for native microbes to utilize various terminal electron acceptors common to the Hanford unconfined aquifer, assessing U & Tc biogeochemical redox reactions, and identifying the nature of end-products and their susceptibility to re-oxidation by dissolved O₂. In addition: enrichment cultures were initiated with 300A IFRC borehole sediments to obtain relevant organisms for more detailed molecular and pore scale mechanistic investigations probing biogeochemical redox reactions; Fe, Ti, and Mn containing microparticles were collected from several locations around the Hanford Site and their characterization initiated; three distinct forms of reactive Hanford metal oxides (titanomagnetites) were generated -including nanoparticles, epitaxial thin films, and bulk materials - to probe the mechanisms and kinetics of heterogeneous redox reactions involving contaminants; core samples were retrieved from the Hanford Z-9 trench to determine the chemical form(s) and potential migration mechanism(s) of transuranics at a key Pu disposal site; and an 11-heme outer membrane c-type cytochrome from a *Shewanella* strain (HRCR-6) isolated from a U seep in the Columbia River adjacent to the Hanford 300A sediments was cloned, purified, and initially characterized.

To define the nature and distribution of biogeochemical reactions in the subsurface, Hanford formation sediments from the 300A IFRC were incubated in synthetic groundwater (SGW1) with and without exogenous electron acceptors or donors. In live sediments provided with the electron donor mix (acetate,

lactate and glucose), nitrate (2 mM) was rapidly consumed (<7 d) in all sediments (Hanford and Ringold) with the transient appearance of nitrite. For microcosm experiments investigating the potential for Fe(III) and sulfate reduction, sediment was incubated in SGW1 without nitrate and with or without exogenous sulfate (0.63 mM) for 104 d. Significant increases in 0.5 N HCl-extractable Fe(II) were measured within 37 d in the live Hanford formation microcosms amended with the electron donor mix, with the highest amount observed in microcosms with exogenous sulfate (Figure 1). Increases in Fe(II) were also observed in microcosms not amended with the electron donor mix but not in the heat-treated microcosms indicating the increase in Fe(II) was due to microbial activity. Significant increases in extractable Fe(II) were not observed in any of the Ringold sediment microcosms following a 59 d incubation period.

Aqueous sulfide increased rapidly in the live Hanford sediment microcosms amended with electron donor and sulfate to a maximum of 4.1 μM at 61d and declined thereafter. Sulfide also increased in the live microcosms amended with sulfate but not electron donor, although to a significantly lower concentration (1.4 μM). After 104 d incubation, acid volatile sulfide was 3.7 $\mu\text{mol/g}$ in the live microcosm amended with organic carbon and sulfate. This indicated that ~63% of the added sulfate was present as sediment-associated sulfide by the end of the incubation period and that the reason for the decline in the aqueous sulfide concentration after 61 d was likely due to precipitation as FeS. ^{57}Fe

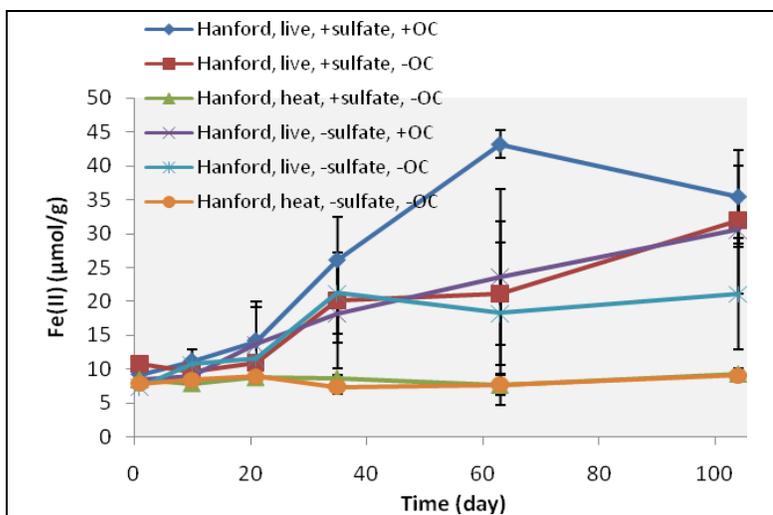


Figure 2. Weak (0.5 N) HCl-extractable Fe(II) concentration in Hanford formation sediment (<4.75 mm) microcosms as an indicator of biogenic Fe(III) reduction.

Mössbauer spectroscopy analyses are underway to characterize the nature of the bioreduced Fe phases in the Hanford sediments. These results clearly indicate the potential for microbial Fe(III) and sulfate reduction in the Hanford formation sediment and suggest that Fe(III) reduction may be facilitated by abiotic reaction with biogenic sulfide. The potential for microbial U(VI) and Tc(VII) reduction was also evaluated in Hanford sediments amended with the organic carbon mix or H₂ and with or without 0.63 mM sulfate. The detailed results from these experiments are described in detail in the Fredrickson project, Appendix (Section 7). In summary, the results from these microcosm experiments revealed diverse metabolic activities associated with the Hanford formation sediments in regards to reduction of O₂ (assumed), nitrate, native sediment Fe(III), and sulfate in addition to Hanford subsurface contaminants U(VI) and Tc(VII).

Near-Term Science Objectives. FY10 science objectives will include: 1) completion of Hanford sediment microcosm experiments and analyses, and generation and submission of associated manuscripts from FY09 activities; 2) initiation of SFA collaborative 300A in-well experiments to assess *in situ* biogeochemical processes and microbial community structure; 3) initiation of collaborative sediment microcosm experiments to simultaneously assess biogeochemical activities (electron acceptor utilization) and community structure (phylogeny), and the utilization of this information to direct cultivation to enrich and isolate representative organisms catalyzing target activities (i.e., Fe³⁺ and sulfate reduction); and (4) identification of Hanford subsurface-based pore-scale physical models for initiation of mechanistic pore-scale biogeochemical research. The latter will be done in conjunction with a new team of investigators focused on developing pore scale models for SFA research.

Collaborative in-well experiments have been designed and will be deployed during early FY10 to probe *in situ* biogeochemical activities in the Hanford formation and across the upper Ringold oxidized-reduced interface. Although groundwater sampled from wells in the Hanford formation in the 300A is oxic, it is believed to be poorly poised in regard to redox state. A key question related to the hypothesis being tested is whether there are reducing “microsites” within the Hanford formation and/or temporal fluctuations in redox state that might be coupled to Columbia River water influx during levels during peak river flow and accompanying high water table. These experiments will begin to address the hypothesis by incubating different Fe minerals and sediments *in situ* to assess biogeochemically-induced changes as a function of time, and to determine whether reducing (or oxidizing in the case of beginning with reduced materials) microsites develop within these materials. The microbial community structure will be simultaneously assessed using in-well incubated materials (Biosep beads). Sediment microcosm experiments will also be initiated whereby activities will be stimulated by addition of low concentrations of electron donor, including DOC concentrated from 300A groundwater. As reduction of native Fe(III) and sulfate proceeds, molecular phylogenetic analyses will be conducted to assay the stimulated microbiota. Based on the biogeochemical measurements and phylogenetic analyses, enrichments will be designed targeting key functional and phylogenetic groups of organisms catalyzing reduction of native Fe(III) phases and sulfate.

In addition to the above 300A IFRC site-related objectives, broader Hanford-related characterization and research activities will continue. These will include: completion of Hanford-relevant subsurface microbial isolations; initiation of physiology/phylogeny/genetic characterization studies on axenic cultures of newly-isolated, metal-biotransforming organisms; investigations of the microbial consortia within the saturated Hanford formation; completion of electrochemical characterization and initiation of crystal trials of UndA-HRCR-6 (11-heme outer membrane cytochrome); initiation of titanomagnetite surface oxidation studies for nanoparticles, thin films, and bulk materials involving conventional and synchrotron-based (EXAFS/XANES, XMCD) spectroscopy studies; application of coupled continuum-KMC atomistic simulations to observed titanomagnetite/Tc(VII) heterogeneous reduction kinetics; construction of molecular models and preliminary simulations of electron transfer within a structurally-characterized OMC complex (for comparison with protein film voltammetry measurements); refine the separation process to isolate enriched samples of other iron containing phyllosilicates from an expanded range of Hanford subsurface materials; extend reactivity studies and characterization of the isolated magnetite particles to determine the true nature of the apparently low reactivity including the role of the identified surface layer (coating or passivation) and impurities; refine the ability to conduct contaminant reactivity and adsorption studies on individual grains of isolated sediment as needed to allow surface measurements to be conducted on Tc and U; determine the chemical form of mobile Pu deep Hanford vadose zone sediments using a combination of autoradiography, XAS analysis of individual particles, and preparation of model compounds that show similar bond distances to those found in the XAS analysis; and extend analysis of the Z-9 core samples to a broader range of subsurface sediments.

Hypothesis 3. Diffusion Controlled Mass Transfer

Description of Scientific Progress. Research addressing this hypothesis includes experimental and modeling studies of mass transfer and diffusion processes of U and Tc at different scales, and their coupling with geochemical and biogeochemical reactions in subsurface sediments. An ongoing research goal has been to understand reactive diffusion at the pore and grain scales that controls U(VI) desorption and adsorption in contaminated 300 A sediments. Reactive diffusion processes are believed to contribute to the unexpectedly long persistence of contaminant U in the groundwater plume at this location. This phenomenon has been a most complicated one to understand, and a collaborative team of four SFA technical leads and collaborators are involved. Research activities have included the calculation of molecular diffusion coefficients for dominant U(VI) aqueous species in Hanford groundwater using molecular dynamics methods; development of pore-scale heuristic models of different type that simulate

reactive intra- and inter-granular diffusion and transport through representative pore networks for explanation of complex observed experimental behaviors; and challenging experimental studies of U(VI) desorption rate, intra-granular porosity, and diffusivity/tortuosity using spectroscopic and wet-chemical methods. These interactive modelling and experimental studies are providing detailed understandings of the reaction-diffusion process, and highly regarded publications.

A Lattice Boltzmann model (LBM) was developed to simulate coupled physical, chemical, and biological processes at the pore scale that control solute reactive transport in microenvironments. The LBM was linked with the grain-scale U(VI) mass transfer processes parameterized from experiment to simulate reactive transport in the Hanford 300A sediments under conditions without advection (Figure 1 left plot) and with advective groundwater flow (Figure 1 right plot). The physical properties of the porous medium were constructed based on the measured porosity, hydraulic conductivity, and hydraulic dispersivity of intact sediments from Hanford 300A IFRC site. X-ray tomographic measurement of pore and grain structures in the intact sediments provided guidelines for the distributions and orientations of pores and grains. U(VI) exchange between solids and the pore fluid was through mass transfer mediated surface complexation. The simulations revealed that: 1) the complex pore structure and their associated interfacial reactions and mass transfer cause large heterogeneities in U(VI) aqueous concentration; 2) the overall rates of U(VI) reactive transport was limited by mass transfer from small, poorly-connected pores to larger, well-connected ones; and 3) simple volume averaging of the grain-scale mass transfer rates did not simulate macroscopic U(VI) transport or breakthrough. Macroscopic rates of geochemical and biogeochemical reactions will consequently be scale-dependent, and incorporating geochemical and biogeochemical reactions in macroscopic reactive transport models will require careful consideration of both the grain-scale and pore-scale mass transfer.

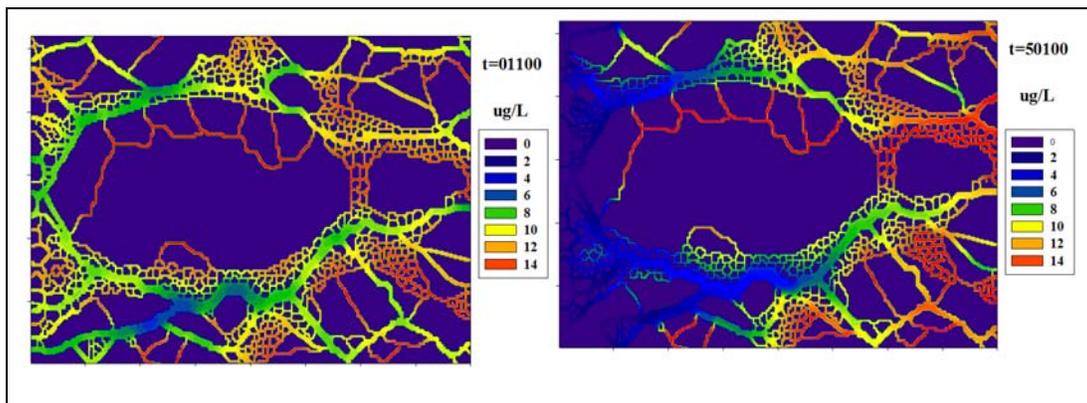


Figure 3. LBM-modeled U(VI) concentration distribution in porous media as a result of the combined effects of the grain-scale mass transfer and pore-scale diffusion (left plot), and the coupled effects of the grain-scale mass transfer and pore-scale advection and diffusion (right plot). The dark blue regions represent the solids. The other color regions are the pore spaces with aqueous U(VI) concentrations as color-code with heat intensity scale. The initial U(VI) was assumed to exist in an adsorbed state; aqueous U(VI) desorbed from solid surfaces by mass transfer at the solid-liquid interface. The U-free solution flowed from left to right in the right plot. The simulation dimension was 10 cm (width) x 6 cm (height).

A variety of new innovative experimental methods are being evaluated to quantify intra-grain porosity, connectivity, diffusivity, tortuosity, and reactivity of Hanford sediments that are fundamental physicochemical controls on the grain-scale mass transfer process shown in Figure 3. These measurements are difficult to perform with few useful examples in the literature. We have been

successful in applying stirred-flow reactors to quantify the shorter time-scale involving well connected pore domains, and column systems equilibrated with $^3\text{H}_2\text{O}$ for extended time periods to quantify the longer-time scale involving poorly connected regions. Pore-network models provide a means for interpretation. Additional research seeks knowledge on the precise nature of the internal porosity (location and reactivity) and other (e.g., chemical) factors that may affect kinetics. For example, predictions obtained using our mass transfer kinetic models suggest that the time to reach equilibrium should be dependent on extraction conditions. If solution conditions strongly favor U(VI) desorption (e.g., high alkalinity), such a system should equilibrate more quickly than one that does not favor desorption as strongly. However, this is not always observed in experiments with Hanford sediments, leading to discrepancies when models calibrated under one set of solution conditions are applied to another. Factors that may complicate the simple reactive diffusion picture include chemical transformation of the adsorbed phase over very long periods (e.g., mineral precipitation or alteration), surface diffusion of U(VI) (rather than purely aqueous-phase diffusion), steric hindrance of large uranyl complexes such as $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3^0$ within small pore spaces, and species-dependent diffusion processes and coupling with surface electrostatics. These issues will be considered in FY 10 and beyond.

Near-Term Scientific Objectives. Research in FY10 will continue the development of modeling and experimental approaches to investigate and understand physical and chemical factors controlling microscopic mass transfer in porous media. The molecular dynamics model developed in FY09 to calculate aqueous U(VI) species diffusion coefficients will be modified and extended to porous media with variable pore size and mineral surface charge properties. The resulting model will be used to evaluate the effects of pore size restriction and mineral surface charge on U(VI) diffusion within micropores of grain coatings and lithic fragment interiors where U(VI) resides in Hanford sediments. Current pore-scale models, including the LBM, cannot simulate coupled multi-species reactive diffusion with variable diffusion coefficients. Consequently, multi-component, species-based reactive diffusion theory will be established at the sub-pore, pore, and continuum scales to incorporate molecular diffusion coefficients for simulating multi-component, diffusive mass transfer. The developed theory will be used to derive scaling relationships between species-specific molecular diffusion coefficients, and apparent rate parameters in porous media with complex pore size and connectivity. Intra-granular diffusion, surface complexation, and other kinetic reactions will also be incorporated into the more versatile and powerful SPH and TE²THYS codes. These will be used for numerical experiments on the macroscopic (porous-media scale) manifestations of these pore-scale processes (for example, how variations in grain size may give rise to an apparent multi-rate mass transfer model) in virtual porous media exhibiting Hanford-relevant properties. A model cross-validation study will also be performed between the SPH, CFD, and LBM codes.

Given the immense complexity found for the physicochemical behavior of U in Hanford 300 A sediments, research will be initiated with synthetic porous material in the laboratory to test hypotheses on causal mechanisms, such as the varying time-to-equilibrium hypothesis described above. A more fundamental understanding of the involved process is sought. Experiments will be initiated using chromatography-grade silica gel as a model system with high internal porosity. A well-characterized, commercially available silica gel with a large grain size (250-500 microns) and small internal pore diameter (60 angstroms) will be used to maximize the diffusion path length. A porous alumina phase will also be used that is more stable to dissolution and mineral alteration, and that exhibits different internal pore structure and chemistry. A comparison of these two matrices should yield insights into adsorption reversibility and effects of U(VI) residence time on desorption, which can be applied to contaminated sediments. We anticipate that these results will be useful in developing more general mass transfer models, and in understanding why such models may break down when applied to complex natural systems.

Hypothesis 4. Process Scale-Dependency

Description of Scientific Progress. The Hanford IFRC site has provided the PNNL SFA with an excellent location for studies of scale dependency. The IFRC project is focused on field-scale transport processes of U(VI) within the vadose and saturated zones. The SFA complements and enriches IFRC research by focus on microscopic and macroscopic geochemical and transport processes and their linkages, and developing relationships between laboratory scale measurements and field-scale properties distributions, geologic features, and reactive transport behavior. Primary objectives for FY 09 research under this hypothesis have been to: i.) evaluate the influence of heterogeneities in physical and geochemical properties of IFRC Ringold Formation sediments on the valence stability of Tc(VII), ii.) determine and understand relationships between laboratory and field scale U(VI) reactive kinetic behaviour in IFRC Hanford formation sediments, and iii.) develop a facies-based model for the IFRC site based on laboratory grain-size and U adsorption measurements as correlated with down-hole geophysical measurements from the IFRC site. Progress toward these objectives is described in the following paragraphs.

The deep characterization borehole described under Hypothesis 1 retrieved sediments from a very interesting redox transition zone within Ringold Formation sediments of the lower unconfined aquifer at the IFRC. Ringold Formation sediments from this same depth interval represent an important aquifer facies along the Columbia River north of the 300 A in areas where 200 A contaminants are expected to break through in the future. These sediments were anoxic, and displayed significant apparent variation in mineralogic, geochemical, and physical properties over a 25 m vertical depth interval. The reactivity of a subset of 7 sediments from the transition zone for both Tc(VII) and O₂ have been evaluated in laboratory stirred-flow reactors, and results interpreted using Mössbauer spectroscopic measurements of Fe(II)/Fe(III) concentration and structural environment before and after reaction. Technetium(VII), an excellent probe of the heterogeneous redox state of the sediments in addition to being a relevant Hanford contaminant, was reduced at highly variable rates with half-lives ranging from days to many months depending on Fe(II) speciation. The nature of redox product Tc(IV) was characterized by x-ray absorption spectroscopy, and was found to be nano-polymeric Tc(IV) in all sediments studied. A multi-site kinetic model, constrained by Mössbauer spectroscopy measurements, has been developed to provide a unified interpretational strategy for both O₂ and Tc(VII) reduction as coupled to Fe(II)/Fe(III) redox behavior. Clearly implied is that Tc(VII)-containing groundwaters that may move laterally through sediments such as these will experience highly varied redox changes and subsequent retardation degree. Some of the sediment depths studied represent meter-plus zones of high reactivity that may dominate field scale Tc migration by reductive collection of large amounts of Tc(IV).

The kinetic adsorption/desorption behavior of U(VI) in Hanford 300 A sediments has long provided an interpretational challenge (note discussion for Hypothesis 3). Ultimately it is critical to understand this kinetic process as it may occur in the field. Two laboratory “upscaling” experimental campaigns were completed during FY 10 in our attempt to connect lab-based research to the field. In the first, stirred-flow reactor studies were performed of U(VI) desorption from the <2 mm size fraction of a reference 300 A sediment at different field-relevant pH values. A distributed rate surface complexation model was developed from that data that was applied to U(VI) desorption effluent data from a small column with < 2 mm materials, and a large 80 kg column with disturbed field textured sediments (85% > 2mm). The average U(VI) desorption rate was observed to be scale dependent, and decreased with each increase in experimental scale. In the second campaign, three intact cores from the IFRC saturated zone were subjected to desorption and adsorption break-through studies using synthetic groundwater to derive surface complexation and kinetic parameters that were representative of “in-situ” behavior.

The intact cores were imaged by x-ray tomography, and statistical properties of physical parameters calculated. The column effluent data showed evidence for i.) rapid transport paths resulting from coring disturbance, and ii.) strongly adsorbing micro-domains with slow kinetics. Data modeling is now underway, but initial fitted parameters show considerable similarity to those obtained from the large laboratory column with disturbed, field textured sediment. Overall, our results and models indicate that kinetic processes governing in-situ U(VI) concentrations at the IFRC site will be fundamentally slower than observed in the laboratory.

The rates and magnitude of the redox and desorption/adsorption processes described above are strongly dependent on geologic facies, that vary with the spatial heterogeneity of large-scale sediment deposition events. Research activities are underway to define and model the relationship between grain-size-distribution statistics and the abundance of natural ^{40}K , ^{238}U , and ^{232}U to allow prediction of field scale facies and associated property distributions from down-hole spectral

gamma logging data. The isotopic compositions and grain size distribution was measured in 100 samples from the Hanford IFRC site. The abundance of ^{40}K , ^{238}U , and ^{232}Th was strongly correlated with mean grain size; isotopic concentrations decreased with increasing grain diameter, and increased with sorting coefficient. Hanford clay showed concentrations of 4 ppm, 5.5 ppm, and 6.5 ppm for K, U, and Th respectively. An increase in geometric mean diameter (d_g) from 0.002 mm (clay) to 45.25 mm (very coarse gravel) showed a decrease in K of over 70% (Figure 1a), 76% for U, and 83% for Th for one of the many boreholes studied. The enrichment of natural isotopes with decreasing grain size suggest that borehole γ -ray spectra could have wide application in characterizing grain separation and sorting, and for estimating flow and reactive transport properties. The resulting correlations are being used to develop 3-D facies models from borehole geophysical measurements that reflect variations in the depositional environment and the associated subsurface heterogeneity. One preliminary realization of the lithofacies distribution along a cross-section running northwest to southeast in the Hanford IFRC well field is shown in Figure 4b.

Near-Term Scientific Objectives. Research in FY 10 for this hypothesis will continue along the three previously discussed subject lines.

Two primary studies are expected on the up-scaling of reactive transport of U(VI) in oxic 300 A sediments. In the first, the < 2 mm mineral size fraction isolated from the three intact saturated cores investigated in FY 09 will be robustly investigated in terms of their reactive mineral properties, and their surface complexation and intragrain mass transfer behaviour for U(VI). Surface complexation and mass

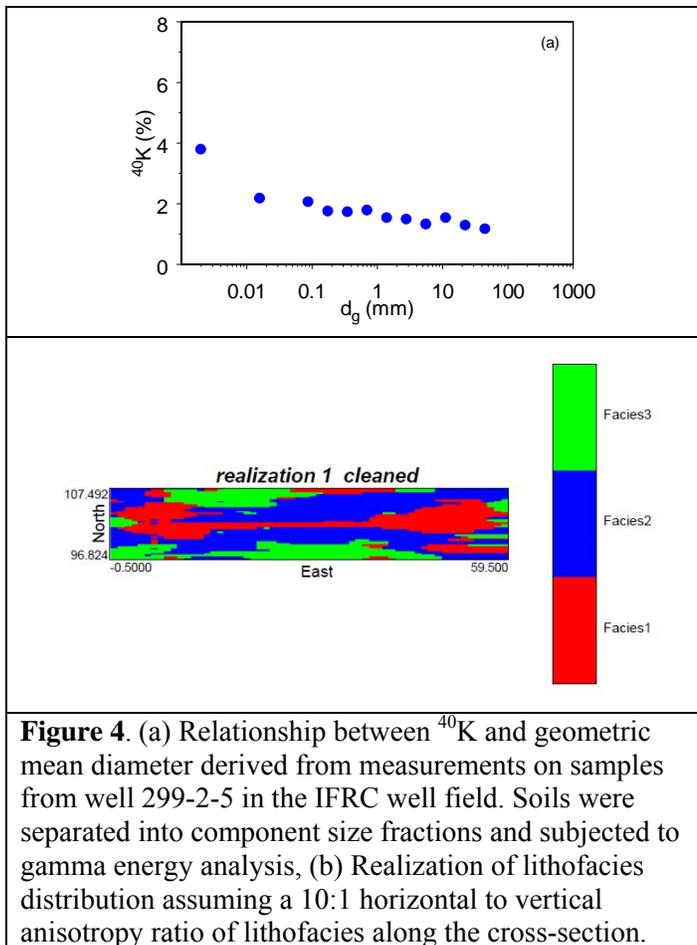


Figure 4. (a) Relationship between ^{40}K and geometric mean diameter derived from measurements on samples from well 299-2-5 in the IFRC well field. Soils were separated into component size fractions and subjected to gamma energy analysis, (b) Realization of lithofacies distribution assuming a 10:1 horizontal to vertical anisotropy ratio of lithofacies along the cross-section.

transfer models will be developed based on experimentation with the < 2 mm fraction in stirred flow reactors with IFRC synthetic groundwater over a range of conditions influencing reaction rate and extent. Various numeric procedures will then be evaluated to reconcile reaction parameters for the isolated <2 mm sediment fraction with the intact core behaviour, noting that all reactivity in the intact core is associated with this fraction. One approach will be to use tomographically-derived pore geometries from intact cores in the SPH and TE²THYS codes to test pore-scale models against column experimental data to determine the effectiveness of proposed upscaling methods. The second study will utilize the EMSL SFTF to investigate the kinetic solubilization of contaminant U(VI) from intact deep IFRC vadose zone cores that are seasonally saturated. An innovative and robust experimental system will be devised to simulate the timescales, advective character, and variable geochemical compositions associated with these complex hydrologic events that are believed to recharge and sustain the persistent 300 A groundwater U plume. Robust experimental observations will drive the development of a kinetic, reaction based simulator for field-scale application.

Research on the implications of redox transition zone biogeochemical heterogeneities will emphasize the oxidation rates of Tc(IV) and Fe(II) in reducing sediments under advective conditions with Hanford-relevant dissolved O₂ concentrations. The seven previously characterized Ringold Formation sediments will be used. The methodologies for these complex, kinetic, stirred-flow reactor experiments have only recently been documented to the point of reproducibility, affording significant opportunities for scientific advance. Macroscopic kinetic studies of O₂ consumption will be closely linked with molecular speciation measurements of Fe by x-ray absorption and Mössbauer spectroscopy and Tc by EXAFS spectroscopy, and local mineral structure by micro-x-ray diffraction. An important goal is the development of linked kinetic models based on experimental measurements that describe the reactivity of Fe(II) in mineralogic pools quantifiable by Mössbauer spectroscopy with both O₂ and Tc(VII); the resultant oxidation rate of Tc(IV) associated with these distinct mineralogic or physical environments, and the mediating role of mass transfer.

New approaches (coupled Markov Chain and transition probability) will be evaluated and applied to simulate the field-scale spatial distribution of lithofacies (e.g., Figure 4b) from local-scale in-situ (core and borehole) and ex-situ laboratory measurements of natural γ -emitting isotopes and broadband electrical properties. Petrophysical models will be developed to translate geophysical attributes or responses to meaningful hydrogeologic parameters relevant to water and U(VI) transport, and/or microbiologic distributions and function. Correlations developed to date have been based on mean grain diameter (e.g., Figure 4a), but many of the properties are sensitive to grain sorting as well. These correlations will be made more robust by incorporating the sorting coefficient and variations in mineral composition of individual size fractions. The statistical uncertainty for field spectral gamma logging and laboratory geoelectric measurements can be high, thus parameter uncertainty will be explicitly considered for all petrophysical correlations and models. Efforts will continue to refine the use x-ray tomography and/or digital, high resolution XRCT imagery for 2-D and 3-D microstructural analysis of intact cores used in reactive transport experiments, and to serve as the basis for computing hydrophysical and geochemical properties using exact finite-element algorithms.

5. Publications and Presentations

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Shi L., D.J. Richardson, T.A. Clarke, Z. Wang, M.J. Marshall, J.M. Zachara, and J.K. Fredrickson. 2009. Functional Characterization of Key Proteins Involved in Metal Redox Transformations. Presented at the ERSP 4th Annual PI Meeting, Lansdowne, VA.

Zachara J.M. 2008. Frontiers in Environment Remediation Research. Keynote Presentation given at Synchrotron Environmental Science (SES)-IV, San Francisco, CA.

Zachara J.M. 2009. Heterogeneous Redox Potential of Different Solid-Phase Fe(II) Forms Defined by Reaction with the Pertechnetate Anion. Invited Presentation given at the American Chemical Society Spring Meeting, Salt Lake City, UT.

Zachara J.M. 2009. Nature and Reactivity of Ferrous Iron Forms Through a Subsurface Redox Transition Zone Probed by Contact with the Pertechnetate Anion. Keynote presentation given at the Goldschmidt Conference, Davos, Switzerland.

Zachara J.M. 2009. Extreme Geochemistry and Mass Transfer Influence Contaminant Migration at the Hanford Site. Keynote presentation given at the Migration 2009, 12th International Conference, Kennewick, WA.

6. Staffing and Budget Summary

Funding allocation by program element (project) and technical lead is listed at the top of Table 1. The originally proposed funding levels are included, as well as the actual funding levels allocated to each project in FY 09. Funding allocations to external collaborators are listed at the bottom of Table 1. Collaborators included multiple Universities, another Federal Agency (e.g., USGS), and other National Laboratories. The collaborators contributed to specific PNNL-led projects as listed in column #1.

Four new staff were hired in FY09 who will contribute to future SFA research. Three of these individuals may be supported as a young investigator team following the internal SFA PI meeting scheduled in late October, 2009. Drs. Carolyn Pearce and Changyong Zhang were hired into the Geochemistry Group to add capability in mineralogy, X-ray methods, and pore scale experiments. Dr. Michael Wilkins was hired into the Microbiology Group to add capability in microbial biogeochemistry and field metaproteomics. Dr. Wilkins is also an investigator in the Rifle IFRC. Dr. Jason Greenwood was hired into the Hydrology Group in the Energy and Environment Division to assist on laboratory and field geophysical research for both the IFRC and the SFA.

Three staff funded by the SFA in FY09 will not have their own projects in FY10, including Drs. Jay Grate, Ravi Kukkadapu, and Dawn Wellman. These three technical leads were funded for the last year of their original ERSP projects. Beginning in FY10, Dr. Matt Marshall will assume duties as technical lead of the project “Biomolecular Studies of Microbiological Processes Controlling Contaminant Fate and Transport from Hanford 300 Area Sediments.” The current technical lead, Dr. Alex Beliaev, will serve as technical consultant on this task and focus his efforts on the PNNL GTL Biofuels SFA.

Capital equipment requests for FY10 will be developed at the internal PNNL SFA workshop in October and submitted to CESD immediately thereafter.

PNNL has started a new Laboratory-level LDRD Initiative called the Microbial Communities Initiative (MCI), which will contribute capabilities related to this SFA. The MCI will develop technologies and approaches to “see the world the microbe sees” and focus on analysis at the spatial scale of microns; analysis of individual cell properties within microbial communities; and move genomic, transcriptomic, and proteomic technologies from descriptive to a functionally predictive approach. An overview of the Microbial Communities LDRD Initiative was given to DOE-BER by Allan Konopka at BER Headquarters on August 26, 2009.

Table 1. ERSP - Scientific Focus Area					
Project #	Project Title	Technical Leads	FY09 Proposed	FY09 Changes	FY09 Actual
Project 1	ERSP Management (includes \$22.172K uncosted from FY08)	John Zachara/Jim Fredrickson	\$275,000	(\$29,222)	\$245,778
		Subcontract overheads	\$83,544	(\$14,650)	\$68,894
		Transfer to capital	\$0	(\$20,000)	(\$20,000)
Project 2	Impact of the Local Environment and Electron-Transfer Initiated Transformation of the Structure and Chemical Properties of Mineral and Contaminate Nanoparticulates	Don Baer	\$250,000	(\$25,000)	\$225,000
Project 3	Biomolecular Studies of Microbiological Processes Controlling Contaminant Fate and Transport	Alex Beliaev	\$300,000	(\$30,000)	\$270,000
Project 4	The Chemical Form and Transformation Reactions of Plutonium in Hanford Soils and Sediments	Andy Felmy	\$300,000	(\$30,000)	\$270,000
Project 5	Pore-Scale Biogeochemical Processes Controlling Contaminant Fate & Transport	Jim Fredrickson	\$500,000	(\$50,000)	\$450,000
Project 6	Microbial Ecology	Alan Konopka	\$550,000	(\$55,000)	\$495,000
Project 7	Microscopic Mass Transfer of U and Tc in Subsurface Sediments	Chongxuan Liu	\$375,000	(\$37,500)	\$337,500
Project 8	Molecular Scale Mechanisms of Biogeochemical Electron Transfer Underlying Subsurface U/Tc	Kevin Rosso	\$300,000	(\$30,000)	\$270,000
Project 9	Multiscale Reactive Transport Modeling	Tim Scheibe	\$450,000	(\$45,000)	\$405,000
Project 10	Functional Characterization of Microbial Macromolecules	Liang Shi	\$300,000	(\$30,000)	\$270,000
Project 11	Facies-based Characterization of Hydrogeologic Structures and Reactive Transport Properties	Andy Ward	\$400,000	(\$27,500)	\$372,500
Project 12	Reactive Transport of U and Tc in Sediments Containing Microenvironments and Transition Zones	John Zachara	\$500,000	(\$50,000)	\$450,000
Project 13	Plutonium Transport Under Unsaturated Groundwater Conditions	Ravi Kukkadapu	\$0	\$94,500	\$94,500
Project 14	ERSP transition funds	Jay Grate/Dawn Wellman	\$300,000	(\$30,000)	\$270,000
	Young Investigator		\$200,000	(\$200,000)	\$0
	Total SFA internal funds		\$5,083,544	-\$609,372	\$4,474,172
Collaborators					
Project 3	University of Wisconsin-Milwaukee	Daad Saffarini	\$90,975	(\$975)	\$90,000
Project 3	Georgia Tech.	Frank Loffler	\$90,890	(\$890)	\$90,000
Project 4	Los Alamos National Laboratory	Steve Conradson	\$100,000	(\$10,000)	\$90,000
Project 5	University of Wisconsin-Madison	Eric Roden	\$90,975	(\$975)	\$90,000
Project 5	Argonne National Laboratory	Ken Kemner	\$150,000	(\$15,000)	\$135,000
Project 6	University of Colorado	Rob Knight	\$90,975	(\$975)	\$90,000
Project 7	Stanford University	Scott Fendorf	\$90,975	(\$975)	\$90,000
Project 9	Oregon State University	Brian Wood	\$113,726	(\$113,726)	\$0
Project 10	University of East Anglia	David Richardson	\$136,470	(\$1,470)	\$135,000
Project 11	Idaho National Laboratory	Roelof Versteeg	\$125,000	(\$25,000)	\$100,000
Project 12	USGS	James Davis	\$136,470	(\$1,470)	\$135,000
Project 12	Oak Ridge National Laboratory	Melanie Mayes	\$200,000	(\$20,000)	\$180,000
	Total University and/or private sector funds (includes USGS)		\$841,456	-\$121,456	\$720,000
	Total other National Laboratory funds		\$575,000	-\$70,000	\$505,000
		Total	\$6,500,000	-\$800,828	\$5,699,172

7. Appendix: Project-Task Summaries for FY 2009

Impact of the Local Environment and Electron-Transfer Initiated Transformations of the Structure and Chemical Properties of Mineral and Contaminant Microparticulates

Principal Investigator: **D. R. Baer**

Internal Participants: K. Krupka, J. Liu

External Collaborators: R. Lee Penn (University of Minnesota)

1. Scientific Progress

The objective of this project is to obtain fundamental information about reactivity and structural, chemical and compositional transformations of sediment-derived and biogenic metal oxide microparticles that are induced by electron transfer reactions and other interactions with their local environment. The initial phase of this activity is focusing on the isolation, identification and characterization of Fe(II), Fe(II/III), Mn(III/IV) and Ti containing microparticulates in Hanford sediments of different ages and facies types. This is essentially a census of the relevant materials and includes assessing existing information about these phases and isolating materials for detailed studies. The second element of the project involves comparison of the reactivity the isolated particles and the performance of mechanistic reactivity studies on isolated microparticles using probe molecules to probe electron transfer reactions and particle transformations. Impacts of surface precipitation and surface passivation on particle reactivity and the time dependence of reactivity are being examined.

Most of the activities during FY09 revolved around the objective to assemble, characterize and catalog Fe, Ti and Mn containing microparticles from Hanford sediments.

- Collecting and summarizing information about Fe particulate distribution around the Hanford Site available from previous studies in published papers or reports has been with focus on the Fe-, Ti-, and Mn-contents of Hanford Site Sediments.
- Using magnetic and density based separation methods to isolate iron containing microparticulates from Hanford sediments. Different levels of separation methods were applied to three different composite sediments from the 200 and 300 areas.
- A variety of characterization methods including SEM, EDS, XRD, and XPS were applied to several portions of the isolated materials
- Collecting significant quantities of representative sediments likely to be relatively rich in Fe particulates for additional separation and study as related to all relevant components of the SFA. Sediment collection was conducted with consultation and assistance of Hanford Site geology expert Bruce Bjornstad.

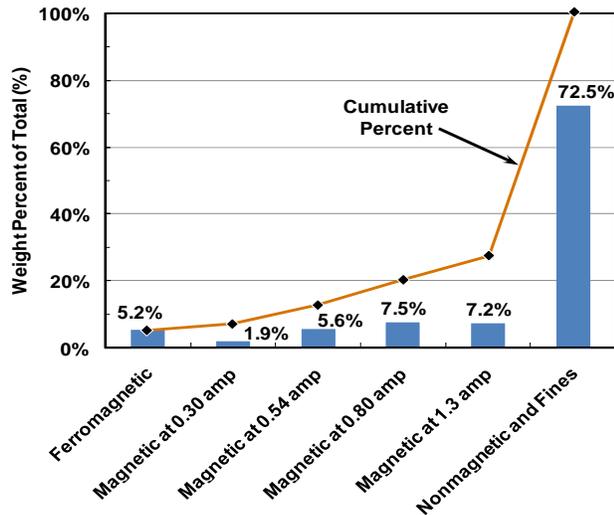
As a start to the mechanistic reaction and kinetic studies, reactivity measurements were made on magnetite particles isolated from the 200 west sediment described below.

The most extensive efforts to isolate and characterize Fe containing particulates were applied to a composite assembled from sediments extracted from a core collected in the 200 west area which appeared to have the best opportunity for efficient separation. With the guidance of Dr. Andrew Grosz from the United States Geological Survey this sediment was separated by both magnetic and density (panning processes) separation processes. A general overview of the results from the magnetic separation process is shown in Fig. 1. Approximately 1/4 of the sediment by weight is ferromagnetic or paramagnetic. Consistent with previous studies, much of the magnetic material is associated with paramagnetic

Fe(II)Fe(III) phyllosilicates which appear enriched due to the magnetic separation process after the ferromagnetic material has been removed. A more detailed characterization of these phyllosilicates is underway.

Between 0.1% and 0.2 % by weight is made up of approximately 100 μm sized crystals predominately of magnetite. This amount of magnetite is generally consistent with previous macroscopic (not with isolated particles) analysis of similar materials. These relatively large isolated crystals, some of which appear to be nearly ideal rhombohedral magnetite crystals, were characterized by XRD, XPS, and SEM and subjected to reactivity studies. Although many of these particles appear to be predominately magnetite by XRD and SEM (EDS), XPS analysis of individual particles indicates they are coated with a Si rich layer that may be 1 or 2 nm thick and that the Fe near the surface is not fully oxidized (e.g. contains a mixture of Fe(II) and Fe(III)). In spite of the Fe(II) near the surface, these particles appear unreactive relative to probe molecules that react with artificial synthesized magnetite. The material remains unreactive relative to synthetic magnetic particles even when the particles were ground to minimize the impact of surface layers.

Weight Percent of Fractions from Frantz® Separator from B8814 Composite Sediment Sample (155 g total)



Based on the amount of surface area needed for some types of solution reactivity studies, it appears potentially useful to examine reductive reaction processes on individual particles. Therefore initial measurements have been conducted examining sorption onto individual particles using XPS. Initial measurements verify that U does adsorb onto the particle surfaces in quantities that can be measured and quantified by XPS. Based on work on related projects, such studies may also be possible for Tc.

To enable separation of particles in quantities sufficiently large to allow a variety of studies, somewhat coarse, medium and fine grained sand/sediment was collected from the ERDF (Environmental Remediation Deposition Facility) and the Submarine Trench. There is approximately 175 kg of the fine and coarse sediment collected from the submarine trench and 350 kg of the medium sand from the ERDF facility. A campaign to separate magnetite particles from this sediment is planned for early FY10 at the USGS. Capabilities to enable such campaigns to be conducted at PNNL are being established.

2. FY10 Goals and Deliverables

The goals for FY 10 expand on the separation and characterization efforts of FY09 and expand the reaction studies initiated. In addition to the studies described below, materials isolated in this project will be used by other projects in the SFA.

Separation and Characterization

- Conduct campaign to isolate sufficient quantity of sediment components for reactive measurements in this project and other related SFA and collaborative projects.
- Refine the separation process to isolate enriched samples of other iron containing phyllosilicates

Reactivity Measurements

- Expand the reactivity studies and characterization of the isolated magnetite particles to determine the true nature of the apparently low reactivity including the role of the identified surface layer (coating or passivation) and impurities.
- Extend the reactivity tests to the iron rich components of a wider range of sediments (including the coarse and fine sediments collected in FY09).
- Refine the ability to conduct contaminant reactivity and adsorption studies on individual grains of isolated sediment as needed to allow surface measurements to be conducted on Tc and U.

Publications on the reactivity of the natural magnetite particles in comparison to synthetic magnetite, the use of surface analysis tools to characterize nanoparticulates, and measurements of sorption properties of individual grains of magnetite are planned for FY10.

3. New Scientific Results that May Shift Emphasis

The measurements indicating that the magnetite particles isolated from the Hanford sediments are much less reactive than laboratory synthesized magnetite will focus some attention on determining the nature and extent of these differences in reactivity between the natural and artificial magnetite. If the larger natural particles are as unreactive as they appear in initial results, attention will likely shift to identifying other more reactive components in the sediment.

4. External Collaborator Research Activities

R. Lee Penn from the University of Minnesota has been testing the use of redox probe molecules on magnetite particles isolated from Hanford sediments. These measurements had been compared to tests that have been conducted on particles synthesized in her laboratory.

Some of the particle isolation and separation activities have been conducted through an informal collaboration with Dr. Andrew Grosz of the US Geological Survey.

Paul Tratnyek from the Oregon Health and Sciences University is not supported as part of the current SFA, but was involved in previous studies of nanoparticles reactivity on the ERSP project from which this SFA project was derived and remains a participant on the linked BES project. Work from the OHSU group has focused on nanoparticulate reactivity as a function of environment and time and the impact of natural organic materials.

5. Capital Equipment Needs

None requested for FY10. The project does rely on existing and new capabilities being developed for EMSL.

6. Awards and Miscellanea

Awards

D. R. Baer elected Fellow of the American Association for the Advancement of Science.

D. R. Baer received the 2009 Albert Nerken Award from the American Vacuum Society “for seminal contributions towards advancing the application of surface-sensitive techniques to understand environmental important materials and interfacial processes.”

Editorial Boards and Symposia

D. R. Baer, Editorial Board and Reviews editor for Surface and Interface Analysis

D. R. Baer on program committee and session chair of “In situ microscopy and spectroscopy” symposium at the American Vacuum Society (2008 and 2009) and session chair for nanotechnology at the European Conference on Applied Surface Analysis, October 2009.

Biomolecular Studies of Microbiological Processes Controlling Contaminant Fate and Transport from Hanford 300 Area Sediments

Principal Investigators: **A.S. Beliaev and M.J. Marshall**

Internal Collaborators: O.V. Geydebekht and D.W. Kennedy

External Collaborators: D.A. Saffarini (U of Wisconsin) and F.E. Löffler (George Tech.)

1. Scientific Progress

This project focuses on genetic and physiological characterization of Hanford site-relevant subsurface microbial isolates and consortia. Emphasis is placed on single-organism investigations to understand the molecular mechanisms involved in metal and radionuclide biotransformation. Consistent with the overall scope of the project, initial studies to isolate relevant subsurface microbial isolates are currently in progress using materials derived from the Microbial Ecology project. Sediment samples from three depths of the “deep characterization borehole”, well 399-2-25 (C6209), were obtained and added to a series of enrichment tubes to isolate relevant microorganisms which are able to reduce Fe(III)- and Mn(IV)-oxides either directly or indirectly. The enrichments were transferred into a defined minimal media containing synthetic ground water based on the composition of Hanford IFRC 300 Area ground water and amended with either synthetic ferrihydrite (FH) or MnO₂ as the sole electron acceptor. Concurrent experiments using sulfate or nitrate as the sole electron acceptor were also performed. At each sediment depth, enrichment tubes were provided with one of fourteen different electron donor sources to stimulate growth and activity of subsurface organisms capable of dissimilatory metal reduction. After our initial studies, we have determined at least ten putative electron donors that appear to be capable of serving as electron donor sources for the reduction of FH. Several tubes reveal the biotransformation of FH to a mineral phase with ferrimagnetic properties, suggestive of biomagnetite. While some electron donors appear to rapidly stimulate FH reduction (~5 days) others require significantly longer (~90 days) to observe biotransformation of the FH. Initially, our studies indicated that the addition of small quantities (0.1 mM) of casamino acids (CAA) into some tubes proved beneficial in stimulating FH biotransformation. We determined that in these tubes, CAA metabolism influencing our FH biotransformation experiments. Subsequently, CAA amendments were eliminated from culture transfers (1% vol). This step has reduced the putative number of electron donor sources which are able to stimulate the biotransformation to about seven different electron donors. We are unable to say if the oxidation of all of these electron donors is being directly coupled with FH reduction or if the oxidation of some of these electron donors, by non-metal reducing, co-contaminating organisms, creates other oxidizable substrates which can then serve as electron donors for FH biotransformation. The putative electron donors include acetate, succinate, CAA, ethanol, glycerol, lactate, and pyruvate. The addition of glycerol or CAA appears to stimulate biotransformation of FH in sediment samples collected from all three depths of the deep characterization borehole. Other electron donor sources only stimulated biotransformation at one or two of the borehole sample depths.

Interestingly, plating experiments with some of these cultures has revealed the formation of a black precipitate on individual colonies. We suspect that the black precipitate results from the generation of FeS after the bioreduction of sulfate, a natural component of the Hanford IFRC 300 Area ground water. This finding has prompted us to further investigate the influence of other components within the IFRC ground water, i.e., sulfate and nitrate. Through collaboration with the Saffarini lab, isolation experiments for sulfate and nitrate reducing bacteria from the Deep Characterization Borehole sediments are in progress.

[Milestone 1 -In collaboration with Hanford microbial ecology effort, complete physiological/genetic characterization of strains isolated from Columbia River; identify and characterize

(physiology/phylogeny/ genetic) new strains from 300 area sediments potentially involved in contaminant transformation]. Our studies to analyze the consortia in each of the positive enrichments and ultimately isolate pure organisms are ongoing. As isolates are obtained, emphasis will first be placed on characterizing the relative rates of biotransformation for both metal and radionuclides. Subsequent studies will be designed to identify genes, pathways, and subsystems involved in those key reactions, which will ultimately result in developing a comprehensive cross-species model of biological mechanisms involved in contaminant biogeochemical transformations with site-relevant organisms.

The Löffler lab has taken a more focused approach at the isolation of dissimilatory metal reducing bacteria from both the Hanford 300 area IFRC core samples and sediment isolated from near-shore region of the Columbia River at known Uranium seep. Using their established anaerobic isolation techniques and a complex electron donor source (acetate and H₂) they have enriched for bacteria within the δ-Proteobacteria group. These studies are focused to isolate both *Geobacter*- and *Anaeromyxobacter*-related organisms. They recently have isolated two new *Anaeromyxobacter* strains from the Columbia River sediment. These strains have been shipped to PNNL for additional physiological characterization.

2. FY10 Goals and Deliverables

FY10 studies will focus on: 1) completion of Hanford-relevant subsurface microbial isolations; 2) initiation of physiology/phylogeny/genetic characterization studies on axenic cultures of newly-isolated, metal-reducing organisms; 3) investigations of the microbial consortia within the saturated Hanford formation.

Our initial isolation studies of the Deep Characterization Borehole did not directly look at the microbial consortia within the saturated Hanford formation. These studies are planned for FY10 in collaboration with the Konopka and Fredrickson SFA tasks. Our goal is to look at the biotransformation of natural sediments by the native microbial consortia. In these studies, we will concentrate the dissolved organic carbon in natural IFRC ground water and use this as a source of reducing equivalents for microbial activity. Microcosm experiments using natural sediment and concentrated dissolved organics will be monitored for the production and composition of Fe(II) containing materials. The reactivity of Fe(II) containing materials with U(VI) or pertechnetate will be determined. Concurrently, qPCR will be used to ascertain the time-dependent changes within the microbial population changes after the addition of concentrated dissolved organics. Studies to isolate bacteria stimulated within this system will further increase our understanding of the relevant microbial consortia at the Hanford 300 Area IFRC.

We are targeting the following manuscripts for FY10 submission:

[Milestone 1 - Complete physiological/genetic characterization of strains isolated from Columbia River and submit paper]

Columbia River bacterial isolations:

Marshall MJ, Kennedy DW, Plymale, Fredrickson JK, Beliaev AS, *et.al.* An Auecological Study of *Shewanella* spp. in the Hanford Reach of the Columbia River. Journal TBD.

Thomas SH, Löffler FE, Geydebekht OV, Marshall MJ, Kennedy DW, Fredrickson JK, and Beliaev AS. Isolation of novel strains of *Anaeromyxobacter* spp from the Hanford Reach of the Columbia River. Journal TBD.

3. New Scientific Results that May Shift Emphasis

There are no major shifts in technical emphasis anticipated in FY10. The primary emphasis will continue to be the isolation of Hanford-relevant metal-reducing organisms from the 300 area IFRC boreholes. In addition to the isolation of metal-reducing organisms, we anticipate that we will also isolate both Hanford-relevant sulfate- and nitrate-reducing organisms. The isolation of these cultures and from the Hanford subsurface will allow for lab-based biomechanistic research in FY11.

4. External Collaborator Research Activities

Subcontracts for external collaborators were not placed until well into FY09, hence the extent of collaborator technical progress to date has been limited. [Milestone 2 - Initiate and carry out physiological and genetic investigations of microbial redox reactions involved in contaminant transformations from strains indigenous to Hanford subsurface and Columbia River] The Saffarini lab (U of Wisconsin-Milwaukee) has begun to investigate techniques for genetic manipulation of *Anaeromyxobacter* spp. They are currently working on solid media combinations which allow for conjugation and mutant colony selection. Their goal is to isolate mutants that are impaired in their ability to reduce metals and radionuclides. Their mutagenesis process will soon be employed on the two newly-isolated *Anaeromyxobacter* spp. from the Hanford Reach of the Columbia River (300 Area Spring 9). In addition, during the a graduate student, Namita Shroff, was sent to PNNL for seven weeks to learn proper techniques for the anaerobic cultivation of bacterial cultures and also to learn the PNNL techniques used for metal-reduction kinetic assays.

Research at the Georgia Institute of Technology (Löffler) has focused on the isolation of dissimilatory metal reducing bacteria from sediment samples collected from well 399-2-29 (C6206), an IFRC well located ~45 meters away from the “deep characterization borehole” (C6209). They have begun to isolate metal reducing bacteria from Using their established anaerobic isolation techniques and a complex electron donor source (acetate and H₂) they have enriched for bacteria within the δ -Proteobacteria group. These studies are focused to isolate both *Geobacter*- and *Anaeromyxobacter*-related organisms. Currently, these isolation studies are still in progress. The goal is to isolate and characterize indigenous microorganisms capable of redox transformation of Fe(III)-containing mineral phases from the Hanford site.

5. Capital Equipment Needs

None requested for FY10.

The Chemical Form and Transformation Reactions of Plutonium in Hanford Soils and Sediments

Principal Investigator: A.R. Felmy

External Collaborators: S.D. Conradson

1. Scientific Progress

As part of PNNL's Scientific Focus Area "Role of Microenvironments and Transition Zones in Reactive Contaminant Transport" this project is characterizing the chemical form and subsurface migration mechanisms of transuranics in Hanford soils and sediments. Emphasis is currently being placed on Pu and Am migration mechanisms in the 200West Area near the Plutonium Finishing Plant (PFP), specifically at the Z-9 and Z-12 crib disposal sites. The studies completed in FY09 are described below.

In FY09 we were able to obtain possession of the core samples from the Fluor Hanford Z-9 trench drilling campaigns which were conducted in 2004 and 2006. These materials represent a unique opportunity to determine the chemical form and migration mechanism of transuranics at one of the key disposal sites at Hanford where up to 160kg of Pu were disposed and the Pu and Am have migrated to depths of up to 120 feet below ground surface. A complete inventory and visual assessment of each of the core sample was conducted and selected samples were sent to SSRL for analysis by XAS spectroscopy. At the same time, samples from the near surface were also sent to SSRL to compare any differences in chemical form between the surface and subsurface sediments. The surface sediments are also unique in that they were collected in the late 1970's before a mining operation removed the top 60 cm of sediments from the Z-9 trench. As a result these samples represent the last known representatives of the original source term material. The XAS analysis identified PuO_{2+x} as being present in the original surface sediments at the Z-9 trench but found that the chemical form of Pu in the deep sediments samples, and well as that in the Z-12 did not appear to be consistent with PuO_{2+x} . To the best of our knowledge this is the first time in an environmental sample that the chemical form of Pu was sufficiently high to be identified by XAS spectroscopy but was found not be PuO_{2+x} . Studies are currently underway to both prepare compounds (such as Pu-F phases see below) as reference materials and isolate the specific components of the soils that contain the Pu and Am. Beamtime at SSRL is scheduled for next year to analyze these samples. In addition, with help from Don Baer and Bruce Bjornstad, we also obtained uncontaminated sediments from the ERDF at the same depth as a key silt layer containing the highest Pu concentration at the Z-9 trench. These uncontaminated materials will be useful in future studies of Pu/Am migration to verify hypothesis on subsurface migration determined from studies of the contaminated materials.

In FY09 we also completed an extensive literature review on transuranic contamination at Hanford. This broad assessment was part of our original plan to examine transuranic contamination more broadly at Hanford than just in the 200 west area near PFP. Over 300 individual sites were inventoried for transuranic contamination. During the review an emphasis was placed on isotopes of Pu, Am, and Np. Approximately 80 different locations were identified that had at least 1 curie of Pu-239 disposed. Interestingly the highest Pu isotope in terms of activity that was disposed was Pu-241 not Pu-239. Pu-241 decays to Am-241 (14 year half life) so there is a larger source of Am-241 in the subsurface than estimated from the original releases. To the best of our knowledge this "source" of Am-241 has not been accounted for in inventory estimates. The inventory models do decay the original Pu-241 but do not appear to account for the formation of Am-241. In any event, both the current distribution of Am-241 in the subsurface and the subsurface migration mechanism of Am-241 must be considered in light of this result. If the Pu-241 is present as an insoluble oxide the resulting decay to Am-241 could result in a chemical form of the Am in Pu particles that is far different from that determined from an aqueous release

of Am-241. This could impact the formulation of models for subsurface fate and transport, especially for Am-241.

Experiments were also initiated to determine the influence of complexants on the aqueous complexation and transport of Pu in the subsurface. The primary complexants of concern are dibutylphosphate (DBP), monobutylphosphate (MBP) and fluoride. The DBP and MBP are breakdown products of tributylphosphate (TBP) that was used as a complexant for Pu(IV) in the solvent extraction process used in the PFP. Fluoride was a product of the conversion of PuO₂ to PuF₄. During FY09 we obtained and purified solutions of both DBP and MBP. We also completed initial solvent extraction studies of Pu(IV)-DBP complexation that extend to higher pH values than earlier work, and scoping studies of Th(IV)-DBP complexation via solubility methods. Interestingly, the Th(IV) solubility studies seem to indicate the formation of a Th(DBP)₄ solid phase that is more insoluble than ThO₂ at pH values below 5. If also true for Pu(IV), it would indicate that DBP is more likely to immobilize Pu(IV) as a solid phase than solubilize Pu(IV) as an aqueous complex. More extensive studies with both Th(IV) and Pu(IV) are planned for the next FY. These studies will also be extended to include MBP. We also initiated experiments on the complexation reactions of Pu(IV) with fluoride. Our initial efforts were focused on preparing a PuF₄(c) phase as a model phase for XAS analysis to compare with the unknown phase in the deep subsurface. Unfortunately, these initial experiments were unsuccessful in preparing PuF₄(c) even though we followed our earlier procedures for preparing ThF₄(c) and calculated the PuF₄(c) solubility with the NEA thermodynamic data base. Specifically, we discovered that the NEA database for Pu(IV)-F is inadequate to model our systems of interest. The NEA review only accepted data for PuF³⁺ and PuF₂²⁺, the reviewers rejected the data from the only studies on the formation of higher Pu-F complexes [i.e., PuF³⁺ and PuF₄(aq)]. The constant for PuF₄(aq) is quite large and if true will completely dominate the speciation in our chemical systems. Our initial efforts to precipitate PuF₄(c) seem to indicate that this species is in factor quite strong since we were unable to prepare PuF₄(c) when the NEA based thermodynamic models predicted it should form. This system needs to be redone given the potential importance of Pu-F complexation on the subsurface mobility of Pu in the 200 west area.

2. FY10 Goals and Deliverables

FY10 will focus on the following tasks. One, determination of the chemical form of Pu in the deep subsurface using a combination of autoradiography, XAS analysis of individual particles, and preparation of ideal compounds that show similar bond distances to those found in the XAS analysis. This task will be a major emphasis. Two we need to extend our analysis of the Z-9 core samples to a broader range of subsurface sediments than those examined in FY09. Specifically, during our sample inventory we identified several samples that were collected at sample depths not examined in the initial limited characterizations supported by Fluor Hanford. The Fluor sponsored work picked samples for analysis at different depth intervals often ten or more feet apart. Unfortunately this leaves major gaps in our understanding of even the total transuranic concentrations. For example, the sample from the slant borehole (W-48), previous analyzed by PNNL investigators, found high Pu/Am concentrations at depths of 60-70' bgs, very low concentrations at 80' and then very high concentrations at 120'. An unusual finding indicating the Pu/Am is not retained in the subsurface sediments between 70' and 120'. However, the low concentrations at 80' are based entirely on one analysis. We have now located several more samples in the depth interval 70 -120' that can be analyzed to determine the exact depth distributions. Analysis of these materials will be important to our final assessment of the subsurface migration mechanisms of Am and Pu. Obviously, these efforts need to be scaled with the available funding. Three, we plan to complete our studies on the impact of DBP and MBP on Pu(IV)/Th(IV) complexation. This will be a major effort but can settle the key issue of the impact of these complexants on the transport of Pu(IV) in the deep subsurface. Our initial indications are that the hypothesis of Pu(IV) migration facilitated by organic complexation maybe erroneous. We also hope to complete our initial

experimental studies of Pu(IV)-F complexation at high F. Besides the organic complexants DBP and MBP, fluoride is the other single most likely ligand to form strong aqueous complexes and transport Pu(IV) into the subsurface. We have also determined that the current thermodynamic models are inadequate to predict this migration.

We are targeting the following manuscripts for FY10 submission:

Conradson S.D. and A.R. Felmy. The chemical form of Pu in Hanford sediments: Identification of phases in the deep subsurface.

A.R. Felmy et al. The solubility and complexation reaction of Pu(IV) and Th(IV) by DBP and MBP to high pH. Impact on the subsurface mobility of Pu(IV).

A.R. Felmy et al. The complexation of Pu(IV) with fluoride to high concentration.

3. New Scientific Results that May Shift Emphasis

The most fascinating scientific finding was that the chemical form of the Pu in the deep subsurface does not appear to be PuO_{2+x} . This has long been thought to be the only environmentally important solid phase of Pu(IV) owing to its very low solubility. In the next FY, identification of the chemical form of Pu will be a major emphasis. The finding that Th(IV) forms very insoluble phases with DBP at higher pH values also casts doubt on the hypothesis that the TBP breakdown products DBP and MBP could mobilize and transport Pu(IV) in the subsurface.

4. External Collaborator Research Activities

S. D. Conradson has conducted the XAS analysis of the soils and sediments from the cribs and the deep subsurface cores described above.

5. Capital Equipment Needs

We request \$85K to replace an old Pu contaminated atmospheric chamber in the 331 building.

Pore-Scale Biogeochemical Processes Controlling Contaminant Fate & Transport: Characterization of Biogeochemical Redox Transformations in Hanford 300 Area Subsurface Sediments

Principal Investigator: **J.K. Fredrickson**

Internal Participants: J.-H. Lee (Post-doc), A.E. Plymale, D.W. Kennedy, A. Dohnalkova, X. Lin

External Collaborators: E. Roden, K. Kemner, S. Heald

1. Scientific Progress

As part of PNNL's Scientific Focus Area "Role of Microenvironments and Transition Zones in Reactive Contaminant Transport" this project is characterizing biogeochemical processes in Hanford 300 Area subsurface sediments with an emphasis on defining redox reactions at the pore scale projected to influence the subsurface fate and transport of U and Tc. In coordination with the Hanford Integrated Field Research Challenge (IFRC), a series of subsurface core samples were collected during July 2008 from the 300A IFRC site as part of site characterization efforts. Using these materials, a series of sediment microcosm experiments was initiated to explore the biogeochemical processes that may impact contaminant fate and transport in the Hanford subsurface. In FY09, the major objectives were to define the nature and distribution of biogeochemical processes in Hanford and upper Ringold formation subsurface sediments collected from the 300A IFRC site, determine the potential for native microbes to utilize various terminal electron acceptors (EAs: oxygen; nitrate; ferric iron; sulfate) common to the Hanford unconfined aquifer, assess U & Tc biogeochemical redox reactions in the Hanford subsurface (pore-scale microcosms study), and identify the nature of end-products and their susceptibility to re-oxidation by dissolved O₂.

Sediment was collected from multiple 300A IFRC borehole cores from the Hanford formation at the 30-31 ft. interval and then consolidated, homogenized, and sieved to <4.75 mm. Upper Ringold Formation sediment from the oxidized (59-60 ft.) and reduced (60-61 ft.) regions was collected in a similar manner but was not sieved due to its fine-grained texture. These materials were characterized in regards to total organic carbon, total inorganic carbon, and total Fe in addition to weak acid-extractable (0.5 N HCl), OH-amine HCl, and DCB extractable forms of Fe. The OH-amine HCl and DCB extractable Fe concentrations in the three sediments were in a similar range with 29-55 and 30-56 µmol/g sediment OH-amine HCl and DCB forms, respectively. The 0.5 N HCl extractable Fe(II) concentrations (1h) were 9.7, 3.2 and 34.7 µmol/g for the Hanford, Ringold oxidized, and Ringold reduced sediment, respectively. The Fe(II) concentration increased with increasing acid extraction time (24h) as did Al concentration, perhaps due to dissolution of phyllosilicates.

Hanford subsurface sediments were incubated in synthetic groundwater (SGW1) with and without exogenous electron acceptors or donors. One gram of sediment was added to 10 mL of SGW1 - formulated based on 300A groundwater geochemical analyses (pH 7.8) and was incubated at 22°C with an anoxic headspace and provided an electron donor mix consisting of 0.9 mM acetate, 0.6 mM lactate, and 0.3 mM glucose or H₂ (10 mL to headspace of pressure tubes). Controls consisted of tyndalized (100°C exposure for 15 min) or autoclaved (nitrate & Tc experiments), each conducted on three consecutive days, with and without electron donor. In live sediments provided with the electron donor mix, nitrate (2 mM) was rapidly consumed (<7d) in all sediments (Hanford and Ringold) with the transient appearance of nitrite. The respiratory pathway may have been denitrification although dissimilatory reduction to ammonium cannot be ruled out. For microcosm experiments investigating the potential for Fe(III) and sulfate reduction, sediment was incubated in SGW1 without nitrate and with or without exogenous sulfate (0.63 mM) for 104d. Significant increases in 0.5 N HCl-extractable Fe(II)

were measured within 37d in the live Hanford formation microcosms amended with the electron donor mix, with the highest amount observed in microcosms with exogenous sulfate (Figure 1). Increases in Fe(II) were also observed in microcosms not amended with the electron donor mix but not in the heat-treated microcosms indicating the increase in Fe(II) was due to microbial activity. Significant increases in extractable Fe(II) were not observed in any of the Ringold sediment microcosms following a 59 d incubation period.

Aqueous sulfide increased rapidly in the live Hanford sediment microcosms amended with electron donor and sulfate to a maximum of 4.1 μM at 61d and declined thereafter. Sulfide also increased in the live microcosms without electron donor but with sulfate although to a significantly lower concentration (1.4 μM). After 104d incubation, acid volatile sulfide was 3.7 $\mu\text{mol/g}$ in the live microcosm amended with organic carbon and sulphate. This indicated that ~63% of the added sulfate was present as sediment-associated sulfide by the end of the incubation period and that the reason for the decline in the aqueous sulfide concentration after 61d was likely due to precipitation as FeS. ^{57}Fe Mössbauer spectroscopy analyses are underway to characterize the nature of the bioreduced Fe phases in the Hanford sediments. These results clearly indicate the potential for microbial Fe(III) and sulphate reduction in the Hanford formation sediment and suggest that Fe(III) reduction may be facilitated by abiotic reaction with biogenic sulfide [Milestone 1 -Incorporation of Hanford-relevant organisms, sediments, and mineral separates into all research elements]. Bioreduced Hanford formation sediments both with and without sulfate (day 104) were analyzed by 16S rRNA gene clone library construction and sequencing to obtain preliminary insights into the stimulated microbial populations. SRB or sulfur disproportionating bacteria were more abundant and diverse in the microcosms amended with sulfate (*Desulfosporosinus*, *Desulfotomaculum*, *Desulfuromonas* etc.) than in the microcosms incubated without sulfate (*Desulfuromonas*). The most dominant bacteria (especially *Clostridia*) in the sediment microcosms were not detected in the Hanford subsurface 16S libraries (Konopka project). *Symbiobacteria*-like sequences (member of *Clostridiales*) was abundant only in sulfate-free microcosm. In general, the microcosm clone libraries share many similar sequences with the Hanford subsurface clone libraries, especially for the β -proteobacteria.

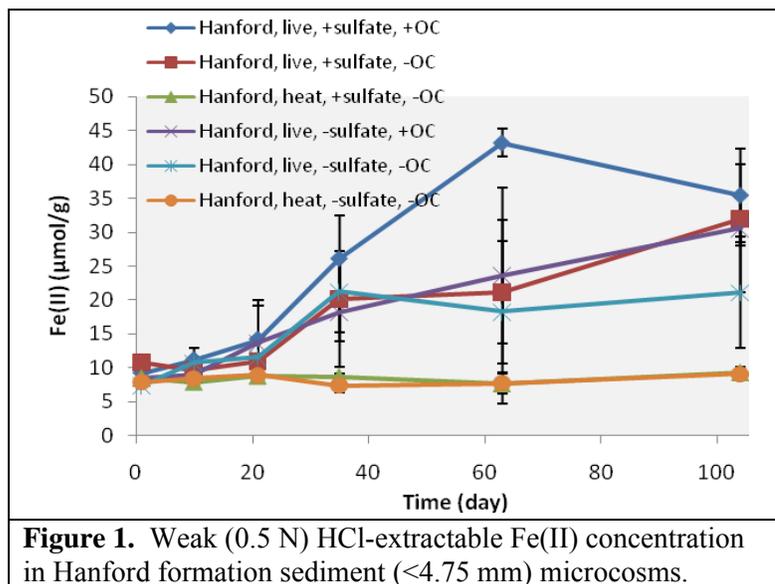


Figure 1. Weak (0.5 N) HCl-extractable Fe(II) concentration in Hanford formation sediment (<4.75 mm) microcosms.

The potential for microbial U(VI) reduction was evaluated in Hanford sediments amended with the organic carbon mix described above and with or without 0.63 mM sulfate. In the preliminary scoping experiment using the same incubation conditions except for a U(VI) concentration of 200 μM , there was no significant U(VI) reduction in Hanford formation and upper Ringold sediments (oxidized or reduced). We speculated that the sediments were inhibited by the high U(VI) concentration. When the U(VI) concentration was decreased to 20 μM , reduction to below or near detection was achieved within 81d in live Hanford sediment amended with organic carbon with or without sulfate. Aqueous sulfide (1.3 μM) was detected in the sulfate amended microcosm. Sediment samples were prepared and sent to ANL/APS (collaborator K. Kemner) for U XAS analyses. The data have been collected and are in the process of being analyzed.

The reduction of pertechnetate, $^{99}\text{Tc(VII)}\text{O}_4^-$ was also extensively evaluated in microcosm experiments. Tc(VII) was extensively reduced in Hanford formation sediment regardless of whether organic carbon was added. Reduction of Tc(VII) was also observed in the heated (tyndalized) sediments suggesting an abiotic redox reaction, possibly with native Fe(II). In a preliminary scoping experiment, no reduction of Tc(VII) was observed in live or autoclaved Ringold oxidized sediments with either H_2 or organic carbon as the electron donor. In this same experiment, Tc(VII) was reduced in Hanford formation microcosms receiving organic carbon or H_2 , regardless of whether the sediments were autoclaved. The reason for this behavior is unclear, and the experiments with H_2 are being repeated. Hanford formation microcosm sediments where aqueous Tc concentration decreased significantly over the course of the incubation period were analysed by Tc K-edge XANES analyses at APS (S. Heald). These analyses revealed that in all samples where Tc loss from solution was extensive the XANES was nearly identical to that for the $\text{TcO}_2 \cdot n\text{H}_2\text{O}$ standard. EXAFS analyses suggest a mixed Tc-Tc and Tc-Fe bonding in 2nd shell or some other type of bonding, possibly Tc-S bonding in 2nd or 3rd shells (minor) although further analysis needed [Milestone 3 - Probe biogeochemical processes impacting ^{99}Tc redox behavior in Hanford Site Ringold Formation redox transition zone].

In summary, the results from these microcosm experiments revealed diverse metabolic activities associated with the Hanford formation sediments in regards to reduction of O_2 (assumed), nitrate, native sediment Fe(III), and sulfate in addition to Hanford subsurface contaminants U(VI) and Tc(VII). While nitrate, and presumably O_2 , respiration were observed in Ringold sediment microcosms, there was no evidence of Fe(III), sulphate, or U(VI) reduction in either the oxidized or reduced Ringold sediment in the experimental condition using synthetic groundwater. We suggest that the reason for the lack of detection of these activities in the Ringold sediments was the relatively low biomass concentration and the fact that sediments were amended only with electron donor and not other potentially growth-limiting nutrients such as N or P. Abiotic reduction of Tc(VII) was observed in the reduced Ringold sediment (see Zachara project); biological reduction was not evaluated in this sediment due to the high concentration of Fe(II) and its potential competition with abiotic reduction pathways.

2. FY10 Goals and Deliverables

FY10 will focus on: 1) completion of Hanford sediment microcosm experiments and analyses and generation and submission of associated manuscripts from FY09 activities; 2) initiation of SFA collaborative 300A in-well experiments to assess *in situ* biogeochemical processes and microbial community structure; 3) initiation of collaborative sediment microcosm experiments to simultaneously assess biogeochemical activities (electron acceptor utilization) and community structure (phylogeny) and the utilization of this information to direct cultivation to enrich and isolate representative organisms catalyzing target activities (i.e., Fe^{3+} and sulfate reduction); and (4) identification of Hanford subsurface-based pore-scale physical models for initiation of mechanistic pore-scale biogeochemical research. The latter will be done in conjunction with a new team of investigators focused on integrating pore scale model research within the SFA.

As the initial microcosm experiments indicated that biotic and abiotic, via Fe(II) and/or S^{2-} , pathways for Tc(VII) reduction are potentially in operation in the Hanford formation sediments, follow-on research in FY10 will focus on delineating these pathways. Experiments and analyses will emphasize identification of reactive Fe(II) in bioreduced Hanford sediment and whether sediment-associated sulfides (AVS) are involved in catalyzing Tc(VII) reduction. We will also assess the potential for hydrogenase-driven Tc(VII) reduction by determining the extent to which genes for these enzymes may change in relative abundance in amended microcosms. Finally, we will assess the reactivity of reduced Tc(IV) toward O_2 by measuring the kinetics of Tc release to solution and, if an oxidation-resistant Tc(IV) phase is present, determine its molecular speciation and mineralogical association.

Collaborative in-well experiments have been designed and will be deployed during early FY10 to probe *in situ* biogeochemical activities in the Hanford formation and across the upper Ringold oxidized-reduced interface. Although groundwater sampled from wells in the Hanford formation in the 300A is oxic, it is believed to be poorly poised in regard to redox. A key question is whether there are reducing “microsites” within the formation and/or temporal fluctuations in redox that might be coupled to Columbia River water levels. These experiments are designed to begin to address these questions by incubating different Fe minerals and sediment *in situ* to assess biogeochemically-induced changes as a function of time, and to determine whether reducing (or oxidizing in the case of beginning with reduced materials) microsites might develop within these materials. The Konopka project will simultaneously assess microbial community structure using in-well incubated materials.

Now that the potential for key biogeochemical processes in Hanford formation subsurface sediments has been established, sediment microcosm experiments will be initiated whereby activities will be stimulated by addition of low concentrations of electron donor, including DOC concentrated from 300A groundwater. As reduction of native Fe(III) and sulfate proceeds, molecular phylogenetic analyses will be conducted to assay the stimulated microbiota (Konopka project). Based on the biogeochemical measurements and phylogenetic analyses, enrichments will be designed targeting key functional and phylogenetic groups of organisms catalyzing reduction of native Fe(III) phases and sulfate (Beliaev/Marshall project).

We are targeting the following manuscripts for FY10 submission:

Hanford 300A Sediments:

Lee J-H., A.E. Plymale, D.W. Kennedy, K. Kemner, and J.K. Fredrickson. Biogeochemical process in Hanford unconfined aquifer sediments. Journal TBD.

Tc(VII) Biogeochemistry:

Plymale A.E., J.K. Fredrickson, J.M. Zachara, A.C. Dohnalkova, S.M. Heald, D.A. Moore, M.J. Marshall, and P. Nachimuthi. Competitive reduction of pertechnetate ($^{99}\text{TcO}_4^-$) by dissimilatory metal reducing bacteria and biogenic Fe(II). Drafted, to be submitted to *Environmental Science & Technology*. [Milestone 2: Determine redox behavior of ^{99}Tc in ferrihydrite – DMRB suspensions and submit publication]

Lee J.-H., A.E. Plymale, J.K. Fredrickson, J.M. Zachara, and S.M. Heald. Biotic and abiotic pertechnetate reduction pathways in Hanford subsurface sediments. Journal TBD.

3. New Scientific Results that May Shift Emphasis

In general, there are no major shifts in technical emphasis anticipated in FY10. The emphasis will continue to be on experiments and analyses with Hanford formation sediments as these are most relevant to contaminant transport at the 300A IFRF and other sensitive environments at Hanford adjacent to the Columbia River. We anticipate that the development of relevant cultures and consortia from the Hanford subsurface and development of relevant microenvironment models will allow for lab-based mechanistic research in FY11.

4. External Collaborator Research Activities

Subcontracts for external collaborators were not placed until well into FY09, hence the extent of collaborator technical progress to date has been limited. Kemner (ANL/APS) has conducted U XAS

analyses on Hanford sediments with and without sulphate where U(VI) appeared to have been reduced, to establish U oxidation state and molecular speciation. Discussions have also been initiated to identify joint experiments between ANL (Kemner) and PNNL (Fredrickson and Liu projects) to investigate U biogeochemical transformation at the pore scale in sediments using synchrotron-based measurements.

Research at the University of Wisconsin (Roden) has investigated the bioavailability of Ringold oxidized material during repeated reduction cycles (3 transfers so far) by *Geobacter sulfurreducens* under growth conditions with 10 mM acetate provided as the electron donor. A total of 9.6 – 10 mmol L⁻¹ of Fe(III) was reduced from 4 % (wt/vol) suspension as measured by 1 hr 0.5M HCl extraction. Reduced Ringold materials were repeatedly oxidized (2 transfers so far) by a model lithoautotrophic Fe(II)-oxidizing, nitrate-reducing enrichment culture with 5 mM nitrate provided as the electron acceptor. A total of 2.3 – 3.1 mmol L⁻¹ of Fe(II) was oxidized from a 4 % (wt/vol) suspension as measured by 1 hr 0.5M HCl extraction. These results indicate that the Ringold materials contain solid phase Fe that can be used as the electron acceptor or donor by model Fe(III)-reducing or Fe(II)-oxidizing microorganisms. The Roden laboratory has also established the following enrichments cultures with reduced and oxidized Ringold material (4 wt % addition to selective media): aerobic (neutral pH) Fe(II) oxidizers with biotite as the source of Fe(II) - positive enrichments were obtained from both oxidized and reduced sediments; nitrate-reducing Fe(II) oxidizers with chemically-reduced NAu-2 nontronite as the source of Fe(II) - positive enrichments were obtained from both oxidized and reduced sediments; and Fe(III) reducers with structural Fe(III) in NAu-2 nontronite serving as the electron acceptor and hydrogen+acetate serving as potential electron donors - positive enrichments were obtained only from the reduced Ringold sediment. The Roden laboratory is also planning on contributing to the 300A IFRC in well experiments by incubating i-chips, designed by Slava Epstein at Northeastern University, loaded with fresh groundwater (with viable cell number adjusted to ca. 10⁵ cells/ml) and natural and/or model phyllosilicate phases. The goal is to isolate indigenous microorganisms capable of redox transformation of structural Fe in phyllosilicates.

5. Capital Equipment Needs

None requested for FY10.

Microbial Ecology

Principal Investigator: **A.E. Konopka**

Internal Participants: X. Lin, M. Lipton, D. Kennedy, A. Plymale

External Collaborators: R. Knight, (U. Colorado-Boulder)

1. Scientific progress

One goal of the PNNL Scientific Focus Area is to develop a conceptual model for microbial ecology in Hanford's unconfined aquifer with specific emphasis on implications for long-term contaminant fate and transport. The first essential element is a characterization of microbial biomass, phylogenetic diversity, and biogeochemically-relevant activities. In coordination with the Hanford IFRC, core samples were collected during drilling of the 52 m "deep characterization borehole" (Well C6209) in July 2008.

Detailed analyses were made on 17 strata traversing multiple geological formations: the oxic saturated zone of the Hanford formation (9-10 m), the oxic fine-grained upper Ringold formation (17.4-18.2 m), and reduced sediments within the Ringold formation. A series of enrichment cultures were set up from these samples to isolate Hanford-relevant organisms. These materials were then passed to Beliaev and Marshall, for them to progress toward the isolation of pure cultures.

Biomass and functional gene analysis. Viable microbial biomass ranged from $1\text{-}29 \times 10^7$ per g, with the highest counts found in the Hanford sediments. Both lipid biomarker analysis and real time quantitative PCR (qPCR) assays showed a 15-fold decline in biomass from Hanford to Ringold formation, with less than 10-fold variation in the Ringold sediment. Most probable number (MPN) of cultivatable aerobes were $1.3 \pm 2.2\%$ of total lipid-based cell estimates, whereas the MPN of fermenters, denitrifiers, metal

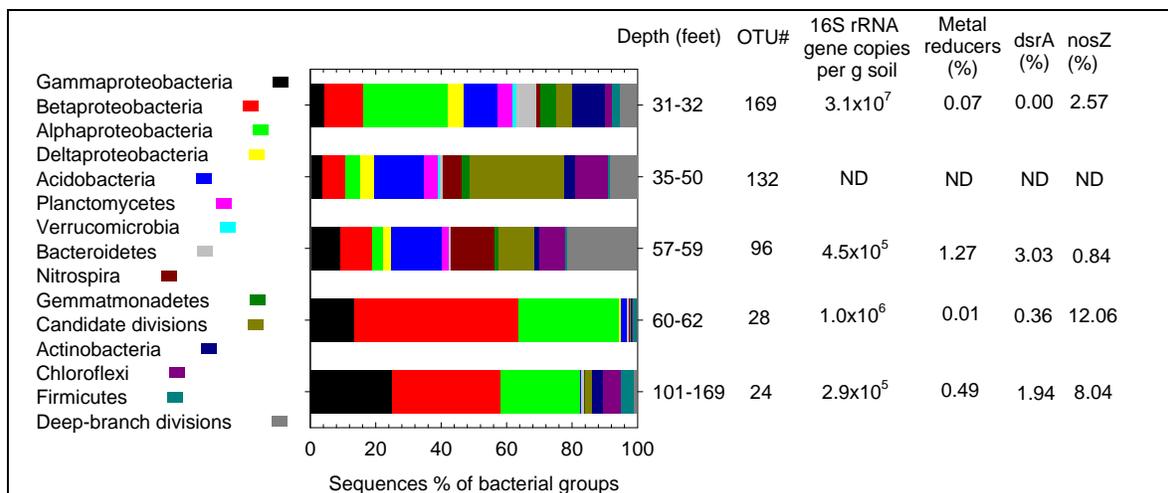


Figure 1. Abundance and diversity of the Hanford Site subsurface Bacteria assayed using 16S rRNA gene sequencing and real time quantitative PCR. Data shown are mean values averaged from a range of sampling depths in the Deep Characterization Borehole (DCB): Hanford 31-32', Hanford 35-50' (non-DCB samples), Ringold oxic 57-59', Ringold anoxic interface 60-62', and deeper Ringold 101-169'. Operational taxonomic unit (OTU) is defined using a cutoff value of 97% sequence similarity. The percentage of each physiological group is relative to the total 16S rRNA gene copy numbers. Metal reducers are the sum of Geobacteraceae, Anaeromyxobacter, and Shewanella. The *dsrA* and *nosZ* were used as biomarkers for detecting sulfate reducers and denitrifiers, respectively.

and sulfate reducers were less than 0.5% of total lipid biomass. Archaea were less than 8% of total microbial community, with elevated relative abundances found near the Ringold oxic-reducing interface. The *nosZ* gene, associated with denitrification, had an abundance of 5-17% of total 16S rRNA genes below 18.3 m and <5% in samples above 18.1 m. Most of *nosZ* gene sequences were affiliated with *Ochrobactrum anthropi* (97% sequence similarity) or *Achromobacter xylosoxidans* (90% similarity). Sulfate reducers were assayed by analysis of *dsrA* gene copies, which ranged from below detection in Hanford formation to as high as 7% in the oxic Ringold sediments. The majority of *dsrA* genes were related to *Desulfotomaculum thermosapovorans* in clone libraries constructed from samples near the Ringold oxic-reducing interface. The abundance of metal-reducing *Geobacteraceae*, *Anaeromyxobacter* or *Shewanella* employing phylogenetic primers was generally less than 0.5% of total 16S rRNA genes. The analyses suggest that the highly transmissive Hanford formation are regions of substantial microbial abundance and potentially high metabolic activity.

Microbial community composition was investigated by generating clone libraries after amplification of 16S rRNA genes from Bacteria (21 samples) or Archaea (12 samples). The samples were obtained by isolating high-quality DNA from subsurface sediment samples. Three hundred and eighty four clones were sequenced from each library, at the Joint Genome Institute. The results demonstrate that the Hanford Site subsurface microbial community is highly diverse, with 1,233 unique bacterial operational taxonomic units (OTUs) and 99 archaeal OTUs determined at 97% sequence similarity of 16S rRNA gene. Similar to microbial biomass distribution, bacterial diversity significantly declined from Hanford formation to the Ringold reduced sediments. In contrast, Archaea were most diverse in the oxic Ringold sediments, and some of them are affiliated with ammonia-oxidizing *Nitrososphaera sp.* and *Nitrosopumilus sp.* Bacterial OTUs were distributed in 42 divisions, with the most abundant ten divisions (from most abundant to least: *Proteobacteria*, *Acidobacteria*, GAL15, *Nitrospira*, *Chloroflexi*, NC10, *Actinobacteria*, SPAM, *Planctomycetes*, and *Gemmatimonadetes*) representing 88% of the bacterial clones in the Hanford formation and Ringold oxic sediments. In the reduced Ringold formation, the microbial community was dominated by proteobacteria, which accounted for 88.8% of total bacterial clones. The Hanford Site subsurface was comparatively rich in candidate divisions (e.g., GAL15, NC10, and SPAM) and other novel clades at various taxonomic cutoffs. This novelty and uniqueness may reflect unique local geochemical settings.

Biogeochemical potential. In collaboration with Fredrickson et al., microcosm experiments were set up to measure the potential for terminal electron acceptor utilization in these sediments. The results of these experiments will be reported in the Pore-Scale Biogeochemical Processes project, and further experiments are under design for early FY10. We have designed experiments for in situ incubation of BioSep beads or sediment materials within boreholes of the IFRC wellfield, and are waiting upon the fabrication of appropriate equipment for deployment and retrieval of these samples.

Proteomics. Characterization of geomicrobial protein expression (proteomics) provides a richer understanding of the unique biological pathways that make remediation processes possible. This knowledge base will permit mitigation and nutrient enrichment of contaminated sites, increasing microbial production of factors important in heavy metal reduction. A significant challenge in studying sediment microbe protein is their initial dissociation from the sediment particles. Removal of intact bacterial cells, prior to lysis, has proven to be ineffective as this process limits characterization of the complete representation of the microbial community. However, more problematic, direct *in-situ* lysis of bacterial cells leads to extreme protein adsorption due to the heterogeneous nature of sediment and the physiochemical properties of protein.

Ultimately for the complete proteomic characterization of the microbial community, we believe that the latter process of in-situ lysis will provide a broader and more representative view of the microbial

community. Therefore we have designed experiments aimed at the addition of chemicals to block potential protein adsorption sites on sediment prior to lysis of bacterial cells *in-situ*. Since the genomic sequence of the native microbial community was not available at the time, we used a solution of lysed *Escherichia coli* cells to represent the lysed proteins. In all experiments, the sediments were pretreated with the chemical mixture and then *E. coli* lysates was added. The metric used to gauge adsorption was the number of peptides or proteins that were extracted from the sediment as compared to both the *E. coli* lysates that was not added to the sediment (upper control) and the *E. coli* added to the untreated sediment (lower control).

Due to the significant heterogeneity of the soil composition, aspects of proteins: isoelectric point (pI), mass and hydrophobicity as well as sediment properties, such as surface charge, sediment particle variation and pore space heterogeneity all play major roles in achieving effective protein removal from sediment for proteomic analysis. For this reason, a variety of compounds and parameters were tested for their impact on protein recovery. Pretreatment of the sediments with a sets of amino acids mixtures improved the recovery of protein 10 fold (for polar positive amino acids) over non treated sediments, however still demonstrated a 50% loss in protein from *E. coli* alone. Secondly, temperature, time of incubation, pH, dispersion and amount of amino acids were also varied to determine the best method for protein extraction. While pH and temperature have an effect, departing from physiological pH and ambient temperature were not conducive for protein recovery. Oddly, an increase in the amount of amino acid to 50% from 10% also produced a decrease in the amount of protein that was recovered. Addition of desorption buffer is useful in removing protein from sediment but a significant amount of protein adsorption is taking place (prior to the addition of the buffer) that cannot be released. However, the highest recovery of proteins from the sediment was only 55% of the total protein contained in the cell, and it is clear that these methods require additional refinement.

2. Future scientific goals and deliverables for FY10

- Complete analysis of molecular data (16S rRNA gene clone libraries, 16S rRNA gene pyrosequencing data, and quantitative PCR data on functional genes), and carry out an integrated analysis with geological, geochemical and hydrological data from Hanford IFRC to generate hypotheses regarding environmental drivers of community change.
- Utilize assays of microbial activity to test hypotheses regarding environmental factors that drive changes in community composition and activity. These drivers include both geochemical nutrient resources and hydrological regimes. The experimental analyses include both microcosm experiments with sediment materials and in-well incubations of sediment surrogates.
- Future improvement on the protein extraction procedures by extension of the current studies to other compounds (milk extract, BSA, other proteins and detergents), followed by extension of proteomics analysis into Hanford and other sediments of interest, as well as to microbial biofilms grown on sediment surfaces.

3. New scientific results that may shift your current research focus area and/or identified knowledge gaps in the program

- Data on the hydrological heterogeneities within the IFRC 300 Area well field create a unique opportunity to determine the effects of local hydrological regimes upon microbial community composition and activity.
- Better methods for the proteomic characterize the microbial communities that are attached to soils will allow for a more in depth understanding of the biological mechanisms present in the sub surface and how the organisms react to or react with the metal contaminants.

4. A summary of collaborative research activities with your external collaborators

The collaboration with Dr. Rob Knight (University of Colorado, Boulder (UCB)) is to take advantage of their pyrosequencing and metadata analysis expertise for understanding vertical and horizontal changes in the subsurface microbial community similarity and composition, and relating to geological/geochemical/geophysical properties of the strata. Ninety-six samples from the deep borehole as well as sediments across IFRC well field array have been sent to UCB and the pyrosequencing of 16S rRNA gene is completed. Data analysis and interpretation is pending; the capacity to integrate other environmental datasets that have been collected on material from sediment boreholes will be a significant factor in the quality of product from this activity.

5. Capital equipment needs for FY10 including item title, a short description of the item and its use on the SFA, and burdened cost

Microscopic Mass Transfer of U and Tc in Subsurface Sediments

Principal Investigator: C. Liu

Internal Participants: S. Kerisit, Z. Shi

External Collaborators: Robert (Toby) Ewing (ISU), Scott Fendorf (Stanford U.)

1. Scientific Progress

This project focuses on investigating microscopic mass transfer processes of U and Tc, and their influence on, and coupling with, geochemical and biogeochemical reactions in subsurface sediments. Major goals are: 1) to characterize and model reactive diffusion at the pore and grain scales that exerts a fundamental kinetic control on the microscopic mass transfer and chemical reactivity of U and Tc in subsurface microenvironments; and 2) to derive scaling relationships of mass transfer parameters from the molecular to the pore, and from the pore to continuum scales in porous media. This project collaborates with other SFA projects investigating geochemical and biogeochemical processes (Rosso, Fredrickson) and macroscopic reactive transport (Zachara). Research in FY09 has been focused on measuring and developing models to describe U(VI) mass transfer in Hanford 300A sediments at different scales. The major part of research task 1 (milestone 1) and the uranium part of research task 5 (milestone 5) for this project has been finished. A good progress has been made toward milestones 2 and 3. FY10 will start research toward milestone 4.

A molecular dynamics (MD) model has been established to calculate the molecular diffusion coefficients of the aqueous uranyl species that are expected to be dominant in Hanford groundwater (Table 1). The method has been validated using those chemical species with known diffusion coefficients. This study found that the MD-calculated diffusion coefficients deviated from the Stokes-Einstein theory because of the complex molecular structures and hydration properties of these uranyl species. The Stokes-Einstein theory is commonly used to calculate the diffusion coefficients of aqueous species, but is apparently not suitable for uranyl species. The apparent diffusion coefficient of total dissolved U(VI), which was measured in a simulated Hanford groundwater in Hanford sediment (see publication 3), was $1.6\text{-}1.7 \times 10^{-10}$ m²/s, which was lower than the diffusion coefficients of the individual uranyl species (Table 1). Further research is ongoing to investigate two factors potentially responsible for this discrepancy. One is the apparent tortuosity effect that manifested the pore connectivity in porous media. The other is the species mass and charge coupling in aqueous solution and on solid surfaces that collectively slowed uranyl diffusion.

Table 1. Molecular diffusion coefficients calculated from MD simulations

Species	UO ₂ CO ₃	UO ₂ (CO ₃) ₂ ²⁻	UO ₂ (CO ₃) ₃ ⁴⁻	CaUO ₂ (CO ₃) ₃ ²⁻	Ca ₂ UO ₂ (CO ₃) ₃	MgUO ₂ (CO ₃) ₃ ²⁻	Mg ₂ UO ₂ (CO ₃) ₃
D (10 ⁻⁹ m ² /s)	0.49	0.39	0.29	0.27	0.25	0.29	0.25

Stirred-flow cell experiments were performed to investigate grain-scale U(VI) mass transfer rates under variable geochemical conditions in Hanford 300A sediments. The experimental results were used to evaluate the applicability of a grain-scale, multi-rate, surface complexation model (GS-MR-SCM) to describe uranyl adsorption and desorption kinetics under variable geochemical conditions (see publication 3). The GS-MR-SCM was designed to approximate coupled uranyl diffusion and surface complexation reactions within intragrain regions with heterogeneous distributions of pore size and length, pore connectivity, and surface properties in sediments. This study found that the model with a fixed set of mass transfer parameters was able to match the general trend of the mass transfer-limited uranyl

adsorption/desorption reactions under variable geochemical conditions that are relevant to Hanford groundwater conditions. However, the mass transfer parameters were found not to be completely independent of geochemical conditions. Further researches are ongoing to provide insights into these findings and to derive alternative grain-scale mass transfer models through theoretical analysis and experimental characterization.

A Lattice Boltzmann model was developed that can simulate coupled physical, chemical, and biological processes at the pore scale in controlling solute and contaminant reactive transport in microenvironments. The LBM was linked with the grain-scale uranyl mass transfer processes to simulate the mass transfer-limited uranyl reactive transport in the Hanford 300A sediments under conditions without advection (Figure 1 left plot) and with groundwater flow (Figure 1 right plot). The porous medium in Figure 1 was constructed based on the measured macroscopic porosity, hydraulic conductivity, and hydraulic dispersivity in the “intact core” sediments collected from Hanford 300A IFRC site. The x-ray tomography of the pore and grain structures in the sediments were used to provide insights for detail distribution of pores and grains. The uranium exchange between solid and pore fluid was through the grain-scale mass transfer at the liquid-solid interfaces. Simulations showed several important uranyl transport properties that were first demonstrated at the pore scale: 1) significantly heterogeneous distribution of aqueous uranyl concentration that was affected by complex pore structure and fluid-solid interfacial reactions; 2) besides the rate limitation of the grain-scale mass transfer, the rates of uranyl reactive transport was also limited by the pore-scale mass transfer from small, poorly connected pores to larger, well connected pores; and 3) simple volume averaging of the grain-scale mass transfer rates was not able to simulate macroscopic uranyl reactive transport and uranyl breakthrough profiles. An important implication is that the macroscopic rates of geochemical and biogeochemical reactions will be scale-dependent, and incorporating geochemical and biogeochemical reactions in macroscopic reactive transport models will require careful consideration of both the grain-scale and pore-scale mass transfer processes.

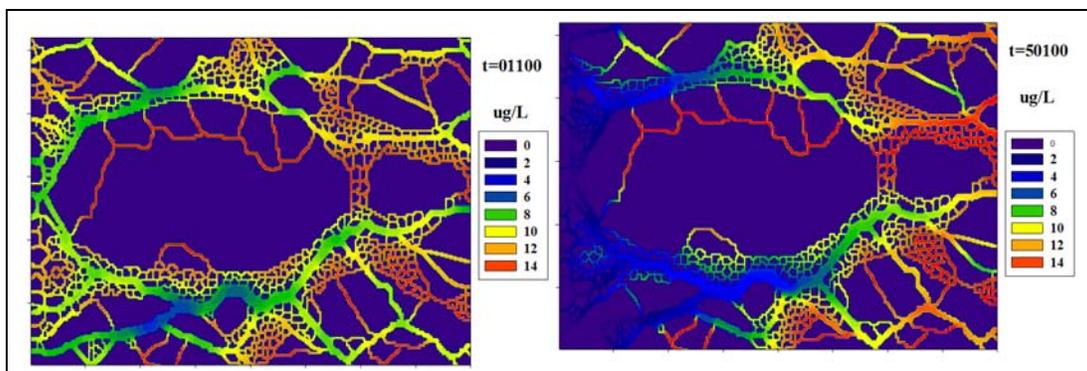


Figure 1. A snapshot of uranyl concentration distribution in porous media as a result of the combined effects of the grain-scale uranyl mass transfer and pore-scale uranyl diffusion (left plot), and the coupled effects of the grain-scale uranyl mass transfer and pore-scale uranyl advection and diffusion (right plot). The dark blue regions represent the solid grains. The other color regions are the pore spaces with aqueous uranium concentrations denoted in the figure legends. Initial U(VI) was assumed to be only associated with solid phase, and aqueous U(VI) was desorbed from the solid grains through the mass transfer process at the solid-liquid interfaces. The U-free solution flowed from left to right in the right plot. Simulation dimension is 10 cm (width) x 6 cm (height).

2. FY10 Goals and Deliverables

This project in FY10 will continuously investigate physical and chemical factors controlling the microscopic mass transfer rates in porous media. The molecular model established in FY09 to calculate aqueous uranyl species diffusion will be modified and extended to calculate uranyl species diffusion in porous media with variable pore size and mineral surface charge properties. The results will be used to evaluate the effects of pore size restriction and mineral surface charge coupling on U(VI) diffusion coefficients in the microporous regions with which uranium is associated in the Hanford 300A and 200A sediments. Multi-component, species-based reactive diffusion theory will be established at the sub-pore and pore scales (e.g., in a format of LBM or percolation-based particle tracking model) and at the continuum scale to incorporate the molecular diffusion coefficients for simulating multi-component, diffusive mass transfer processes. Current pore-scale models, including LBM, are not able to simulate coupled multi-species reactive diffusion with variable diffusion coefficients. The developed theory will be used to derive scaling relationships between the apparent and molecular diffusion processes and rate parameters in porous media with complex pore size and connectivity. The established scale relationships will be used to establish alternative, grain-scale mass transfer models. The established mass transfer models will be validated by experimentally measuring uranyl diffusive mass transfer in the Hanford 300A, 200A, and Oak Ridge saprolite sediments under variable geochemical conditions. Stirred flow cell experiments and diffusion cell apparatus will be used to derive experimental data for model validation. These models will be compared in their applicability to describe uranyl reactive transport in the Hanford sediments using the Hanford 300A “intact column” results in collaboration with Zachara’s SFA project. The following manuscripts are planned as FY10 deliverables:

Ewing R., Q. Hu, and C. Liu. 2009. Scale dependence of intra-granular porosity, diffusivity, and tortuosity. *Water Resources Research*.

Liu C., L. Zhong, and J.M. Zachara. 2009. Uranyl diffusion in low permeability materials. *Radiochimica Acta*.

Kerisit S.N. and C. Liu. 2010. Molecular dynamics calculation of uranyl diffusion coefficients in groundwater. *Environmental Science & Technology*.

Liu C. 2010. Lattice Boltzmann modeling of coupled grain-scale adsorption/desorption kinetics and advective uranyl reactive transport in porous media. *Water Resources Research*.

3. New Scientific Results that May Shift Emphasis

There are no major shifts for this project in FY10. The project will focus a bit more on the pore scale investigation on the coupling of microscopic mass transfer processes with geochemical and biogeochemical reactions with in microenvironments given the important findings from FY09 LBM modeling. Also the pore-scale measurements of coupled geochemical and mass transfer processes may become available in FY10 (e.g., microfluidics measurements at PNNL, and microscopic measurements of coupled redox reactions and diffusion at Stanford University).

4. External Collaborator Research Activity

Collaboration with Toby Ewing at Iowa State University (ISU) proceeds well. This collaborative effort is to investigate the effect of pore connectivity, pore size distribution, and grain size on the apparent diffusion rates of uranyl species in the Hanford 300A sediment. ISU is applying the percolation theory in developing particle tracking models to investigate the grain-scale manifestation of the molecular diffusion

processes within intragrain microporous regions. The goal of this work is to derive theoretical scaling relationships to link the molecular diffusion coefficients, microscopic diffusion length, and pore connectivity to the measurable macroscopic, grain-scale effective diffusion coefficients and apparent tortuosity. The FY09 research at ISU has been focused on deriving the theoretical expression of apparent solute diffusion coefficient as a function of molecular diffusion coefficient, intragrain pore connectivity, and grain diameter under well defined conditions (e.g., spherical grains, uniform pore size, and no interactions between diffusing ions and mineral surfaces). FY10 research will continue along this line, but will focus on real sediment grains with complex particle geometry, measured pore size and pore connectivity in the Hanford 300A sediments.

FY10 will start to collaborate with Scott Fendorf at Stanford University in investigating the coupled redox reactions and diffusion in Hanford fine-grained materials collected from the Ringold/Hanford formation interfaces. Stanford U. will use synchrotron-based reactive diffusion systems to collect microscopic data of coupled O-Fe redox reactions and O₂ diffusion in the sediments, and PNNL will perform pore-scale (LBM) numerical analysis. The research is to provide microscopic insights into the mass transfer limitation on the O₂ consumption and its effect on the development of redox microenvironments in subsurface sediments. This collaboration was initially planned for FY09, but was delayed because of the delay in establishing subcontract with Stanford U.

5. Capital Equipment Needs

None requested for FY10.

Molecular Scale Mechanisms of Biogeochemical Electron Transfer Underlying Subsurface U/Tc Valence Transformation and Stability

Principal Investigator: **K.M. Rosso**

Internal Collaborators: C.A. Pearce (New staff), J. Liu (New post-doc), O. Qafoku, S. Kerisit, L. Shi, Z. Wang

External Collaborators: K. Kemner (ANL), D. Richardson (U of East Anglia, UK)

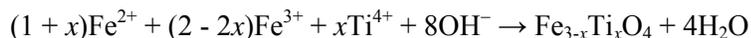
1. Scientific Progress

This project is focused on developing a molecular-scale understanding of biogeochemical electron transfer reactions affecting the stability of U and Tc in Hanford-specific microenvironments towards long-term resolution of SFA Global Hypothesis #2. Major goals include examining three Hanford-relevant U(VI)/Tc(VII) reduction pathways: i.) reduction by abiotic or biogenic Fe(II) sorbed to mineral matter, ii.) direct enzymatic reduction by cytochromes on the outer-membranes of bacteria, and iii.) reduction by structural Fe(II) available in relevant mineral phases. In FY09 this project focused on the third goal first so as to establish representative model Hanford minerals for laboratory and field reactivity studies, and for utility to other SFA projects. We successfully developed three distinct forms of metal oxide materials (nanoparticles, epitaxial thin films, and bulk materials) along the selected compositional series $Ti_xFe_{3-x}O_4$ (titanomagnetite). The research goal for these materials is that they will enable us to probe in detail mechanisms and kinetics of heterogeneous redox reactions affecting the stability of contaminants such as U and ^{99}Tc , and they will enable us to probe the effects of surface oxidation/passivation on accessibility of Fe(II) reducing equivalents in solid phases, for comparison with the behaviour natural sample isolates. Ultimately this research is meant to enable development of mechanistic rate expressions for heterogeneous Tc(VII) reduction by Fe(II)-bearing reactive phases more broadly for use in pore-scale and coupled mass transfer biogeochemical models for Hanford site problems and beyond.

The selected oxide composition was chosen for relevance to Fe(II)-bearing metal oxides in Hanford sediments. Sediments at the Hanford Site contain significant total Fe, of which a large percentage exists in the reactive ferrous state. The ferrous component in part arises in the form of magnetites crystallized within the basaltic source material that are present in the sediments both as subhedral to euhedral micron-sized free crystal grains and as mineral components of basaltic lithic fragments. These magnetites naturally contain variable amounts of Ti as a dominant constituent and can be described with the titanomagnetite formula $Fe_{3-x}Ti_xO_4$, which falls between endmember magnetite ($x=0$) and ulvöspinel ($x=1$). In the absence of surface passivation, ferrous minerals such as these have been shown to be redox-active polyvalent metal reductants. At Hanford, it has been speculated that grain interfaces of such minerals are reducing microenvironments, especially if oxygen is scavenged by associated oxygen-consuming mineral reactions or by microbial activity. Of particular importance in this regard is the availability of electron equivalents for metal reduction by the ferrous mineral phases. Titanomagnetites have the feature that substitution of Ti^{4+} into the metal sublattice is charge compensated by a proportional increase in the Fe^{2+} content. This built-in natural ‘tunability’ of the Fe^{2+}/Fe^{3+} ratio in principle adjusts the reducing capacity of the solid, and is therefore central to its redox-reactivity with respect to contaminant reduction.

There are no previous reports of titanomagnetite particle synthesis that were complete in detail and conclusiveness. We selected a general approach reported previously and optimized it for synthesis of titanomagnetite nanoparticles. The approach involves dissolving suitable amounts of ferrous, ferric, and titanium chloride in an HCl solution under anoxic conditions at room temperature. This mixture is added

to a strongly basic ammonia hydroxide solution leading to instantaneous precipitation of crystalline titanomagnetites according to the following equation:

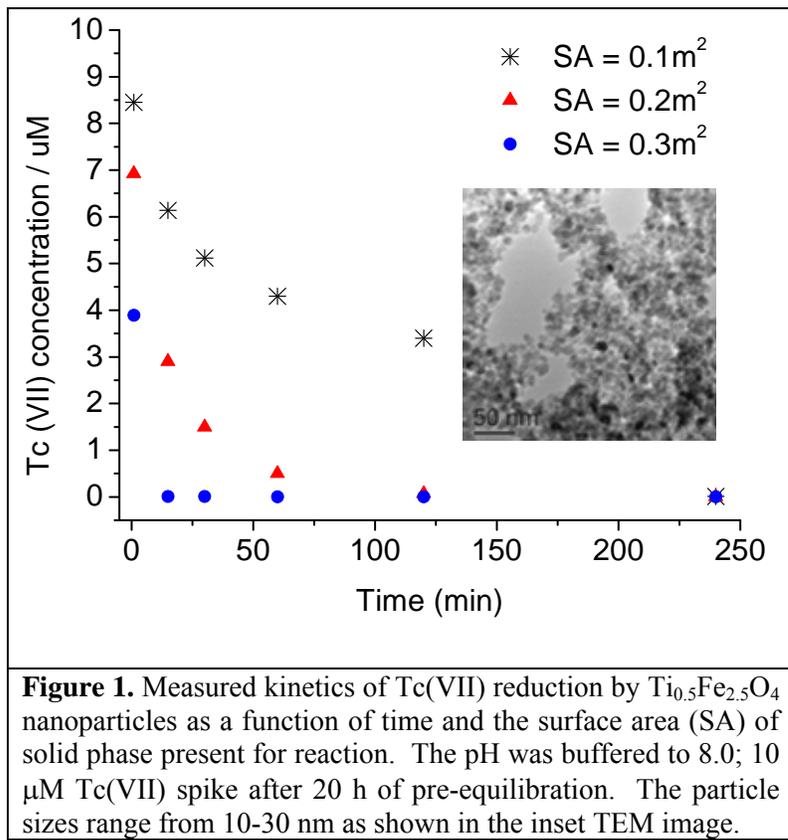


Grown nanoparticles were washed and stored in anoxic pH 7 solution. The Fe^{2+} , total Fe and total Ti content of the nanoparticle samples is determined by acid digestion, followed by a combined ferrozine assay/ICP-MS analysis. Characterization of the samples also involves primarily XRD (in situ on the μ -XRD) to assess the crystallinity, particle size, and lattice spacing, TEM for particle size, shape, and uniformity, BET analysis for surface area, and Mössbauer spectroscopy for magnetic structure and iron valence characterization.

Bulk titanomagnetite powders were prepared by sintering stoichiometric mixtures of hematite, titanium dioxide and iron metal in silver-foil lined evacuated silica-glass tubes at 900°C for 7 days in the presence of an appropriate oxygen fugacity buffer. We acquired this expertise and capability through the hire of staff member Carolyn Pearce and associated equipment purchases. This approach precisely achieves the desired composition and structure, and provides a useful reference material for judging the quality of the other forms of titanomagnetites developed under this project.

Growth of epitaxial titanomagnetite thin films was approached using pulsed laser deposition, which involves ablation of magnetite and titanium dioxide targets onto a crystallographically oriented magnesium oxide (100) substrate at 300°C to form an epitaxial layer with single surface expression and of controlled thickness. As for the nanoparticles, there are no previous reports of thin films successfully synthesized with this composition.

A major objective of FY09 work for all three materials was to determine the extent to which Ti^{4+} was being incorporated into the magnetite structure in the desired location (octahedral sublattice) in the desired amount, accompanied by proportional increases in the structural Fe^{2+} content. Compositional analysis of digested nanoparticles show a good correlation between desired Ti^{4+} content and $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio. TEM shows that the nanoparticles are relatively uniform with particle sizes in the range 10-30 nm. In situ μ -XRD of anoxic nanoparticle suspensions show an increase in the lattice parameter with increasing Ti^{4+} content up to approximately $x=0.3$, whereupon the trend diverges somewhat from the expected relationship displayed by the bulk material due to possible exclusion of



some of the Ti^{4+} into an amorphous phase. The analyses give us good confidence that the desired titanomagnetite characteristics are attainable from $x=0$ up to approximately $x=0.3$, above which the samples require further study. Thin films epitaxially grown by PLD on $\text{MgO}(100)$ were characterized by XRD, K-edge XANES/EXAFS, XPS, and RHEED and found to possess the desired compositional and structural characteristics from $x=0$ up to $x=1.0$ (ulvöspinel). The nanoparticles and thin films are first-of-a-kind in this compositional series and are publish-caliber results from FY09.

In FY09 we also began titanomagnetite reactivity studies with Tc(VII). Nanoparticles for compositions $x=0$, $x=0.25$, and $x=0.5$ were used as solid phase electron donors for heterogeneous reduction of Tc(VII) in anoxic solution (**Fig. 1**). These studies show that structural Fe(II) in these materials is capable of reducing pertechnetate rapidly, and that the rate increases systematically with increasing Ti content and the solid:solution ratio. The results are consistent with Ti-enhanced Fe(II) content in the octahedral sublattice of titanomagnetite is the electron donor and suggest accessibility to electron equivalents in the crystal structure is of key importance. This work validates progress towards stated FY09 milestones for this project, specifically that we would provide molecular-scale insights into initial stages of heterogeneous Tc(VII) reduction by Fe(II) that is structural or otherwise sorbed to Hanford-relevant mineral phases.

Other FY09 activities include experimental design and cell construction for anoxic cryogenic XANES/EXAFS measurements of nanoparticles at APS with Steve Heald, collaborative discussions with Ken Kemner for EXAFS data analysis, submission of an ALS user proposal for FY10 XMCD measurements on all three forms of titanomagnetite materials, development of a radiological work protocol for Tc(VII)/thin film experiments and XPS measurements in the EMSL, and measurements of the acid dissolution kinetics and reaction extent for titanomagnetite nanoparticles. This project has also been developing a state-of-the-science atomistic simulation tool coupling kinetic Monte Carlo with continuum expressions that will enable dynamics simulations of electron exchange and surface passivation reactions at model mineral/fluid interfaces. The code was written in FY09 and is now available for FY10 application to our Tc(VII) reduction kinetics data set. Furthermore, this project has maintained a continuing dialogue with the Shi project regarding coupled measurements and modelling of protein-mineral electron transfer kinetics. Preliminary experiments have been designed and attempted to perform protein film voltammetry of STC adsorbed onto epitaxially grown hematite (001) thin film electrodes. These experiments are ongoing, and could be augmented in FY10 with outer-membrane cytochromes currently under structural study by the Richardson group (e.g., MtrA, MtrF) when sufficiently resolved structures become available (anticipated early FY10).

2. FY10 Goals and Deliverables

FY10 will focus on 1). Data set completion and submission of associated manuscripts from FY09 activities, 2). Initiation of titanomagnetite surface oxidation studies for nanoparticles, thin films, and bulk materials, with new staff member Carolyn Pearce involving conventional and synchrotron-based (EXAFS/XANES, XMCD) spectroscopy studies, 3). Application of coupled continuum-KMC atomistic simulations to observed titanomagnetite/Tc(VII) heterogeneous reduction kinetics, and, if possible, 4). Construction of molecular models and preliminary simulations of electron transfer within MtrF and/or an MtrDEF complex, ultimately for comparison with protein film voltammetry measurements.

In order to understand potential differences in the redox reactivity of the natural titanomagnetites in aqueous environments in Hanford site sediments relative to the more pristine synthetic materials, we will investigate oxidation/passivation of the synthetic materials by dissolved oxygen. Among other measurements, XMCD will be used to monitor the change in surface composition, in terms of $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratios, as the bulk material, nanoparticles, and thin films are exposed to aqueous solutions having a range

of dissolved oxygen activities. We will do this as a function of systematically varied Ti^{4+} content in the solid to proportionally adjust the Fe^{2+} content and assess the importance of this effect on passivation layer structure and thickness. The findings will be used to develop an atomically resolved conceptual structure model for passivation layers on titanomagnetite. Simultaneously, this activity will also produce partially oxidized model materials for reactivity comparisons to the natural crystals with respect to contaminant reduction.

Furthermore, we will continue Tc(VII) reduction studies towards a complete data set. Titanomagnetite samples in various forms with a range of $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$ compositions will be exposed to solutions of different TcO_4^- concentrations to determine the Ti^{4+} effect of ‘tuning’ the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio on redox reactivity with respect to pertechnetate reduction. The reaction behaviour of the natural crystals will be compared to that of the pristine materials but also to that of pre-oxidized materials generated from the previous activity above. Of particular interest is to determine the extent to which electron equivalents ‘trapped’ in the core of the particles are available for contaminant reduction either through the passive layer or via its dissolution. Furthermore, we will characterize the Fe valence and site occupancy of the surface reaction product, ostensibly a $\text{Fe}^{3+}\text{-Tc}^{4+}$ phase of unknown mixed-metal proportions and crystallinity. The findings will allow us to begin to understand reduction extent, its relationship to Ti^{4+} -based adjustment of Fe^{2+} content in the solid, and to propose atomically resolved conceptual models of this reduction process with associated implications for Tc contamination at Hanford.

We are targeting the following manuscripts for FY10 submission:

Hanford model material development:

Pearce C.A., O. Qafoku, J. Liu, K.M. Rosso, S.M. Heald, and T.C. Droubay. 2010. Synthesis and characterization of titanomagnetite ($\text{Ti}_x\text{Fe}_{3-x}\text{O}_4$) nanoparticles and bulk materials for model reactivity studies. To be submitted to *Geochimica et Cosmochimica Acta*.

Droubay T.C., S.M. Heald, W. Jiang, C.M. Wang, and K.M. Rosso. 2010. Pulsed laser deposition of epitaxial $\text{Ti}_x\text{Fe}_{3-x}\text{O}_4$: From magnetite to ulvöspinel. To be submitted to *Physical Review B*.

Heterogeneous reduction kinetics of Tc(VII):

Liu J., O. Qafoku, C.A. Pearce, K.M. Rosso, S.M. Heald, and T.C. Droubay. 2010. Tc(VII) reduction kinetics by magnetite and titanomagnetite nanoparticles: A tunable Fe(II)/Fe(III) redox potential system. To be submitted to *Environmental Science & Technology*.

Biomolecular electron transfer:

Rosso K.M., N.S. Wigginton, Z. Wang, L. Shi, S.N. Kerisit, M. Toney, J.K. Fredrickson, and J.M. Zachara. 2010. Electron tunneling characteristics of bacterial multiheme cytochromes. To be submitted to *Proceedings of the National Academy of Sciences*.

3. New Scientific Results that May Shift Focus

The Richardson group is getting close to a fully resolved crystal structure for MtrF (currently down to 4 Å with a full structure expected by Dec 2009) and MtrA. If successful, it may soon become possible to construct a molecular model to investigate intramolecular electron transfer kinetics in MtrF and possibly also an approximate model for like simulations within a supercomplex of key cytochromes such as MtrDEF. This activity is fully dependent on the findings and success of the Richardson group. Any

pursuit of this activity would be handled in parallel with our continuing Tc(VII) heterogeneous electron transfer line of research.

4. Collaborative Research Activities

In FY09 we have 1). Written and submitted a (still pending) user proposal for beamtime at the ALS for XMCD studies of our model titanomagnetite materials with Elke Arenholz, 2). Had collaborative exchanges of samples and data with Steve Heald, and have plans for APS experiments in early October, 3). Have had face-to-face collaborative discussions with Ken Kemner for assistance with EXAFS data analysis, and APS measurements pertaining to possible U/titanomagnetite experiments.

5. Capital Equipment Needs

None requested for FY10.

6. Awards and Miscellanea

Awards

K. Rosso selected for 2008-2009 Outstanding Recent Alumnus Award, College of Science, Virginia Tech.

K. Rosso selected for Best Paper Award – U.S. DOE OBES Geosciences Symposium, 2009.
Pearce appointed Honorary Senior Lecturer within the School of Earth, Atmospheric & Environmental Science, University of Manchester.

Multiscale Reactive Transport Modeling

Principal Investigator: **T.D. Scheibe**

Internal Participants: A. Tartakovsky, G. Tartakovsky, M. Richmond, W. Perkins, C. Rakowski, J. Serkowski, Y. Fang

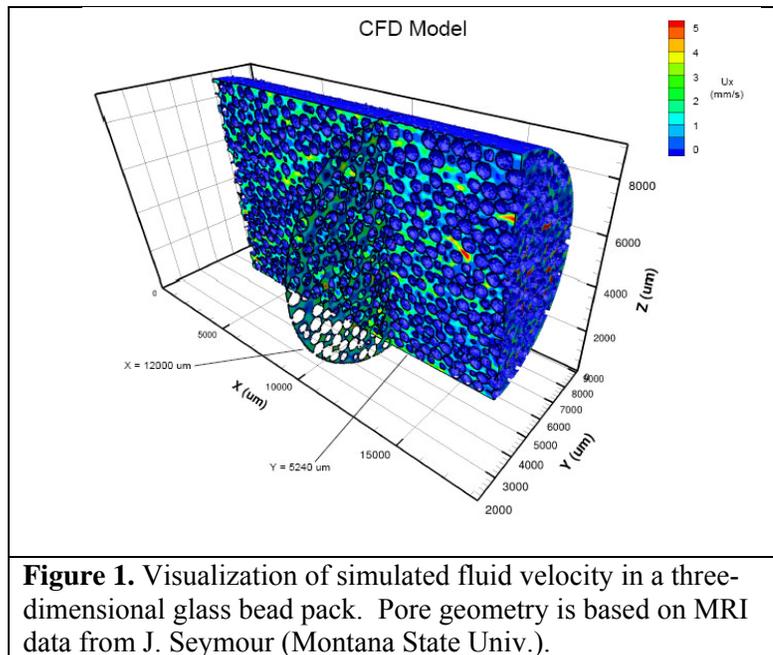
External Collaborators: B.D. Wood (Oregon State University)

1. Scientific Progress

This project is focused on development and application of pore-scale reactive transport models and upscaling of pore-scale simulations to obtain models of U(VI) reaction and advective transport relevant to Hanford site sediments. The major goal is to provide integration between laboratory and field experimental elements of the SFA by development and application of pore-scale models and upscaling methods. In particular, pore-scale models provide a platform for translating new molecular-scale process understanding and properties to model forms that can be utilized at larger scales to make quantitative predictions. We have focused efforts in FY09 on enhancement of existing pore-scale models to incorporate new process descriptions relevant to the 300-Area IFRC, and developed novel approaches to upscaling that will facilitate their application at the field scale. Primary interactions within the SFA are with projects providing molecular-scale information to pore-scale simulations (e.g., Liu), pore-scale characterization of Hanford sediments and analysis of column-scale transport experiments (Zachara), identification of field-scale facies distributions (Ward), and the Hanford IFRC. Research in FY 2009 has followed three primary thrusts described further below: i) identification of key elements of a conceptual model of pore- and field-scale transport at the Hanford 300 Area IFRC; ii) development and validation of pore-scale modeling tools to incorporate key processes; iii) development and preliminary application of pore-scale numerical volume averaging (upscaling) techniques.

Conceptual Model Identification. Based on review of the extensive recent literature and technical reports on the 300-Area hydrogeology and geochemistry, surface sorption, intragranular diffusion, and diurnal changes in flow direction/velocity were identified as the critical processes of interest. Subsequent enhancement of pore-scale modeling tools has focused on these critical processes.

Development and Validation of Pore-Scale Modeling Tools. We are building on existing capabilities for pore-scale modeling using two methods: 1) Smoothed Particle Hydrodynamics (SPH), a mesh-free lagrangian particle method, and 2) mesh-based computational fluid dynamics (CFD) methods. Under separate funding (SciDAC), a 3D parallel SPH code has been developed. This code runs on the EMSL Chinook supercomputer and the Franklin supercomputer at NERSC. We have performed simulations with up to 14 million computational particles, and are continuing to increase



the size of simulations. Also under separate funding (university-led ERSP), we have developed a method for representing microbially-mediated reactions at grain surfaces; the same approach can also be used for surface sorption reactions. This SFA project leverages those related efforts; here we have focused our attention on the simulation of intragranular diffusive mass transfer. A modeling plan was developed and reviewed by SFA collaborators (T. Scheibe, unpublished internal report) and is the basis for ongoing incorporation of intragranular diffusion into SPH codes. This work is expected to be completed by December 2009 and will meet the project deliverable: “*Implement Smooth particle hydrodynamics model of diffusive intragranular mass transfer and surface sorption of U(VI) with input from molecular-scale studies.*”

PNNL’s TE²THYS code (CFD) was successfully ported to the Franklin supercomputer and has been used for large 3D pore-scale flow simulations. Figure 1 shows simulated flow in a 3D beadpack geometry provided by ERSP-funded collaborators at Montana State University (J. Seymour, personal communication). This simulation demonstrates our unique capability to perform pore-scale simulations in domains large enough (10 mm diam; 15 mm length; thousands of grains) to be considered a representative volume of porous medium.

Because the methods used for 3D pore-scale simulation are relatively novel and complex, and because there exist few direct observations for verification, careful code validation is needed to establish the veracity of code predictions. We have performed extensive code validation tests with both TE²THYS and SPH codes (see, e.g., <http://subsurface.pnl.gov/software/SALSSA/SlitValidation/SlitValidation.shtml>). We have also developed a set of common benchmark problems to which both methods will be applied for cross-validation.

Development of Numerical Upscaling Capability. A key element of FY09 work, conducted in collaboration with our SFA external collaborator, Dr. Brian Wood of Oregon State University, is to incorporate numerical volume averaging (VA) methods into our CFD code TE²THYS and perform validation tests and preliminary applications. The VA solution for effective dispersion has been coded into TE²THYS and tested successfully on parallel plate and flow in straight and wavy tube problems (compared to analytical and particle tracking solutions). We are currently completing testing the code on arrays of cylinders (which incorporates an additional term in the VA equations), after which it will be fully ready for use in arbitrary pore geometry. This work directly addresses the project deliverable “*Develop and test combined numerical/theoretical upscaling approach for uranium mass transfer / sorption / dispersion.*” As a preliminary application, we have performed an extensive set of numerical experiments in a wavy tube geometry (analog for pore throats and bodies) to explore the nature of dispersion over a range of Reynolds numbers (*Re*) ranging from low (Stokes flow) to intermediate (inertial flow to unsteady laminar flow). At the upper end of this range, dispersion phenomena are poorly understood and existing upscaled solutions do not match experimental observations. By applying this numerical approach to VA, we have eliminated a number of restrictive assumptions inherent to analytical VA solutions. Our simulations computing effective dispersion over the specified range of *Re* will be completed by the end of FY 2009 and a draft manuscript for submission to *Physical Review E* will be completed by the end of October.

Other upscaling methods are being explored in addition to VA, as recommended by the SFA review panel. An upscaling analysis of pore-scale solute mixing performed using the SPH model led to a manuscript that has been accepted for publication in *Advances in Water Resources*. We have also made enhancements to and performed validation tests on a particle-tracking code used in conjunction with TE²THYS that can be used to develop upscaling strategies based on random-walk methods. We provided a collaborative letter of support to a new university-led ERSP proposal by Dr. Arash Massoudieh

(Catholic University of America) that would, if funded, explore the application of new lagrangian upscaling methods to our pore-scale simulations.

Valdes-Parada J.F., M.L. Porter, and B.D. Wood. 2009. The role of tortuosity in upscaling. *Transport in Porous Media* (In review).

Presentations

Scheibe T.D., A.M. Tartakovsky, B.J. Palmer, M.C. Richmond, B.D. Wood, I. Battiato, and D.M. Tartakovsky. 2009. Linking Pore- and Darcy-Scale Models of Reactive and Non-Reactive Transport. *Eos Trans. AGU*, 89(53), Fall Meet. Suppl., Abstract H13F-0993. Invited presentation at the American Geophysical Union Fall Meeting, San Francisco, CA.

Scheibe T.D., M.C. Richmond, B.D. Wood, M. Rockhold, Y. Fang, A.M. Tartakovsky, and B.J. Palmer. 2009. Reactive Transport Modeling for the PNNL Science Focus Area. Poster presentation at the ERSP 4th Annual PI Meeting, Lansdowne, VA.

Scheibe T.D., A.M. Tartakovsky, and B.J. Palmer. 2009. Pore-Scale Simulation and Upscaling of Uranium Transport in Groundwater Incorporating Intra-Grain Mass Transfer Processes. Poster presented at the Migration 2009, 12th International Conference, Kennewick, WA.

Scheibe T.D., A.M. Tartakovsky, and B.J. Palmer. 2009. Pore-Scale Simulation and Upscaling of Solute Transport in Groundwater Incorporating Intra-Grain Mass Transfer Processes. Abstract submitted to American Geophysical Union Fall Meeting, San Francisco, CA.

2. FY10 Goals and Deliverables

FY 2010 research will complete the work initiated under thrust areas (ii) and (iii) as described above and initiate research in two new areas.

Under thrust area (ii), we will complete incorporation of intragranular diffusion, surface sorption, and equilibrium and kinetic reactions into the SPH and TE²THYS codes. We will use the resulting code to perform numerical experiments on the macroscopic (porous-media scale) manifestations of these pore-scale processes (for example, how variations in grain size may give rise to an apparent multi-rate mass transfer model). We will also complete a model cross-validation study between the SPH and CFD codes (and possibly a Lattice-Boltzmann code). Under thrust area (iii), we will perform numerical volume averaging studies of dispersion in generalized pore geometry under a range of Reynolds numbers and compare with experimental results from the literature.

In addition, we will initiate research in two new areas. (iv) In collaboration with the Ward project, we will develop a quantitative 3D model of hydrofacies spatial distributions at the 300-Area IFRC, conditioned to geophysical and hydrologic field observations. This model will provide the large-scale framework within which upscaled representations of pore-scale models are to be applied, and will meet the project deliverables “*Define field-scale hydrofacies framework for 300 Area IFC. Compile existing field data and develop preliminary geostatistical model of hydrofacies distribution. Begin initial numerical model development*” and “*Integrate geophysical observations into the geostatistical model of hydrofacies distributions for 300 Area IFC.*” (v) Secondly, in collaboration with the Zachara project, we will apply the SPH and TE²THYS codes to perform pore-scale simulations based on tomographically-derived pore geometries from column sediments, and will test the models against column experimental

data to determine the effectiveness of proposed upscaling methods. This will address the deliverable under the Zachara project: “Begin system modeling with Scheibe at the pore scale to include responsible reaction networks and transport processes (1/10).”

We are targeting the following manuscripts as deliverables for FY10 submission:

Richmond M.C., W.A. Perkins, B.D. Wood, A. Lambert, J. Serkowski, and T.D. Scheibe. 2010. Dispersion in a wavy tube under inertial and unsteady laminar flow conditions. To be submitted to *Physical Review E*.

Palmer B. J., A.M. Tartakovsky, T.D. Scheibe, M.C. Richmond, and M. Sukop. 2010. A comparative evaluation of alternative pore-scale flow and transport simulation methods. To be submitted to *Advances in Water Resources*.

Scheibe T. D., B.J. Palmer, A.M. Tartakovsky, and G.D. Tartakovsky. 2010. Pore-scale simulation and upscaling analysis of solute transport with intragranular diffusion. To be submitted to *Water Resources Research*.

Ochiai N., M.C. Richmond, M. Dragila, T.D. Scheibe, J. Serkowski, and C.L. Rakowski. 2010. Delineation of low velocity zones near orthogonal grain-to-grain contacts: Implications for non-Brownian colloid transport in porous media. To be submitted to *Environmental Science and Technology*.

Valdes-Parada F.J., M.L. Porter, and B.D. Wood. Interpretation of closure variables in volume averaging. To be submitted to *Transport in Porous Media*.

3. New Scientific Results that May Shift Emphasis

There are no major shifts in technical emphasis anticipated in FY10.

4. External Collaborator Research Activities

Our external collaboration through the SFA is with Dr. Brian Wood of Oregon State University. Dr. Wood was funded in FY09 under the third and final year of the previous ERSP project, “Mechanistically-Based Field-Scale Models of Uranium Biogeochemistry from Upscaling Pore-Scale Experiments and Models”. In FY 2010, this will transition to funding through an SFA subcontract. This collaboration focuses on the implementation of numerical volume averaging in arbitrary pore geometry (thrust area (iii) above). Dr. Wood has supported PNNL staff in implementing volume averaging numerical solutions within the TE²THYS code, and we have collaborated on the analysis of dispersion in a wavy tube (used both as an analytical validation test and for numerical experimentation on dispersion across a range of flow conditions). As part of that collaboration, we have hosted a doctoral student from OSU (Adam Lambert) for extended visits at PNNL to work on code and analyses collaboratively. PNNL is performing an analysis of dispersion for a range of Reynolds numbers in one particular wavy tube geometry; the OSU student is extending that analysis to consider a variety of wavy tube geometries. Initial results indicate that the structure of the flow field changes dramatically when inertial effects become significant (at Reynolds numbers above 10). These results are relevant to remediation efforts at Hanford, where the structure of the porous materials is such that relatively high Reynolds numbers would be routinely encountered during pumping, and potentially even during natural gradient flows in some highly-conductive geologic materials (e.g., 300 Area Hanford formation gravels subject to river stage fluctuations). When inertial effects are significant, the nature of the upscaled transport process changes

from one that is described by a conventional convection-dispersion process to one that is no longer Fickian in the dispersion.

In addition to the formal SFA collaboration, we have also collaborated with Dr. Joseph Seymour (Montana State University) who was also funded through a final year of the previous ERSP project. Dr. Seymour has developed magnetic resonance imaging (MRI) methods for porous media, and has been providing us with novel datasets describing pore geometry and flow velocity in glass beadpacks. These datasets are extremely useful in validating our pore-scale models. We have also collaborated with Dr. Maria Dragila of Oregon State University (OSU) and her doctoral student, Naoyuki Ochiai, on analysis (using our CFD pore-scale codes) of micromodel experiments on colloidal transport.

5. Capital Equipment Needs

None requested for FY10.

Functional Characterization of Microbial Macromolecules

Principal Investigator: L. Shi

Internal Participants: S. Belchik, Z. Wang, D.W. Kennedy, M.J. Marshall, J.M. Zachara and J.K. Fredrickson

External Collaborator: D.J. Richardson (University of East Anglia, UK)

1. Scientific Progress

The overall objective of this research project is to understand the molecular mechanisms by which microbial macromolecules (e.g. redox proteins) engage and react with uranium (U) and technetium (Tc) contaminants and with redox-active metals (e.g. Fe, Mn). The research has focused on characterizations of the redox proteins located in the microbial cell envelope. The characterization results will be used in the development and evaluation of molecular model of electron transfer (ET) developed by the Rosso project. In turn, the predictions of the model will help generate new hypotheses and design new characterization experiments. The initial focus of research is on characterizations of the proteins used by the *Shewanella* strains isolated from the Hanford Reach of the Columbia River (HRCR) as well as by the model strain of *Shewanella*, *S. oneidensis* MR-1, for extracellular reduction of U(VI), Tc(VII) and Fe(III) oxides. The research activities include i.) purification of the redox proteins from HRCR isolates, ii) determination of ET mechanisms across the microbial cell envelope, iii.) structural determination of purified proteins, and iv.) characterizations of purified proteins. In the future, the focus of this project will be shifted to characterizations of the biomolecules identified from other microorganisms isolated from the Hanford site (Konopka and Beliaev).

Purify the identified redox-active biomolecules from Columbia River isolates. As part of the overall SFA effort to enhance Hanford relevance, we have initiated the characterizations of the *c*-type cytochromes (*c*-Cyts) from 7 *Shewanella* strains isolated from the HRCR. PCR and sequencing results showed that all 7 tested strains possessed *mtrC*-like genes, while 3 strains had *omcA*-like genes and the remaining 4 strains contained *undA/undA1*-like genes, which is consistent with previous finding that *MtrC* genes are more conserved than *OmcA* genes. We sequenced the entire coding region of an *undA/undA1*-like gene from HRCR isolate 6 (HRCR-6). The results showed that this *c*-Cyt i.) contained 843 amino acid residues, ii.) had 11 putative heme-binding sites, and iii.) was 73% and 93% identical to the *UndA1* of *S. putrefaciens* CN-32 and the *UndA* of *S. baltica* OS223, respectively. Based on these results, we named this protein as *UndA-HRCR-6*. We purified *UndA-HRCR-6* from the membrane fraction of *S. oneidensis* MR-1 cells after its overexpression, consistent with the prediction that *UndA-HRCR-6* is an outer membrane (OM) cytochrome. Our preliminary data also showed that *UndA-HRCR-6* could complement, at least in part, *S. oneidensis* MR-1 mutants lacking *MtrC* and *OmcA* in Fe(III)/Mn(IV) oxides reduction assays, suggesting that *UndA-HRCR-6* most likely has a functional role in extracellular metal reduction.

Determine electron transfer mechanisms across the microbial cell envelope. To understand how electrons are translocated across bacterial OM (the outer layer of cell envelope of the Gram-negative bacteria), we characterized a trans-OM icosa-heme complex, *MtrABC* of *S. oneidensis* MR-1. The results showed that a stable *MtrAB* sub-complex could assemble in the absence of *MtrC*, but that an *MtrBC* sub-complex was not assembled in the absence of *MtrA*. Protein film voltammetric analyses revealed the functional capacity of *MtrABC*. We propose a model for the modular organization of the *MtrABC* complex in which *MtrC* is an extracellular element that mediates electron transfer to extracellular substrates

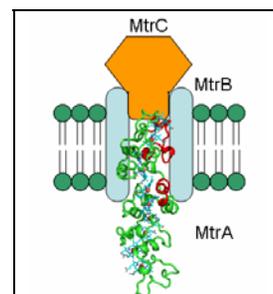


Figure 1. Structure of *MtrA* and a model for integration into the *MtrABC* complex.

and MtrB is a trans-OM spanning β -barrel protein that serves as a sheath to embed MtrA subunit in the membrane where it forms a trans-membrane electron delivery module that services MtrC (Figure 1). This is the first molecular model of electron translocation across bacterial OM, which could also apply more widely to the mechanism of electron exchange of a number of bacterial genera with extracellular electron sources and sinks.

Determine three-dimensional structures of the purified molecules. We recently improved our structural model for MtrA, which was ~ 80 Å in length with 10 hemes whose porphyrin rings were within 7 Å of each other. By using the cross-linking method developed at PNNL, we also found that i.) MtrA physically interacted with MtrB and MtrC and ii.) ~ 50 % of the MtrA heme chain was embedded within MtrB. All these results support our MtrABC model (Figure 1). At present, we have full datasets at less than 4 Å resolution for MtrF (a MtrC homologue). The overall fold of MtrF was totally novel. The N-terminus of MtrF, which was ~ 200 amino acids, formed a β -barrel that most likely anchored MtrF into the OM. We believe that MtrC will have a similar β -barrel where MtrC may interact with MtrB. The remaining C-terminal portion of MtrF formed two penta-heme domains that were connected via a hinge region. Again a similar structure is predicted for MtrC. One of these MtrC/MtrF penta-heme domains may interact with MtrA/MtrD in the MtrB/MtrE β -barrel. Another penta-heme domain could then serve as an electron exit module from the MtrABC/MtrDEF complex via direct contact with metal ions (solid or solution phase) or electron shuttles (Figure 1). The heme arrangements within each domain are very similar to each other, consistent with each domain arising from a gene duplication event.

Determine redox properties of the purified molecules especially their electron transfer rate to U and Tc and Hanford-relevant minerals and the effects of environmental conditions, such as O_2 , on their redox properties. To investigate the mechanism by which [NiFe]-hydrogenases ([NiFe]-H₂ases) reduce Tc(VII), we developed a system for overexpressing [NiFe]-H₂ase of *S. oneidensis* MR-1 (SO-[NiFe]-H₂ase). The genes encoding SO-[NiFe]-H₂ase were cloned into an expression vector. The construct was transformed into a *S. oneidensis* MR-1 mutant that did not produce H₂. The resulting *S. oneidensis* MR-1 strain produced H₂ at the level that was 36% of that of wild-type cells. The recombinant SO-[NiFe]-H₂ase was isolated and the isolated recombinant protein contained H₂ase activity that was inhibited by O₂. Together, these results show for the first time overexpression and isolation of a functional SO-[NiFe]-H₂ase. Successful development of this *Shewanella*-based system for overexpressing SO-[NiFe]-H₂ase lays the foundation for future characterization of [NiFe]-H₂ase-mediated Tc(VII) reduction. In collaboration with the Rosso project, we measured the direct electron transfer between purified *S. oneidensis* MR-1 *c*-Cyt STC (selected because a high-resolution crystal structure is available) and hematite (α -Fe₂O₃) with cyclic voltammetry. Although it did not form a stable film on the hematite surface, STC could still transfer electron to hematite most likely via transient interactions with the oxide particles. Recently, *Shewanella* species were found to secrete riboflavins, such as flavin mononucleotide (FMN), which were thought to function as electron shuttles for facilitating extracellular reduction of Fe(III) oxides. By using protein film voltammetric and stopped-flow-based methods, we found that MtrC reduced FMN, a finding that is consistent with recent results published by other groups. Our results also showed that the binding affinity of MtrC to FMN was an order of magnitude higher than that to Fe(III)-citrate. These results suggest that MtrC may use FMN as a diffusible co-factor to reduce Fe(III) oxides.

2. FY10 Goals and Deliverables

In FY10, we will continue the research activities that were initiated in FY09.

Because lack of genetic system for HRCR-6 prevents us to characterize UndA-HRCR-6 function in HRCR-6 cells, we will use *S. oneidensis* MR-1 as a surrogate host to test the metal reductase activity of UndA-HRCR-6 inside bacterial cells. We will complete the measurements of the ability of UndA-

HRCR-6 to complement *S. oneidensis* MR-1 mutant lacking MtrC and OmcA in Fe(III)/Mn(IV) oxide and U(VI) reductions assays. To determine whether UndA-HRCR-6 can function extracellularly, the extracellular translocation of UndA-HRCR-6 via bacterial type II secretion system will be investigated in *S. oneidensis* MR-1 cells. *In vitro* reductions of Fe(III) oxide, Fe(III)-NTA, FMN, U(VI) and Tc(VII) by purified UndA-HRCR-6 will be measured. Polyclonal antibodies against UndA-HRCR-6 will also be generated. All these planned works will be performed at PNNL.

To continue investigation of the electron transfer mechanisms across the envelope of *S. oneidensis* MR-1 cells, we plan to clone the gene for CymA at PNNL and sent the construct to the Richardson's group at University of East Anglia (UEA). CymA will be purified. Whether purified CymA can interact with purified MtrA physically will be determined by the sedimentation equilibrium analysis.

The structural models of MtrF and MtrA will be further improved at UEA. This includes mapping surface peptides of MtrA. MtrA will be chemically modified at UEA. The modified MtrA will be sent to PNNL for mass spectrometric identification of the modified peptides. To initiate the structural determinations of MtrD (a MtrA homologue), MtrE (a MtrB homologue) and MtrDE and MtrDEF complexes, the respective constructs will be made at PNNL. The constructs, including that for UndA-HRCR-6, will be sent to UEA for protein purification. The crystal trials for the purified proteins will be initiated.

The redox properties of MtrF will be determined at UEA with electron paramagnetic resonance spectroscopy, UV-vis spectropotentiometric titration and protein film voltammetry. In PNNL, SO-[NiFe]-H₂ase will be purified and the reduction of Tc(VII) by purified SO-[NiFe]-H₂ase will be measured. By using the structural templates available in protein data base, the homologous structural model of SO-[NiFe]-H₂ase will be generated. In collaboration with the Rosso project, we will begin to investigate how SO-[NiFe]-H₂ase reduces Tc(VII) at molecular level. Characterization of interaction between purified MtrC/OmcA and FMN will be continued. The redox properties of FMN on Fe(III) oxide surface will be measured.

The following manuscripts are targeted as deliverables for FY10 submission:

Belchik S., A.Z. Wang, D.W. Kennedy, M.J. Marshall, J.M. Zachara, J.K. Fredrickson and L Shi. 2010. Identification, gene cloning and protein purification and characterization of UndA-HRCR-6. 2010. To be submitted to Environmental Microbiology.

MtrF structure/spectroscopy/electrochemistry (manuscript 2 planned to be submitted to Science/PNAS).

MtrA structure/mapping of peptide interactions between MtrA-MtrC and MtrA-MtrB (manuscript 3 planned to be submitted to PNAS).

3. New Scientific Results that May Shift Emphasis

The success with MtrF structure and our confidence that we can deliver a structure by Dec 2009 suggests that we should focus additional resources on structural studies on MtrD, MtrE and the MtrDE or MtrDEF as a complex. This may be a productive route for obtaining a structure of a family member of the MtrABC complex. This would require a slight shift in our research plans as we would need expression constructs for MtrD, MtrE, MtrDE and MtrDEF. We believe that this represents a significant scientific opportunity for the SFA.

H₂ases play a critical role in Tc(VII) reduction. The molecular mechanisms by which H₂ases reduce Tc(VII), however, remain uncharacterized. Successful developments of a *S. oneidensis* MR-1-based

system for overexpressing [NiFe]-H₂ase and [FeFe]-H₂ase by us at PNNL as well as other groups open new opportunities to investigate the mechanisms used by H₂ases to reduce Tc(VII). In FY10, we propose to expand our research activities by including i.) characterization of SO-[NiFe]-H₂ase-mediated Tc(VII) reduction and ii.) purification of SO-[FeFe]-H₂ase.

4. External Collaborator Research Activities

Dr. Richardson's group at UEA has collaborated with us in the area of the ET properties as well as the molecular structures of the redox proteins that are directly involved in extracellular ET processes in the cell envelope of *S. oneidensis* MR-1 for five years. This collaboration was supported previously by EMSL Biogeochemistry Grand Challenge research project. The main task for the UEA team in this SFA is the structural determination of purified proteins at high resolution by crystallography-based method. In FY09, the UEA team obtained the low-resolution structures of MtrA and MtrF. In FY10, the UEA team is expected to finish the determination of the high-resolution structures of MtrA and MtrF. To investigate the ET mechanisms across the envelope of *S. oneidensis* MR-1 cell, the UEA team characterized MtrABC complex with different methods, including UV-vis spectropotentiometric titration, protein film voltammetry and sedimentation equilibrium analysis. The results from the MtrABC functional characterizations as well as those from the PNNL team were integrated in a manuscript that is accepted by PNAS.

5. Capital Equipment Needs

An Auto-iTC200 Isothermal Titration Calorimetry (<http://www.microcal.com/products/itc/auto-itc200.asp>) will be needed for FY10.

Auto-iTC200 Isothermal Titration Calorimetry is specially designed for measuring biological samples. Its applications include: i.) characterization of molecular interactions of small molecules, proteins, and other biomolecules, ii.) enzyme kinetics, iii.) assessment of the effect of molecular structure changes on binding mechanisms, and iv.) assessment of biological activity. It will be used for quantitative measurement of the interactions between purified proteins and their ligands, such as U, Tc, Fe(III)-NTA and flavins. The estimated cost is \$250 K.

In Situ Hydrogeologic Structures and Reactive Transport Properties

Principal Investigator: **A.L. Ward**

Internal Participants: C. Murray, A. Tartakovsky, S. Yabusaki, K. Draper, N. Hasan, and R. Kiddy

External Collaborators: R. Versteeg (INL)

1. Scientific Progress

This project is focused on developing quantitative links between sedimentary facies and the primary hydrogeochemical and geophysical properties. It is hypothesized that the primary properties of sedimentary structures and their directional dependence are largely controlled by the distribution of facies, which is in turn controlled by the depositional environment and grain size distributions. Thus, a major goal is to understand and model the relationship between fundamental sediment properties and the parameters of interest such that the resulting correlations can be applied to different source materials and locations. Research emphasis has been on the characterization of whole and fractionated sediments, including geochemical, hydraulic, geophysical, and geophysical measurements coupled with numerical modeling of the relationships between sediment characteristics (size distributions, shape indices, packing efficiency) and different responses. Interactions thus far have been with SFA projects investigating pore scale reactive transport models (Scheibe/Tartakovsky) and with the Hanford and Rifle IFRCs. Research in FY09 pursued two primary Subject Lines: i.) defining and modeling the relationship between grain-size-distribution statistics and the abundance of natural isotopes (^{40}K , ^{238}U , and ^{232}U) using sediment samples from the Hanford and Rifle IFRC sites, and ii.) an improved understanding of the nature of the geoelectric response of Hanford IFRC sediments.

In support of Subject Line 1, 100 whole samples from Hanford's IFRC well field and 25 from the Rifle IFRC site were characterized to determine grain size distributions and the γ -ray spectra. Measurements have been completed on whole sediments and on their component size fractions (10 per sample) separated according to the Udden-Wentworth scale. Similar measurements were made on samples collected from the Rifle IFRC site. The research objectives were to establish a basis for using the abundance of natural isotopes to quantify grain size distributions and to use resulting relationships as a means of identifying lithofacies using γ -ray log and the uncertainty in their distribution. In the Hanford sediments, the abundance of ^{40}K , ^{238}U , and ^{232}Th is strongly correlated with the extent of weathering and with mean grain size with abundances decreasing with increasing grain diameter and increasing with sorting coefficient. Hanford clay showed concentrations of 4%, 5.5 ppm, and 6.5 ppm for K, U, and Th respectively. Figure 1a shows a plot of K (%) as a function geometric mean diameter (d_g) derived from well C5708 in the IFRC well field. An increase in geometric mean diameter from 0.002 mm (clay) to 45.25 mm (very coarse gravel) showed a decrease in K of over 70% (Figure 1a), 76% for U, and 83% for Th. In Rifle IFRC sediments ^{40}K is uniformly spread throughout principal rock-forming minerals and showed no correlation with grain size. However, there was an 81% decrease in U and a 73 % decrease in Th from clay to gravel. Cross plots of Th/U and Th/K also showed strong correlations with grain size. The enrichment of natural isotopes with decreasing grain size suggest that borehole γ -ray spectra could have a much wider application in characterizing grain separation and sorting and ultimately flow and reactive transport properties.

The resulting correlations are being used as a basis for developing 3-D facies models that reflect variations in the depositional environment and the associated subsurface heterogeneity from surface and borehole geophysical measurements. Thus far, identification of lithofacies has been based entirely on the total γ -ray log using an approach that approach assumes that the global histogram of γ -ray is the sum of several normal populations. An optimization strategy was then used to identify the combination of

possible normal sub-populations that best fit the global. Three main lithofacies have been identified using this approach, with mean γ -ray values of 151.2, 161.5, and 177.5 API units. The proportions of those lithofacies represented 32%, 40%, and 29%, respectively, and represent 3 lithofacies with varying amounts of fine-grained material.

A number of different approaches are being evaluated for simulating the spatial distribution of lithofacies between the boreholes. Figure 1b shows one realization of a simulation of the lithofacies distribution along a cross-section running northwest to southeast in the Hanford IFRC well field.

The research objective of Subject Line 2 is to develop an understanding of the effects of microstructure and the grain parameters controlling particle arrangement and packing on the geophysical response of unconsolidated sediments. Such an understanding is needed to develop relationships between electrical properties and reactive transport properties. In support of this subject line, a pore-scale model has been developed to predict the broadband electrical properties of porous media. Significant progress has been made in simulating the packing of aspherical particles (in collaboration with DOE FaST Fellow Dr. R. Al-Raoush from Louisiana State University), prediction of fluid distributions using smooth particle hydrodynamics (SPH). A finite-element based code that incorporates algorithms to account for the effects of varying in surface conductivity, has successfully applied to spherical and aspherical packs, with the associated SPH-generated flow fields, to predict the electrical response of in relation of lithology, saturation, and pore fluid composition. The resulting responses are being used to physics-based relationships between textural/structural properties, the electrical response of different sedimentary facies, and geochemical properties. Results thus far show the electrical response to be sensitive to grain parameters controlling particle arrangement and packing, particle shape, cation exchange capacity, and the surface area to pore volume ratio.

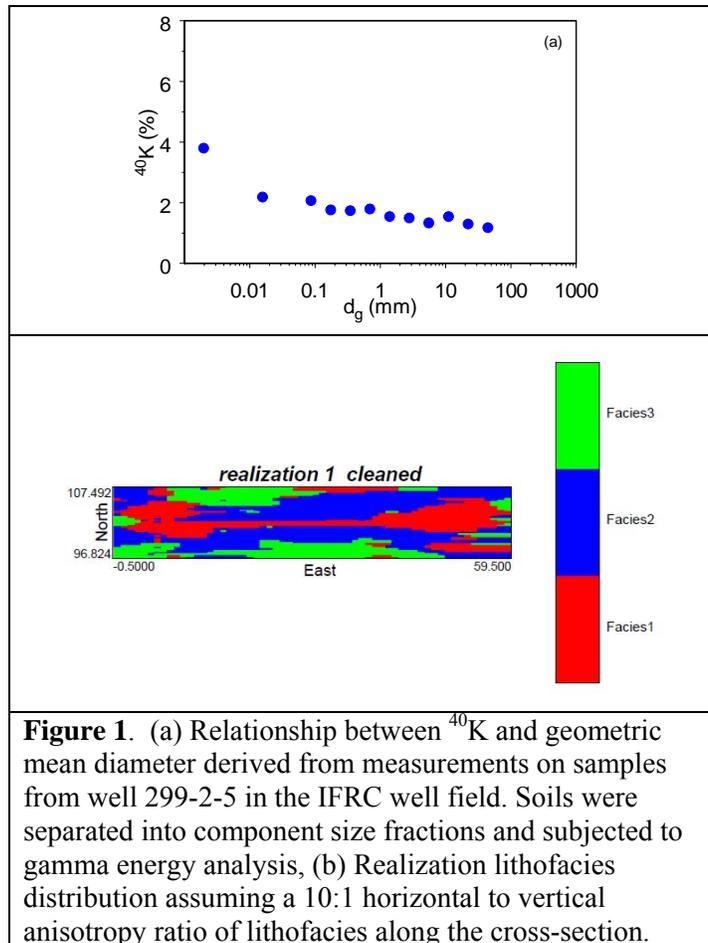


Figure 1. (a) Relationship between ^{40}K and geometric mean diameter derived from measurements on samples from well 299-2-5 in the IFRC well field. Soils were separated into component size fractions and subjected to gamma energy analysis, (b) Realization lithofacies distribution assuming a 10:1 horizontal to vertical anisotropy ratio of lithofacies along the cross-section.

2. FY10 Goals and Deliverables

FY10 research will continue emphasis along Subject Lines 1 and 2 that were initiated in FY 09 and initiate a third Subject Line 3, which will focus on developing methods to predict hydraulic and thermal properties of unconsolidated sediments from grain size distributions.

Subject Line 1. Research will extend the current approach to the full spectral gamma data set, calibrated with grain-size data, for lithofacies identification and two primary studies are planned. We have evaluated several approaches that might be used to simulate the field-scale spatial distribution of lithofacies from local-scale (core and borehole) measurements. These included sequential indicator

simulation, the coupled Markov Chain approach, and the transition probability approach. Sequential indicator simulation is well known and widely used in the simulation of categorical data like lithofacies but has the disadvantage that each lithofacies is simulated independently, so that the spatial relationships between lithofacies cannot be captured. A related issue is that the probability of transition from one lithofacies to another is symmetric so that the tendency for certain types of facies transitions to dominate (e.g., fining upward sequences) are not reproduced. Work in FY10, will focus on the applying coupled Markov Chain approach and the transition probability approaches, which were designed to overcome those shortcomings. However, the coupled Markov Chain approach has only been implemented in two dimensions but a fully functioning 3-D approach is currently under development and will be applied in FY10. The transition probability approach addresses the major problems with sequential indicator simulation, and a 3D implementation of the code is available, so it will be used for lithofacies mapping in this project. The second aspect of the study will focus on completing the petrophysical models needed to translate geophysical attributes in the parameters needed as input to the reactive transport models. Correlations to date have been based entirely on mean grain diameter but many of the properties are sensitive to grain sorting as well. These correlations will be made more robust by incorporating the sorting coefficient and variations in mineral composition of individual size fractions. The statistical uncertainty for γ -rays emitted from low abundances of natural isotopes can be quite high for the 200-s acquisition time used in logging the IFRC wells and the resulting depth profiles show high levels of uncertainty. Lab-based correlations will therefore account for the uncertainty in field measurements. Completion of the petrophysical models to estimate ion of hydrologic and geochemical properties from the lithofacies distributions.

Subject Line 2. In FY 10, work will continue on the development of correlations between lab- and field-measured geoelectric responses and the primary sediment physical, hydrologic, geochemical, and microbiologic properties quantified by ex-situ characterization of whole sediments and their size fractions. Work will continue on using X-ray tomographs and/or digital images to define the 3-D and 3-D microstructure to serve as the basis for computing various hydrophysical and geochemical properties using exact finite-element algorithms. Two basic assumptions implicit in this line are that : 1) the microstructure of sediments can accurately represented as digital image with each pixel representing a homogeneous single phase, and 2) the electrical response of each individual phase in a composites is known at arbitrary frequencies. Thus, we will initially focus on the laboratory characterization of the broadband electrical properties of separated fractions of sediments and model mixtures from the IFRC with the resulting data being used to fit appropriate circuit models to represent the single phase properties. This work will be spearheaded by our new SFA geophysics hire, Jason Greenwood with additional data coming from the INL external collaborator. An important goal to develop methods to upscale the resulting correlations for application to the field and this will be accomplished using grain size distributions.

Subject Line 3. Hydraulic properties (water retention and hydraulic conductivity) measurements on some IFRC samples (Hanford and Rifle) will be impractical because of the gravel content and wide range of particle sizes. We hypothesize that the water retention properties of soil separates contributes to soil water retention of the composite soil in proportion to the volumes of pore subspaces related to each fraction. The objective of subject Line 3 is to test this hypothesis. Water retention and hydraulic conductivity will be measured in repacked cores consisting of soil separates and on model mixtures of these separates using the evaporation method. A component additivity model will be developed for predicting the hydraulic properties of whole sediments and evaluated using laboratory measurements on fractions and model mixtures. Possible sources of errors will be identified and quantified and conclude that the attained accuracy warrants testing the additivity model with other soil data and improving this model to accommodate various types of soil structure and additional soil components.

We are targeting the following manuscripts as deliverables for FY10 submission:

Subject Line 1: Relation of Natural Isotope Abundance to Grain Distributions

Draper K.E., A.L. Ward, and W.J. Greenwood. 2010. Influence of sediment texture on the distribution and availability natural isotopes ^{40}K , ^{238}U , and ^{232}Th in Hanford Sediments. To be submitted to *Journal of Environmental Radioactivity*.

Ward A.L., K.E. Draper, and W.J. Greenwood. 2010. Estimating soil particle size distribution of composite materials from γ -ray spectra using a soil component additivity model. To be submitted to *Geoderma*.

Murray C.J., A.L. Ward, and Y. Xie. 2010. Multi-scale, multi-resolution synthesis of subsurface heterogeneity in flow and reactive transport parameters using γ -ray spectra. To be submitted to *Water Resources Research*.

Ward A.L., C.J. Murray, and Y. Xie. 2010. 3-D quantification of grain size and mineral distributions based on γ -ray spectra. To be submitted to *Sedimentary Geology*.

Subject Line 2: Linking Electrofacies to Sedimentary Facies for Hanford 300A sediments

Greenwood, W.J., A.L. Ward, and N. Hasan. 2010. Complex resistivity of Post Ringold Hanford Sediments and their components. To be submitted to *Geophysics*.

Hasan N., A.L. Ward, and W.J. Greenwood. 2010. A numerical study on the grain boundary electrical properties of composites. To be submitted to *Geophysics*.

Hasan N., A.L. Ward, and W.J. Greenwood. 2010. Characterization of Sorption and Precipitation Processes by means of dynamic impedance spectroscopy. To be submitted to *Environmental Science & Technology*.

Subject Line 3: Linking Electrofacies to Hydrofacies for Hanford 300A sediments

Strickland C.E., A.L. Ward, and N. Hasan. 2010. An improved transient evaporation method for determining soil hydraulic properties for gravelly sediments. To be submitted to *Soil Science Society of America*.

Strickland C.E., A.L. Ward, and N. Hasan. 2010. Predicting the water retention and hydraulic conductivity of unconsolidated sediments using a component additivity model. To be submitted to *Soil Science Society of America*.

3. New Scientific Results that May Shift Emphasis

There are no major shifts in technical emphasis anticipated in FY10. However we will seek to strengthen collaboration with other SFA projects and involvement in the development of methods to interrogate controlled reactive transport experiments using geophysical methods for both the Hanford and Rifle IFRCs. A new SFA project hire, Jason Greenwood, a geophysicist, will work collaboratively between this and the IFRC project performing laboratory measurements and numerical modelling to quantify the

relationship between lithostratigraphy and electrical properties. His emphasis will be on measuring and modelling the broadband electrical properties in variably saturated Hanford sediments.

4. External Collaborator Research Activities

Subcontracts for external collaborators were not placed until well into FY09, hence the extent of collaborator technical progress to date has been limited. Further delays have resulted from the inability of INL to accept IFRC cores from PNNL for laboratory characterization. Nonetheless, these measurements once started will contribute to Subject Line 2.

Versteeg (INL) – INL will focus on the execution of broadband (.001-1000 Hz) electrical experiments to characterize undisturbed soil cores collected during installation of the well field at the 300-Area Integrated Field Challenge Research (IFRC) site. Electrical measurements will be made under static and dynamic conditions and interpretive models will be applied to identify linkages between the electrical response and sediment properties that control or influence reactive transport properties. Also included in this work scope is the adaptation and application of predictive and interpretative models for interpretation of the data and to link the broadband measurements to sediment properties and subsurface processes. These models will initially be based on simple circuit models (Cole Cole and similar) that are currently available in the open literature for interpreting impedance/dielectric spectroscopy measurements.

5. Capital Equipment Needs

None requested for FY10.

Reactive Transport of U and Tc in Sediment Systems Containing Microenvironments and Transition Zones

Principal Investigator: **J.M. Zachara**

Internal Participants: T. Peretyazhko, M. Oostrom, C. Liu, C. Pearce, J. McKinley, D. Moore, and T. Wietsma

External Collaborators: J. Davis (USGS), S. Brooks (ORNL)

1. Scientific Progress

This project is focused on macroscopic-scale studies of U(VI) and Tc(IV/VII) reaction and advective transport in Hanford site sediments from different vadose and saturated zone locations in the Columbia River corridor (e.g., 300 A IFRC) and the 200 A plateau. Major goals are to understand and model kinetic processes controlling Tc and U solid liquid distribution and associated transport in sediments with complex or variable geochemical or mineralogic conditions, and in-situ structures and heterogeneities. Results and models are to be upscaled to field systems, such as the Hanford IFRC, where these important risk-driving contaminants are problematic. Research emphasis is placed on experimental studies of coupled, non-linear processes including geochemistry, hydrology, and microbiology; with rigorous multi-component kinetic modeling for interpretation and system scale conceptual model development. Strong interactions are had with SFA projects investigating pore scale processes (Liu and Fredrickson) and reactive transport models (Scheibe) as well as with the Hanford IFRC. Research in FY09 has pursued two primary Subject Lines that are described below: i.) defining and modeling the desorption-adsorption and mass transfer behaviour of U(VI) in intact, oxic vadose zone and saturated zone cores from the IFRC site, and ii.) determining the geochemical nature of the biogeochemically active Fe(II) fraction in Ringold formation sediments from a natural redox transition zone and their reaction kinetics with O₂ and Tc(VII).

Advective transport experiments were performed in Subject Line 1 with three intact, U(VI) contaminated saturated zone cores (30 cm x 10 cm) from the Hanford IFRC in the EMSL Subsurface Flow and Transport Facility (SFTF). The cores were obtained from the top, intermediate, and lower sections of the Hanford formation saturated zone that have been observed through field characterization to exhibit different hydraulic conductivities. The research objectives were to determine how reaction properties (surface complexation strength, adsorption/desorption kinetics) measured on the < 2mm sediment fraction scale to the structured aquifer materials that contain a high fraction of non-reactive river gravel. The experiment contained three phases, all performed with a synthetic groundwater and advective velocities representative of the IFRC: i.) desorption of contaminant U(VI), ii.) transport behaviour of non-reactive tracers with different diffusivities (Br⁻; pentafluorobenzoic acid, PFBA), and iii.) adsorption and desorption of a spiked U(VI) pulse (@60 ug/L). Over 1000 aqueous samples were collected and analyzed for each core providing a robust experimental data set for evaluation of different model types. The hydrologic properties (k_{sat} and pressure-saturation relations) of the cores were measured by the multi-step method in the SFTF, and high resolution computed x-ray tomography to define in-situ physical structure was performed at the University of Texas, NSF user facility. U(VI) adsorption-desorption behaviour was found to be similar in all three columns, and to exhibit strong kinetic behaviour associated with mass transfer to and from fine-textured domains. A dual domain, multi-rate surface complexation model could well describe the noted U(VI) fluxes with a common set of parameters for each core. These model parameters are being used to premodel a fall 2009 U(VI) desorption experiment at the Hanford IFRC. The cores have now been dissected and the isolated < 2 mm fractions are being subject to a number of physical, mineralogic, and geochemical measurements as necessary to complete upscaling study objectives.

A comprehensive laboratory experimental series was initiated in Subject Line 2 with a unique series of seven Ringold Formation sediments collected across a complex redox transition zone in the deep, near-river, unconfined aquifer at the Hanford 300A IFRC. All Hanford risk analyses assume that Tc(VII) originating from tank waste disposal and leakage is stable and fully mobile in Ringold Formation sediments of the unconfined aquifer; it is consequently one of the site's most important risk-driving contaminants. This research sought to evaluate the redox stability of Tc(VII) in naturally anoxic Ringold sediments. The samples were obtained from the lower half of the deep microbiology characterization borehole at the IFRC; and because of their apparent reduced character, were carefully stored to prevent oxidation.

The following activities were performed: i.) total chemical and mineralogical characterization, ii.) variable temperature Mossbauer spectroscopy to determine Fe(II) and Fe(III) forms and their concentrations, iii.) reduction kinetics of Tc(VII) in batch reactors and EXAFS characterization of redox products, iv.) oxygen consumption/reaction kinetics in stirred-flow reactors, and v.) Tc(IV) oxidation kinetics in presence of reactive Fe(II) in stirred-flow reactors (in progress). Very significant results have been obtained with the following noted as key findings: i.) all of the sediments contain significant reactive Fe(II), with Fe(II)-containing phyllosilicates being common, ii.) most of the anoxic Ringold sediments reduce and immobilize Tc(VII) as nano-clusters of TcO₂; reduction rates are rapid in certain sediments, iii.) the most reactive sediments contain discrete Fe(II) precipitates that are visible by Mossbauer spectroscopy but have not yet been identified, iv.) molecular oxygen reacts preferentially [over Tc(IV)], albeit slowly, with Fe(II) in the sediments; and v.) the influx of O₂-saturated groundwater (e.g., 8 mg/L) generates Tc effluent concentrations of approximately 50 nM/L (Figure 1, the nominal solubility of TcO₂) in the first day of contact, with

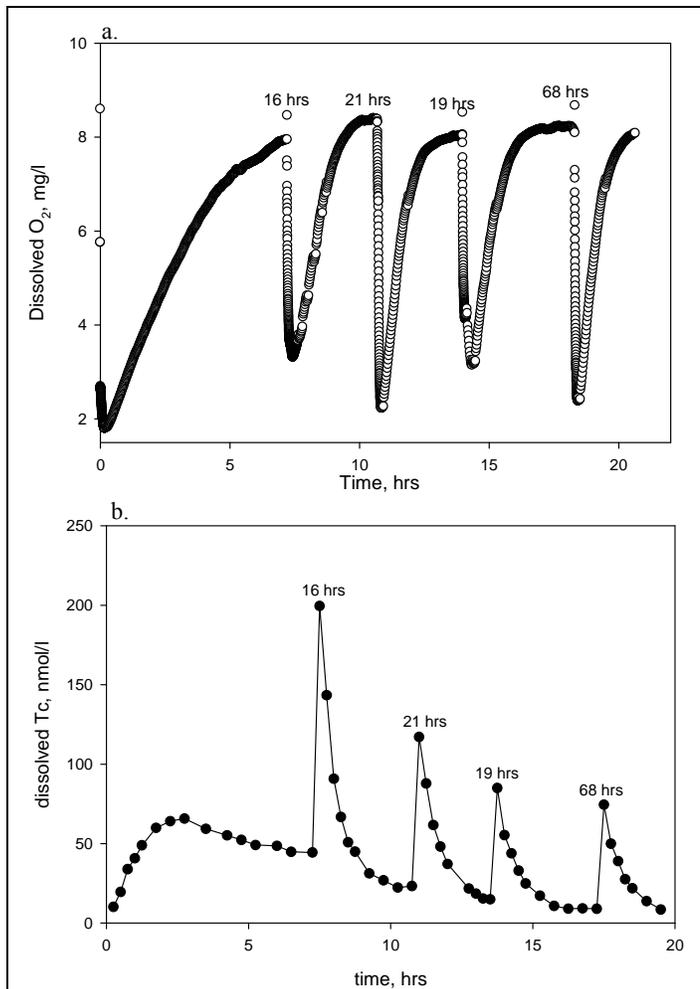


Figure 1. Stirred flow reactor results for oxygen consumption (a) and Tc release (b) from a naturally anoxic Ringold sediment that had been spiked with 10 $\mu\text{M/L}$ Tc(VII) and allowed to react for 2 weeks. The Tc(VII) spike was reduced, yielding a final Tc(IV) aqueous concentration of 4.6×10^{-8} mol/L. The sediment was centrifuged before addition to the flow cell. The experiment was initiated by placing moist, Tc-containing sediment into the flow cell reactor, and beginning the inflow of O₂ saturated groundwater at time 0. At 7 h the inflow solution was stopped and the flow cell allowed to react in batch mode for 16 h. Inflow was again started at 7.5-11 h, after which another 21 h static period was allowed, and so on. Decreases in O₂ concentration below 8 mg/L result primarily from Fe(II) oxidation. While interpretation is dependent on modeling, the results indicate that Fe(II) is oxidized in preference to Tc(IV), the Tc(IV) oxidation rate is slow, and the total Tc(IV) that can be oxidized is < 40% of the total.

elevated concentrations occurring during stop flow events. Research on Tc(IV) oxidation rate in presence of reactive Fe(II) and advective O₂ supply (Figure 1) is ongoing, with very complex results noted to date. Establishing macroscopic mechanisms and dependencies for this important of this process under advective conditions will be an emphasis of FY 10 research.

2. FY10 Goals and Deliverables

FY10 research will continue emphasis along Subject Lines 1 and 2 that were initiated in FY 09.

Subject Line 1 research will continue emphasis on the reactive transport of U(VI) in oxic 300 A sediments. Two primary studies are expected. In the first, the < 2 mm mineral size fraction isolated from the three intact saturated cores investigated in FY 09 will be robustly investigated in terms of their reactive mineral properties, and their surface complexation and intragrain mass transfer behaviour for U(VI). Surface complexation and mass transfer models will be developed based on experimentation with the < 2 mm fraction in stirred flow reactors with IFRC synthetic groundwater over a range of conditions influencing reaction rate and extent. Various numeric procedures will then be evaluated to reconcile reaction parameters for the isolated <2 mm sediment fraction with the intact core behaviour, noting that all reactivity in the intact core is associated with this fraction. The second study will utilize the EMSL SFTF to investigate the kinetic solubilisation process of contaminant U(VI) from intact deep vadose zone core samples from the IFRC that are seasonally saturated by multiple and falling water table events. An innovative and robust experimental system will be devised to simulate the timescales, advective character, and variable geochemical compositions associated with these complex hydrologic events that are believed to recharge and sustain the persistent 300 A groundwater U plume. Robust experimental observations will drive the development of a kinetic, reaction based simulator for field-scale application.

Subject Line 2 research will continue investigation of Tc(IV/VII) oxidation and reduction kinetics and O₂ consumption rates by ferrous iron containing mineral phases in Hanford sediments under advective conditions and dissolved O₂ concentrations relevant to groundwater flow in different Hanford locations. The methodologies of these complex, kinetic, stirred-flow reactor experiments have only recently been documented to the point of complete reproducibility, affording significant opportunities for scientific advance. The macroscopic kinetic studies will be closely linked with molecular speciation measurements of Fe by x-ray absorption and Mossbauer spectroscopy, and Tc by EXAFS spectroscopy. An important goal is the development of linked kinetic models based on experimental measurement that describe the reactivity of Fe(II) in mineralogic pools quantifiable by Mossbauer spectroscopy with both O₂ and Tc(VII); and the resultant oxidation rate of Tc(IV) associated with these distinct mineralogic or physical environments.

We are targeting the following manuscripts as deliverables for FY10 submission:

Subject Line 1: U(VI) dissolution, surface complexation, and mass transfer in Hanford 300A sediments

Ilton E.S., J.M. Zachara, D. Moore, J. McKinley, C.L. Cahill, and A.R. Felmy. 2009. A dissolution study of metatorbernite: Thermodynamic properties and the effect of pH and phosphate. To be submitted to *Environmental Science & Technology*.

Wang Z., J.F. Boily, J.M. Zachara, Y. Xia, R.K. Kukkadapu, C.T. Resch, D.A. Moore, and C. Liu. 2009. Determining individual mineral contributions to U(VI) adsorption in a contaminated aquifer sediment: A fluorescence spectroscopy study. To be submitted to *Geochimica et Cosmochimica Acta*.

Zachara J.M., C. Liu, M. Oostrom, J.P. McKinley, T. Wietsma, and C.T. Resch. 2010. Reactive transport of U(VI) in intact, contaminated aquifer sediments. To be submitted to *Environmental Science & Technology*.

Zachara J.M., C. Liu, M. Oostrom, J.P. McKinley, T. Wietsma, and C.T. Resch. 2010. Scaling surface complexation and mass transfer parameters from stirred flow reactors to intact sediment columns. To be submitted to *Environmental Science & Technology*.

Subject Line 2: Tc(VII) Biogeochemistry

Plymale A.E., J.K. Fredrickson, J.M. Zachara, A.C. Dohnalkova, S.M. Heald, D.A. Moore, M.J. Marshall, and P. Nachimuthi. 2009. Competitive reduction of pertechnetate ($^{99}\text{TcO}_4^-$) by dissimilatory metal reducing bacteria and biogenic Fe(II). To be submitted to *Environmental Science & Technology*.

Peretyazhko T., J.M. Zachara, R. Kukkadapu, C. Liu, D. Moore, and A. Plymale. 2010. Pertechnetate reduction and reactive ferrous iron forms in naturally anoxic, redox transition zone sediments from the Hanford Site, USA. To be submitted to *Geochimica et Cosmochimica Acta*.

Peretyazhko T., J.M. Zachara, C. Liu, D. Moore, and A. Plymale. 2010. Rates of oxygen consumption and Tc(IV) oxidation in naturally anoxic sediments under advective conditions: Mineralogic and mass transfer controls. To be submitted to *Geochimica et Cosmochimica Acta*.

3. New Scientific Results that May Shift Emphasis

There are no major shifts in technical emphasis anticipated in FY10. We do expect greater teaming and collaboration with other SFA projects as their research matures, as well as the initiation of collaborative experiments with a new SFA investigator team/project that will develop Hanford-inspired pore scale models for controlled reactive transport experiments. Our new SFA project hire, Carolyn Pearce will work collaboratively between this and the Rosso projects performing and modelling X-ray experiments on Fe and Tc biogeochemistry and mineralogy. Her emphasis in this project will be on Tc and Fe molecular speciation in mineralogically complex Hanford sediments that vary in their natural redox potential.

4. External Collaborator Research Activities

Subcontracts for external collaborators were not placed until well into FY09, hence the extent of collaborator technical progress to date has been limited. However, the research of both collaborators is now well underway. Both are contributing to Subject Line 1.

Davis (USGS) – The USGS is applying a number of innovative experimental and modelling techniques to characterize the reactive microporosity of 300 A vadose zone and aquifer sediments under advective conditions. The basic approach is to allow long term contact of the sediments with non-reactive tracers of different form in a water-saturated column setting without flow. During long contact, the dissolved tracers diffuse into all internal pore or fracture volumes. Reversing this process, and following tracer release to unspiked waters for long periods provides a data set from which the volumes and effective diffusivities of different pore domains can be calculated or fit with different models. Significant collaboration is underway between this activity and the Liu project.

Brooks (ORNL) – The ORNL team is investigating the kinetics of contaminant U(VI) release from IFRC vadose zone sediments under unsaturated conditions. The group has been provided with vadose zone grab samples and intact cores from the IFRC from the depth range of 5-7m, where residual contamination from process pond operation remains. These generally contain relatively high concentrations of adsorbed U(VI) (> 25 mg/kg). Their experiments will investigate the influence of water content on the rate and extent of contaminant U desorption, and the concentrations of U(VI) that evolve in the resulting unsaturated porewaters. This experimental series directly complements those performed at PNNL with saturated zone sediments.

5. Capital Equipment Needs

None requested for FY10.