Argonne National Laboratory Subsurface Science SFA
FY2017 Annual Progress Report
For the BER Subsurface Biogeochemical Research Program
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1. Program overview
Water is vital for sustaining life on Earth; its movement, whether through oceans, lakes, rivers, streams, or underground, is responsible for transporting most of the biologically catalyzed and many of the chemically reactive elements that cycle through the environment. The movements of water and the constituents dissolved in it, along with the biogeochemically catalyzed transformations of those constituents, determine the mobility of contaminants, atmospheric greenhouse gas (e.g., CO₂, CH₄, and N₂O) emissions, carbon cycling and sequestration in subsurface environments, nutrient (e.g., carbon and nitrogen) mobility, and the quality of water itself. Iron (Fe) is a highly abundant element in the lithosphere; its biogeochemistry in many aquatic and terrestrial environments is driven largely by microbial activity. In Fe-rich soils and sediments, particularly, Fe redox cycling by microorganisms is a significant component of major and minor elemental cycling and energy flux. Similarly, sulfur (S) is commonly found in groundwater; its transformations, often driven by microbially catalyzed redox reactions, can also greatly affect the cycling of elements. Redox-dynamic environments where Fe or S biogeochemical cycling can be a major driver of other elemental cycles include terrestrial-aquatic interfaces and subsurface environments (e.g., marshes, wetlands, floodplains, soil aggregates, plant root-soil interfaces, and locations where groundwater and surface waters mix). These environments are often part of polar, temperate, or tropical systems containing continuously or temporarily water-saturated soils and sediments. Although much is known about the respective biogeochemical cycles of Fe and S, the interplay between the two cycles is less understood. Thus, understanding the interplay of the Fe and S biogeochemical cycles with the water cycle is critical for prediction of the mobility of contaminants; atmospheric greenhouse gas emissions; carbon cycling and sequestration in subsurface environments; and nutrient mobility in near-surface and subsurface polar, temperate, or tropical systems.

The objective of the Argonne Subsurface Biogeochemical Research Program (SBR) Scientific Focus Area (SFA) is to identify and understand coupled biotic-abiotic molecular- to core-scale transformations of Fe and S within redox-dynamic environments and understand the effects of Fe and S biogeochemistry on transformation and mobility of major/minor elements and contaminants. To accomplish this objective, the Argonne SBR SFA integrates two unique strengths at Argonne — the Advanced Photon Source (APS) for synchrotron-based interrogation of systems and next-generation DNA sequencing and bioinformatics approaches for microbial community and metabolic pathway analysis — with biogeochemistry and microbial ecology. Drawing on these strengths, we pursue the long-term scientific goal of elucidating the interplay, from the molecular to core scale, between specific microbial metabolic activities, solution chemistry, and mineralogy contributing to the transformations of Fe, S, nutrients, and contaminants in subsurface environments. Addressing this objective contributes directly to the goal of the United States Department of Energy (DOE), Office of Biological and Environmental Research (BER), Climate and Environmental Sciences Division (CESD) to “advance fundamental understanding of coupled biogeochemical processes in complex subsurface environments to enable system-level environmental prediction and decision support” (http://science.energy.gov/ber/research/cesd/) (DOE 2012). In addition, the work of the Argonne SBR SFA specifically addresses the SBR priority of “understanding and predicting biogeochemical processes in subsurface environments” (DOE 2012). By focusing research efforts on understanding the effects of coupled Fe and S biogeochemical cycles on carbon, nutrient, and
contaminant transport, the Argonne SBR SFA will continue contributing to the CESD’s identified core capability (DOE 2012) that includes “focused research on key Earth system processes that represent significant uncertainties and currently limit predictive understanding.” Similarly, by continuing the well-integrated use of synchrotron radiation at the APS to investigate coupled Fe and S biogeochemical processes, the Argonne SBR SFA will contribute to CESD’s identified core capabilities and needs for “exploiting synchrotron radiation light sources provided by the SC Office of Basic Energy Sciences” (DOE 2012) and providing “foundational knowledge of these molecular scale processes” (DOE 2014).

Hypotheses directed toward achieving the goal of the Argonne SBR SFA are tested by experiments that capitalize on the talents of Argonne researchers and unique Argonne capabilities. Other members of the Argonne SBR SFA are research scientists at several additional institutions; they bring diverse, relevant expertise and established track records of collaborative and integrated multidisciplinary research for experiments investigating hydrological and biogeochemical processes at multiple spatial and temporal scales, in both field and laboratory settings. All members of the Argonne SBR SFA share a long-term vision of ultimately integrating the new knowledge generated by the SFA into future multiscale modeling approaches to understand and predict relevant environmental processes. Argonne SBR SFA research addresses four critical knowledge gaps related to accomplishing this goal: (1) an in-depth understanding of the molecular processes affecting Fe, S, and contaminant speciation in dynamic redox environments; (2) an understanding of the role of biogenic and abiotic redox-active products and intermediates in Fe, S, and contaminant transformations; (3) a mechanistic understanding of the factors controlling the mass transfer of Fe, S, and contaminants in heterogeneous media; and (4) an in-depth understanding of the relationship of microbial community dynamics and function and coupled biotic-abiotic controls and their effects on major/minor element cycling and contaminant transformations. Addressing these knowledge gaps has driven the development of 14 specific hypotheses to be tested within the Argonne SBR SFA.

At the core of our Science Plan are sophisticated molecular speciation measurements, performed at the APS, and omics analyses, using next-generation sequencing and bioinformatics approaches, that provide insights into biogeochemical reaction mechanisms and pathways as components of larger reaction networks driven by microbial communities. This 3-yr science plan focuses on Fe and S redox transformations, driven by microbial communities, and their effects on the transformation of major and minor elements, nutrients, and contaminants at different spatial scales. Experiments at Argonne (Dion Antonopoulos, Ted Flynn, Ken Kemner, Ed O’Loughlin) — in collaboration with the Bulgarian Academy of Sciences (Max Boyanov); Illinois Institute of Technology (Bhoopesh Mishra, Carlo Segre); University of Iowa (Michelle Scherer, Drew Latta); Korean Institute of Science and Technology (Man-Jae Kwon); North Central College, Naperville, Illinois (Silvia Alvarez-Clare); and Pacific Northwest National Laboratory (Jim Fredrickson, John Zachara, Chongxuan Liu, Tim Schiebe, Kevin Rosso) — requires the integration of microbial ecology, microbiology, molecular biology, geochemistry, physics, and modeling approaches. Members of the Argonne SBR SFA team represent expertise in all of these disciplines. Research will emphasize laboratory-based experiments with single-crystalline-phase Fe oxides (including oxides, oxyhydroxides, and hydroxides) and Fe-containing clays (e.g., smectite, illite, chlorite), fabricated Fe-rich mineral assemblies designed to mimic mineralogical conditions in subsurface environments in the field, and geomaterial collected from field environments (e.g., groundwater-Columbia river water mixing zone
at the Hanford 300 Area at Richland, Washington, and stream sediments around Oak Ridge National Laboratory). Inocula for promoting bioreducing or biooxidizing conditions will include (1) monocultures of well-characterized organisms representing dominant genera with recognized roles in Fe and S redox transformations (e.g., *Geobacter* spp., *Shewanella* spp., *Desulfitobacterium* spp., *Desulfovibrio* spp., *Desulfotomaculum* spp. *Thiobacillus* spp., *Acidithiobacillus* spp.), and (2) natural microbial consortia collected from all of these sites. To improve our ability to identify cryptic biogeochemical processes within larger complex reaction networks, we will use model minerals with mixed microbial communities to reduce the complexity of the mineralogical aspects of the system. Similarly, we will use model microbial monocultures to reduce the complexity of the microbial aspects of the system.

In addition to the unique molecular-scale electronic and chemical information provided by measurements at the APS, the experimental work of the Argonne SBR SFA will drive optimization of techniques at APS beamlines, thus increasing the availability and productivity of x-ray beamlines with the characteristics required for the proposed work and benefitting the larger international synchrotron-based biogeochemistry research community. The focus will be on optimization of synchrotron-based hard x-ray capabilities for *in situ* investigations of coupled microbiological and geochemical processes in free-flowing columns. Additional developments of hard x-ray Raman approaches for bulk sample analysis of C and N chemistries in natural samples will also benefit CESD research activities.

We also have a long-term vision of work to be performed during the six years following the end of the present Science Plan. This includes (1) an ever-increasing emphasis on integrating metagenomic-based analysis approaches for understanding community structural and functional controls on fundamental Fe and S biogeochemical processes, (2) an increasing emphasis on model development to predict the transformations and mobility of nutrients and contaminants in many subsurface and near-surface environments, and (3) expansion of these studies with a greater emphasis on using minerals and microbial communities from a network of field sites encompassing many types of redox-dynamic environments, including arctic and tropical sites and a midwestern field site presently being developed by Argonne.

### 2. Scientific Questions

The scientific questions focusing the work done within the Argonne Subsurface Science SFA are motivated by four major knowledge gaps currently hindering our understanding of the coupled biotic-abiotic molecular- to core-scale transformations of Fe and S in redox-dynamic environments and understanding of the effects of Fe and S biogeochemistry on transformation and mobility of major/minor elements and contaminants. Providing data to fill these knowledge gaps is the central driver for this Science Plan comprising the Argonne Subsurface SFA. We list the Knowledge Gaps below. A detailed description of these Knowledge Gaps and a justification for our focus on them is contained within our Science Plan. Knowledge Gaps 4.2 and 4.3 were removed from this Science Plan because the requested $200K/year increase in support to cover their exploration was not provided.

**Knowledge Gap #1 (KG1):** An in-depth understanding of the molecular processes affecting Fe, S, and contaminant speciation in dynamic redox environments.
**Knowledge Gap #2 (KG2):** An understanding of the role of biogenic and abiotic redox-active products and intermediates in Fe, S, and contaminant transformations.

**Knowledge Gap #3 (KG3):** A mechanistic understanding of the factors controlling the mass transfer of Fe, S, and contaminants in heterogeneous media.

**Knowledge Gap #4 (KG4):** An in-depth understanding of the relationship between microbial community dynamics and function and coupled biotic-abiotic controls and their effects on major/minor element cycling and contaminant transformations.

To summarize the importance of these Knowledge Gaps, a fundamental knowledge of the specific mechanisms of microbial redox processes and the links between microbial community function/dynamics and redox transformations of C, Fe, and S is needed to understand the effects of Fe and S biogeochemistry on transformation and mobility of major/minor elements and contaminants.

We are leveraging decades of total experience in synchrotron-based Molecular Environmental Science (MES) research among members of the Argonne SFA team, the unique capabilities of the APS, and our unique local association with the APS to pursue the development and advancement of a number of synchrotron-based capabilities at the APS, particularly capabilities focused on x-ray absorption spectroscopy (XAS) and x-ray microscopy, that are critical to the Argonne SFA and are of service to the SBR scientific community. In addition, we are leveraging unique capabilities at Argonne that enable the use of DNA sequencing technologies and bioinformatics approaches to determine microbial community structure and function in our samples. The further development and integrated use of synchrotron-based and DNA sequencing and bioinformatics approaches with standard laboratory-based biogeochemical experimental approaches for characterizing microbiological and geochemical processes controlling transformations of major/minor elements and contaminants in the subsurface is central to the Argonne SBR SFA and represents unique capabilities that Argonne contributes to the SBR mission.

Fourteen hypotheses related to these knowledge gaps guide the experimental design of the Argonne Subsurface SFA so as to investigate transformations over a range of spatial scales. Experiments are done using mixed batch reactors (MBRs) and columns.

### 3. Argonne Subsurface SFA Program Structure

The figure below shows the organizational structure of the Argonne Subsurface Science SFA. The research manager, with support from the PI, is responsible for communicating with program managers in the SBR office regarding program direction and progress. The research manager is responsible for transmitting information from the SBR program office to the CELS ALD and the Laboratory Director, ensuring that SBR mission needs are represented in the Argonne strategic plan, and for transmitting information from the SBR program office to the SBR SFA PI and Co-PI. The PI is responsible for communicating with the SFA team members to integrate the experimental work efficiently. This communication occurs through regular group meetings (at least monthly and often weekly) at which the PI, Co-PI, and local members of the SFA discuss research progress in the context of the stated research objectives and deliverables. The research manager attends these meetings when possible; the PI is responsible for providing reports on program progress to the research manager quarterly and assists the research manager with presentations to the SBR program managers, as well as to the CELS ALD.

The key Argonne scientific staff responsibilities for FY2017 are detailed below.
Dr. Ken Kemner’s efforts (50%) are focused on synchrotron beamline development and applications, and as a secondary lead on subsurface biogeochemistry and spectroscopic characterizations. Dr. Kemner also assists with the MBR and column experiments and serves as SFA PI.

Dr. Ed O’Loughlin’s efforts (75%) are focused on subsurface biogeochemistry, and as a secondary lead on metagenomics-enabled science. Dr. O’Loughlin also serves as the SFA Co-PI.

Dr. Antonopoulos efforts (10%) are focused on metagenomics-enabled science.

Dr. Flynn, an early-career scientist, serves as a secondary lead on biogeochemistry and metagenomics-enabled science. He brings value to the SFA at no cost as his effort on projects related to the SBR SFA is supported under a strategic hire LDRD at Argonne.

Key non-Argonne SFA members are described below.

Dr. Boyanov (Bulgarian Academy of Science) is the co-lead for spectroscopic characterization and a secondary lead for beamline development and applications. Dr. Boyanov travels from Bulgaria to Argonne so that he may participate in experimental beam runs at the APS. He remains at Argonne for approximately an additional week after each of the six beam runs per year to participate in face-to-face collaborative discussions with other members of the Argonne SBR SFA team.

Dr. Mishra (Illinois Institute of Technology) was a co-lead on spectroscopic characterization and secondary lead for beamline development and applications through an Argonne Guest Appointment. However, he now has an appointment at the University of Leeds and is no longer supported by Argonne SBR SFA programmatic funds.

Dr. Scherer (University of Iowa) originally was identified as a secondary lead for spectroscopic characterization, and a lead for Mössbauer characterization. Similarly, Dr. Latta (University of Iowa) was identified as a secondary lead on spectroscopic characterization, particularly as it relates to investigations involving clays and U. However, since the funds to cover their efforts were not provided, the contribution from University of Iowa collaborators has been eliminated from this work. Limited contributions from the University of Iowa collaborators in the form of sample preparation have been provided as no-cost collaborations.

The roles of additional no-cost collaborators have related to supplying field materials (Fredrickson, Zachara, and Brooks), model development and integration with experiments (Liu, Schiebe), investigation of fundamental biogeochemical processes related to clays (Rosso, Zachara, Fredrickson), biogeochemical experimental work in the laboratory (Kwon), and operation of the MRCAT/EnviroCAT beamlines at the APS (Segre). Recently, there have been changes to this structure. More details concerning changes in the no-cost collaborators are provided below.
In FY2018, the responsibilities of team members will remain essentially the same as described above for FY2017. However, Dr. Mishra primarily is stationed at the University of Leeds and will no longer be supported by Argonne SBR SFA programmatic funds. Although not supported financially in FY2018, it is expected that Dr. Mishra will finish development and submission of manuscripts related to electron transfer reactions between Hg and Mn. We have investigated the potential to replace Dr. Mishra’s efforts via the support of a postdoctoral appointee. Presently, not enough funds are available to cover a postdoctoral appointee’s efforts. Therefore, we will increase the level of support to Dr. O’Loughlin to enable him to meet the remaining goals previously associated with Dr. Mishra’s efforts. We also have made use of summer undergraduates, whose efforts primarily are covered by Argonne National Laboratory, to assist Dr. O’Loughlin and his efforts.

There have been additional changes to personnel who contribute to the Argonne SFA as external no-cost collaborators. Specifically, Drs. Fredrickson and Zachara (PNNL) have recently retired. Additional, Dr. Liu (PNNL-reactive transport modeling) has left PNNL to work at a University in China. We have maintained connections with Drs. Schiebe and Steegan (PNNL) to facilitate access to sediments from the groundwater–river water interaction zone of the Columbia River and to maintain alignment with the PNNL SBR SFA. During FY2018, we will work to identify another researcher with modeling expertise to contribute to the Argonne SBR SFA.

Dr. Peter Littlewood is no longer the Director of Argonne National Laboratory. Presently, Dr. Paul Kearns is the Interim Laboratory Director.
4. Performance milestones and metrics
   a. FY 2017 Scientific progress
      i. Progress toward milestones

The milestones proposed for FY2017, including the knowledge gap and hypotheses numbers associated with that knowledge gap that the milestones address, along with the names of the SFA team members responsible for that milestone, are listed below in bold letters followed by a brief description of our progress to date. We have met all 9 of our milestones for FY 2017 that were not removed due to reduction in project funding. Finally, in addition to the work described in our original Science Plan, we have continued to make progress in the development of x-ray Raman approaches to characterize the bonding environment of carbon in soil and sediment samples. A description of the opportunity to establish x-ray Raman capabilities at the MRCAT/EnviroCAT at the APS was presented to DOE program officers during Kemner’s and Flynn’s visit to DOE Headquarters on July 11, 2016. A discussion of the results associated with each FY 2017 milestone is included below.

- Investigate the effects of structural Fe$^{II}$ in clays on the reduction of Hg$^{II}$; prepare manuscript (KG1.1). O’Loughlin/Boyanov/Mishra/Kemner

  Aluminosilicate clay minerals are a major component of many soils and sediments and many of these clays contain structural Fe (e.g., smectites and illites). Our previous work and that of others, has shown that Fe$^{II}$-bearing minerals such as magnetite and green rust can reduce Hg$^{II}$ to Hg$^{0}$. However, the ability of other environmentally relevant Fe$^{II}$ phases such as structural Fe$^{II}$ in clays to reduce Hg$^{II}$ is poorly understood. We have conducted experiments examining the potential for reduction of Hg$^{II}$ by 6 different specimen clay minerals containing 0–25 wt% Fe. Fe$^{II}$ in the clays (SHCa-1 hectorite, SWy-2 montmorillonite, NAu-1 and NAu-2 nontronite, and Ward’s nontronite) was reduced to Fe$^{II}$ using the citrate-bicarbonate-dithionite method. Experiments were initiated by adding 500 $\mu$M Hg$^{II}$ to clay suspensions (4 g clay L$^{-1}$) buffered at pH 7.2 in 50 mM MOPS. Analysis of the samples by Hg L$\text{III}$-edge XAFS spectroscopy indicated no reduction of Hg$^{II}$ by barasym (0% Fe) and SHCa-1 (0.15% Fe), while reduction of Hg$^{II}$ to Hg$^{0}$ was observed in the presence of reduced SWy-2, NAu-1, NAu-2, and Ward’s nontronite (2.8%–24.8% Fe). In previous work we have shown that the presence of ligands such as Cl$^{-}$ and SO$_4^{2-}$ can affect the extent of reduction of Hg$^{II}$ to Hg$^{0}$ as well as the formation of Hg$^{I}$ species. Moreover, the presence of DOC can also affect the speciation and reduction of Hg$^{II}$. We found no effect of Cl$^{-}$ concentration (0–10 mM) or DOC (0–100 mg L$^{-1}$ Suwannee River humic acid) on the reduction of 500 $\mu$M Hg$^{II}$ by reduced NAu-1 (4 g clay L$^{-1}$) at pH 7.2 in 50 mM MOPS. Additional experiments are planned for the last quarter of Project Year 2 (PY2) examining pH effects (4–10) on the reduction of Hg$^{II}$ by Fe$^{II}$-bearing clays. These results are being drafted as a manuscript to be submitted to Environmental Science and Technology.

- Complete MBR studies of U$^{VI}$ reduction in the presence of clays: explore the effects of ligands and surface:U ratio on U$^{VI}$ speciation; complete synchrotron x-ray measurements, analyze data, prepare manuscript (KG1.3). Boyanov/Latta/Scherer/O’Loughlin/Mishra/Kemner

  We have completed our studies of the interactions between dissolved U$^{VI}$ and reduced Fe-containing clay minerals of environmental relevance (Syn-1, SWy-2, NAu-1, and NAu-2, which cover a wide range of Fe contents possible in nature, from <0.1% to 32% w/w). Using synchrotron x-ray spectroscopy (U L$\text{III}$-edge XANES and EXAFS) we find that these clay minerals are able to transfer electrons to aqueous U$^{VI}$ under the studied conditions (2-100 g clay L$^{-1}$ loading, 250 $\mu$M U$^{VI}$, 2 mM bicarbonate, pH 7.2 MOPS). However, we found that the end product of the redox reaction depends on the type of clay. Despite the stoichiometric excess of Fe$^{II}$ in the system, U$^{VI}$ is reduced to U$^{IV}$ only at high clay solids loadings, whereas a mixed-valence U$^{V}$-U$^{VI}$ oxide is stabilized at lower loadings. By varying the amounts
of reductant we find that the transformation of U\textsuperscript{VI} to the U\textsuperscript{V} or the U\textsuperscript{IV} state is controlled by the reducing capacity in the system. In the most reducing systems the distribution of U\textsuperscript{IV} between uraninite and adsorbed U\textsuperscript{IV} species depends on surface site availability. The results suggest that Fe-containing clays have multiple and significant roles in controlling both the valence state and the molecular coordination of the reduced uranium. This newly discovered U\textsuperscript{VI} reduction pathway through a U\textsuperscript{V}/U\textsuperscript{VI} phase is currently not included in transport models, which may result in inaccurate predictions of U migration in reduced subsurface and wetland sediments such as those present at DOE field sites. One paper has already been published, “Surface area effects on the reduction of U\textsuperscript{VI} in the presence of synthetic montmorillonite”, M.I. Boyanov, D.E. Latta, M.M. Scherer, E.J. O’Loughlin, K.M. Kemner, Chemical Geology, 464 (2017) 110-117. Another publication, “Clay-mineral controls on the reduction of aqueous U(VI) to a stable U(V)/U(VI) phase”, M.I. Boyanov, D.E. Latta, M.M. Scherer, E.J. O’Loughlin, K.M. Kemner, is in the final stages of preparation for submission to Environmental Science & Technology.

- **Investigate the reduction of Hg\textsuperscript{II} to Hg\textsuperscript{0} by Mn\textsuperscript{II} in the presence of redox-inactive minerals. Prepare manuscript detailing results of ligand and mineral surface effects on Hg\textsuperscript{II} reduction by Mn\textsuperscript{II} (KG1.4). Mishra/Boyanov/O’Loughlin/Kemner**

  Our studies over the past few years demonstrated that Hg\textsuperscript{II} is reduced to Hg\textsuperscript{0} by many forms of Fe\textsuperscript{II} due to the low redox potential of Hg\textsuperscript{II} reduction to Hg\textsuperscript{0}. Hence we expanded our research and tested the ability of Mn to reduce Hg\textsuperscript{II}. Our results from PY1 has shown that at pH 7.5, the majority of Hg\textsuperscript{II} is reduced to Hg\textsuperscript{0} within first three hours, followed by significantly slower rates of reaction. Hg\textsuperscript{II} reduction is coupled with oxidation of soluble Mn\textsuperscript{II} to highly insoluble Mn\textsuperscript{IV} oxide.

  In contrast to our hypothesis that presence of minerals would surface catalyze reduction of Hg\textsuperscript{II} to Hg\textsuperscript{0}, our results suggest that the presence of redox inactive mineral surfaces (e.g. Al\textsubscript{2}O\textsubscript{3} and TiO\textsubscript{2}) does not enhance the rate of Hg\textsuperscript{II} reduction. Additionally, Hg\textsuperscript{II} reduction by Mn\textsuperscript{II} in the presence of complexing ions (e.g., Cl\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−}) results in the formation of meta-stable Hg\textsuperscript{I} species, which slowly transforms to Hg\textsuperscript{0} over an extended period of time. Progress on drafting a manuscript detailing these results for submission to Environmental Science and Technology has been slowed due to transition of Dr. Mishra (primarily responsible for this milestone) to a new position in PY1.

  In addition to investigating cryptic pathways for abiotic Hg redox transformations, we have continued to investigate the role of organic and inorganic ligands and surfaces on Hg speciation in natural environments. Our previous work had shown that thiols bound to bacterial cell envelopes control the speciation and bioavailability of Hg by 1) providing high-affinity binding sites, and 2) mediating redox transformations of Hg. Developing this newly developed paradigm further, we have demonstrated that Hg complexation with intact bacterial cell suspensions, a mechanism which is likely applicable to other chalcophilic metals (e.g. Zn, Cd, and Pb) as well, is strongly dependent on metal loading and that the following conclusions can be drawn: 1) complexation of Hg with cell bound thiols is much more complicated than the formation of a single type of Hg-thiol complex at low Hg:biomass ratios; and 2) Hg can be complexed with cell-bound thiol sites in a variety of stoichiometries depending on the biogeochemical attributes of the ecosystem in question (e.g., the Hg:biomass ratio, the abundance of thiol sites on the bacterial species in question, and whether the species is Hg-methylating or not). It must be emphasized that in contrast with expectation from purely thermodynamic considerations, variation in the complexation behavior of Hg with thiols is not always dictated by the abundance of thiols in a given
bacterial species. Differences in abundance and density of thiol sites on cells of different bacterial species, and the corresponding stoichiometry of Hg-thiol complexes that arise from those differences, could also explain the observed differences in passive oxidation of Hg(0) mediated by cell bound thiols. These cell bound Hg-Sn complexes also form readily in the presence of other strongly competing ligands, such as Cl\(^-\) and natural organic matter (NOM), and were found to be stable in aqueous solutions at room temperature for over a period of 2 months. These results have been recently published, “Stoichiometry of Hg-thiol complexes on bacterial cell envelopes” B. Mishra, E. Shoenfelt, Q. Yu, N. Yee, J. B. Fein, and S.C.B. Myneni, Chemical Geology, 464 (2017) 137-146.

- **Complete MBR studies of U\(^{IV}\) reoxidation in redox-buffered systems:** set up oxidation reactors with reduced clays, perform x-ray measurements, analyze XAFS and chemical data, prepare manuscript (KG2.1). Boyanov/Latta/O’Loughlin/Mishra/Kemner

  We are continuing our investigations on the stability of U\(^{IV}\) phases towards reoxidation, which is an important question related to the mobility of U in subsurface environments. In previous studies, we compared the reoxidation kinetics of U\(^{IV}\) phosphate relative to that of uraninite, and we examined the ability of magnetite to delay or inhibit the reoxidation of U\(^{IV}\) in air-saturated solutions. Here, we are using reduced clay minerals with increasing Fe\(^{II}\) content (SYn-1, SWy-2, NAu-1, and NAu-2) to determine the ability of these environmentally-relevant minerals to serve as a competitive electron acceptor and thus protect U\(^{IV}\) from reoxidation. The clay purification protocols and the specific reduction procedures for this experiment have been developed, and the U\(^{IV}\) phases to be used in the experiments have been synthesized. A series of sacrificial reactors will be set up to determine the extent of U reoxidation over time. Reactor set up and synchrotron measurements are pending during an upcoming beamrun.

- **Initiate MBR studies of redox cycling of clays and sulfur species:** incubate Fe-rich clays with sulfide; characterize Fe/S speciation; cycle oxidation conditions and characterize the changes in Fe speciation (KG2.2). Boyanov/ O’Loughlin/Latta/Scherer/ Mishra/Kemner

  The interaction between Fe in clay minerals and sulfide species produced by bacterial metabolism is an important factor in defining the mineralogical and redox conditions in subsurface environments, which in turn affects the availability of nutrients and the mobility of contaminants. We are currently studying the reduction of Fe-bearing montmorillonite clay minerals (SWy-2, NAu-1, and NAu-2) by dissolved sulfide. The changes in speciation of solid-phase Fe in reactors with fixed amounts of clay (2 g clay L\(^{-1}\)) and increasing amounts of sulfide (0 – 20 mM HS\(^-\) at pH 7.2) were investigated using x-ray absorption spectroscopy (Fe K-edge XANES and EXAFS). Initial results show that sulfide is able to transfer electrons to the Fe atoms in SWy-2 clay, but the extent of Fe reduction is significantly less than the reduction capacity available from stoichiometric oxidation of the added sulfide to elemental sulfur. The reasons for this limitation in redox reactivity are unclear and currently being investigated in follow-up experiments. Despite the presence of both reduced Fe\(^{II}\) and sulfide in these systems, the x-ray spectroscopy data show no evidence of FeS formation even in cases of large excess of sulfide and significant extent (~80%) of Fe\(^{III}\) reduction in the clay. This behavior is unexpected in light of the extremely low solubility of FeS and suggests significant stability of reduced Fe\(^{II}\) in the structure of
montmorillonite clays. Follow-up experiments will be carried out to refine these results and to produce sufficient material for one or two manuscripts. A separate line of experiments is set up to investigate the reversibility of redox processes in Fe-containing clays. Suspensions of montmorillonite clay minerals (SWy-2, NAu-1) are being reduced and then oxidized a number of times to determine whether the extent of Fe reduction/oxidation becomes limited due to loss of Fe$^{II}$ from the clay structure and the formation of secondary minerals. Initial experiments utilized a combination of H$_2$ gas and Pd on alumina catalyst to carry out the reduction step in the suspension. However, this approach introduced artifacts in the oxidation step due to Pd detaching from the alumina pellet and remaining in the suspension. We are in the process of developing a new redox procedure for clays to remove this experimental artifact.

- **Conduct experiments examining the effects of electron shuttles on the microbial oxidation of Fe$^{II}$ in clays and microbial community development in systems inoculated with native microbial communities in soils/sediments (KG2.3 and KG4.1).**
  O'Loughlin/Boyanov/Flynn/Antonopoulos/Kemner

Fe$^{III}$ oxides are poorly soluble at the temperature and pH ranges typical of most critical zone environments, and consequently many Fe$^{III}$-reducing bacteria use soluble electron-shuttling compounds to aid the transfer of electrons to these extracellular electron acceptors. Pure culture studies suggest these electron shuttles enhance the overall rate of iron reduction, yet little is known about how the presence of these compounds affects the complex communities of microorganisms in soils and sediments under iron-reducing conditions. We examined the effect of riboflavin (RIBO) and 7 different quinone-based electron shuttles with redox potentials ranging from (-247 to 217 mV) on the rate of iron reduction, the onset of methanogenesis, and the trajectory of microbial community development in wetland sediment microcosms amended with goethite ($\alpha$-FeOOH) and either acetate or H$_2$. Unlike pure culture experiments, microcosms given 9,10-anthraquinone-2-carboxylic acid (AQC), 5,8-dihydroxy-1,4-naphthoquinone (DHNQ), 5-hydroxy-1,4-naphthoquinone (juglone, NQJ), or 2-hydroxy-1,4-naphthoquinone (lawsone, NQL) showed either no increase or a decrease in the rate of iron reduction relative to control experiments with no shuttle (NS) added. The rate and extent of iron reduction increased significantly however in those given 9,10-anthraquinone-2,6-disulfonate (AQDS), 9,10-anthraquinone-2-sulfonate (AQS), 3,4-dihydroxy-9,10-anthraquinone-2-sulfonate (alizarin red s (AQZRS)), and RIBO. Methanogenesis did not begin in AQDS, AQS, AQZRS, DHNQ, NQJ, NQL, RIBO, or NS microcosms until ferrous iron production ceased, and was inhibited entirely in AQC-amended microcosms. These AQC-amended microcosms also showed a significant divergence from the others in microbial community development. 16S rRNA sequences classified as Geobacteraceae dominated NS control microcosms as well as those amended with electron shuttles other than AQC, though distinct operational taxonomic units (OTUs) were abundant depending on the shuttle used. AQC microcosms were instead dominated by Pelobacteraceae (acetate-amended) or Shewanellaceae (H$_2$-amended). These results suggest both redox potential and electron donor determine in part the extent and mechanism of iron reduction as well as the identity of the bacteria carrying out this process. Moreover, the complete inhibition of methanogenesis by AQC highlights the possibility for electron shuttles to influence microbial processes in addition to those involving respiration with insoluble terminal electron acceptors. These results are being prepared for publication in *Applied and Environmental Microbiology.*

As with Fe$^{III}$ oxides, the presence of electron shuttles generally enhances both the rate and extent of the bioreduction of structural Fe$^{III}$ in clay minerals. However, the ability of shuttles to facilitate the
biooxidation of Fe\textsuperscript{II}-bearing clays and Fe\textsuperscript{II} minerals as well as their impact on microbial community structure and function are largely unknown. We have initiated experiments investigating the role of electron shuttles in these processes, focusing on benzo-, naphtho-, and anthraquinones as putative shuttles. These structurally similar compounds have midpoint potentials ranging from -250 to 700 mV, making them suitable as potential electron shuttles for Fe\textsuperscript{III}/Fe\textsuperscript{II} redox processes. Our initial experiments are focused on the enhancement of the oxidation of Fe\textsuperscript{II} in reduced NAu-1 nontronite by *Rhodopseudomonas palustris* as well as enhanced oxidation of NAu-1 by native Fe\textsuperscript{II}-oxidizing bacteria present in soil collected downstream of the discharge of Fe\textsuperscript{II}-bearing groundwater. We expect that these experiments will be concluded during the last quarter of PY2.

- **Investigate the reduction of Hg\textsuperscript{II} by biogenic Mn\textsuperscript{II} species; prepare manuscript (KG2.4).**
  O'Loughlin/Boyanov/Mishra/Kemner

The reduction of Fe\textsuperscript{III} oxides by metal-reducing bacteria (MRB) can result in the production of a suite of Fe\textsuperscript{II} species, including soluble Fe\textsuperscript{II} complexes and a host of mineral phases containing structural Fe\textsuperscript{II}. Most MRB can also use Mn\textsuperscript{IV}, Mn\textsuperscript{IV,III}, and Mn\textsuperscript{III} oxides as terminal electron acceptors for anaerobic respiration, resulting in the production of aqueous and solid-phase Mn\textsuperscript{II} species [e.g., rhodochrosite (MnCO\textsubscript{3})]. Although many biogenic Fe\textsuperscript{II} phases have been shown to be effective reductants for a range of contaminants, little is known regarding the reactivity of biogenic Mn\textsuperscript{II} species. We have synthesized a series of environmentally relevant Mn\textsuperscript{IV}, Mn\textsuperscript{IV,III}, and Mn\textsuperscript{III} oxides (pyrolusite (β-MnO\textsubscript{2}), vernadite (δ-MnO\textsubscript{2}), birnessite (e.g., Mg\textsubscript{0.29}Mn\textsuperscript{IV}1.42Mn\textsuperscript{III}0.58O\textsubscript{4}•1.7H\textsubscript{2}O), hausmannite (Mn\textsuperscript{III}Mn\textsuperscript{III}2O\textsubscript{4}), feitknechtit (β-MnOOH), and manganite (γ-MnOOH)) and initiated experiments examining their bioreduction by the model MRB *Shewanella putrefaciens* CN32. We will then assess the ability of the resulting Mn\textsuperscript{II} species to reduce Hg\textsuperscript{II}. We anticipate the completion of these experiments by the end of PY2.

- **Perform reactive transport simulations for F, S, and contaminant (U, Hg, Cr) dynamics in x ray accessible columns; initiate column experiments based on the conditions determined from the simulations; perform x-ray measurements to determine the speciation along the column; refine the reactive transport model as needed; iterate. Finish experiments from year 1; prepare manuscript. (KG3.1).**
  Boyanov, Liu, O’Loughlin, Mishra, Kemner

We are continuing to use flow-through columns with x-ray-transparent windows (developed during prior years of the Argonne SBR SFA) to characterize the coupled biological, chemical, and physical factors that influence subsurface biogeochemical processes and the transport of contaminants. In these columns, we monitor key parameters including Fe and S mineral transformations, as well as contaminant spatial distribution and speciation (e.g., adsorbed or precipitated species and their valence state) in heterogeneous regions of the sediments. During PY2 we have examined the transformations in columns constructed with layers of ferrihydrite-coated quartz sand, through which carbonate- or phosphate-containing reductant solutions were passed (1-2 mM reduced AQDS, 10 mM bicarbonate or 2 mM phosphate, 20 mM MOPS, pH 7.2). We observed *in situ* the gradual transformation of the ferrihydrite phase to magnetite with the carbonate-containing influent. Despite the relatively high carbonate content we did not observe the formation of siderite. The Fe-coated sand initially dissolved at the upstream side of the layer and re-precipitated downstream, but once the transformation to magnetite was complete the Fe-
rich regions were stable and remained in place. In similar experiments with a reducing, phosphate-containing influent, we observed in situ the transformation of the ferrihydrite phase to vivianite along the flow path, concurrent with the gradual discoloration of the layer. The x-ray fluorescence data allowed quantification of the Fe lost from the layer, which was limited to less than 30% of the original Fe content over the studied reaction period. The operation of the column is ongoing. The results above deviate from the expected outcome of these experiments. Unfortunately, we are no longer able to pursue a theoretical treatment of these new results. Our collaborative relationship with Dr. Chongxuan Liu for simulations of the spatiotemporal profiles of Fe and S species has ceased due to him leaving PNNL. With the limited resources in our SFA we are also not able to perform the reactive transport simulations. We still plan on submitting a manuscript detailing the experimental results. Now that the Fe dynamics are described experimentally in sufficient detail we are continuing with the addition of contaminants (Cr, Hg, U) by the end of PY2 and with the x-ray spectroscopy characterization of their spatio-temporal distribution and speciation.

- **Initiate column studies of U\textsuperscript{IV} remobilization under anoxic conditions:** compare columns with uraninite or non-uraninite starting U\textsuperscript{IV} phases, with or without ferric iron oxides present; explore the effect of carbonate and phosphate; characterize the spatiotemporal species profiles by using x-ray techniques; characterize the effluents (KG3.2). Boyanov, O'Loughlin, Latta/Scherer, Mishra, Kemner.

With the Fe spatio-temporal speciation profiles defined and with the experimental procedures in place from prior column work (see above), we are proceeding with the column studies of the mobility of reduced U phases in porous media under advective flow. The U\textsuperscript{IV} phases to be used in the experiments have been synthesized (uraninite UO\textsubscript{2}, and non-uraninite U\textsuperscript{IV}-phosphate). The solids will be mixed with clean or ferrihydrite-coated quartz sand in order to be used as the initial stationary phase in our x-ray accessible flow-through columns. To minimize waste, a series of small samples with varying U:solids ratios will be prepared first for an upcoming beamrun to determine the range for sufficient sensitivity in the column measurements and to test for the solubility of the U solids in the planned influent solutions. Following these control measurements, we will set up flow-through columns with uraninite and U\textsuperscript{IV}-phosphate amended sand (clean or ferrihydrite-coated). X-ray spectroscopy investigations of the U transformations and of the changes in spatio-temporal distribution of U are planned for later in the year.

**ii. Science Highlights**

Argonne SFA team members have given at least 35 presentations, including 14 invited talks and 21 contributed talks (see Appendix B). Contributed presentations included four international conferences or universities (Advanced Techniques in Actinide Spectroscopy, Pacific Northwest National Laboratory; University of Vienna; University of Newcastle; 8th Workshop on Speciation, Techniques, and Facilities for Radioactive Materials at Synchrotron Light Sources, University of Oxford). In addition to national scientific conferences, SFA scientific staff also presented invited talks at various universities and national laboratories, as well as invited talks for local community outreach opportunities.

Since our last SFA progress report (June 2016), 6 papers related to the SFA research efforts were accepted for publication (see Appendix A). Two additional manuscripts are presently in review. Specific Argonne SFA scientific highlights summarized from five of these publications are given below to illustrate some of the foci of the SFA. The names of authors who are members of the Argonne SBR SFA are listed in bold letters.

- **Surface area effects on the reduction of U\textsuperscript{VI} in the presence of synthetic montmorillonite.** Boyanov MI, Latta DE, Scherer MM, O’Loughlin EJ, Kemner KM (2017) Chemical Geology, Vol. 464, p. 110-
The redox transformations that affect the environmental mobility of metal or radionuclide contaminants take place in the presence of mineral or biological surfaces. Adsorption can alter the atomic coordination and the free energy of a dissolved ion and can thus change the occurrence, rate, or products of redox reactions relative to those expected in homogeneous solution. It is thus imperative to understand the interactions of contaminants with environmentally-relevant surfaces (e.g., metal oxides, clay minerals, bacteria, etc.) in order to be able to model contaminant transport and behavior. We have finished our investigations on the effect of SYn-1, a redox-inactive synthetic montmorillonite clay mineral, on the reduction of U\(^{VI}\) by dihydroanthraquinone-2,6 disulfonate (AH\(_2\)QDS), the reduced form of the soluble electron shuttle AQDS. We varied the surface:U ratio in a circumneutral bicarbonate solution and measured the valence and atomic coordination of U in the solids using X-ray absorption spectroscopy (XANES and EXAFS). The spectra show that U\(^{IV}\) was the predominant product both in the presence or absence of the clay mineral, indicating that adsorption of U\(^{VI}\) to clay mineral surface sites does not affect its ability to be reduced by AH\(_2\)QDS. In the absence of the clay mineral and at low-to-intermediate surface:U ratios the predominant U\(^{IV}\) product was nanoparticulate uraninite, UO\(_2\). At the highest surface:U ratio tested (100 g/L clay mineral and 50 μM U) we observe a decrease in the proportion of uraninite down to 50%, with the remaining U\(^{IV}\) present as adsorbed, non-uraninite species. The observed trend is similar to our previous findings with magnetite and rutile, where adsorbed U\(^{IV}\) were the predominant species below a specific surface coverage and U in excess of this coverage was precipitated as uraninite. However, the threshold coverage determined here for the SYn-1 clay mineral is 10-100x lower than that determined for magnetite and rutile, suggesting that clay mineral surfaces may be less important than metal oxides for stabilizing non-uraninite U\(^{IV}\) species in natural sediments. These results have significant implications for the development of the reactive framework in contaminant transport models by elucidating the important reactions preventing uraninite formation and resulting in the adsorbed U\(^{IV}\) species that are observed in natural sediments.

Transformation of zinc-concentrate in surface and subsurface environments: Implications for assessing zinc mobility/toxicity and choosing an optimal remediation strategy, Kwon MJ, Boyanov MI, Yang J-S, Lee S, Hwang YH, Lee JY, Mishra B, Kemner KM (2017) Environmental Pollution, Vol. 226, p. 346-355. Zinc contamination in near- and sub-surface environments is a serious threat to many ecosystems and to public health. Sufficient understanding of Zn speciation and transport mechanisms is therefore critical to evaluating its risk to the environment and to developing remediation strategies. The geochemical and mineralogical characteristics of contaminated soils in the vicinity of a Zn ore transportation route were thoroughly investigated using a variety of analytical techniques (sequential extraction, XRF, XRD, SEM, and XAFS). Zn-concentrate (in the form of ZnS) was deposited in a receiving facility and dispersed over time to the surrounding roadside areas and soils. Subsequent physical and chemical weathering resulted in dispersal into the subsurface. The species identified in the contaminated areas included Zn-sulfide, Zn-carbonate, other O-coordinated Zn-minerals, and Zn species bound to Fe/Mn oxides or clays, as confirmed by XAFS spectroscopy and sequential extraction. The observed transformation from S-coordinated Zn to O-coordinated Zn associated with minerals suggests that this contaminant can change into more soluble and labile forms as a result of weathering. Acid-extraction efficiency increased with the increase of O-coordinated Zn relative to S-coordinated Zn in the sediment. This study demonstrates that improved understanding of Zn speciation in contaminated soils is essential for well-informed decision making regarding metal mobility and toxicity, as well as for choosing an appropriate remediation strategy.
ammonio)bimane (qBBr) to block thiol sites, and potentiometric titrations of biomass with and without spectroscopy at the Hg L edge. The abundance of thiols on intact cells was determined by a fluorescence-spectroscopy based method using a soluble bromobimane, monobromo(trimethyl-ammonio)bimane (qBBr) to block thiol sites, and potentiometric titrations of biomass with and without qBBr treatment. The chemical forms of S on intact bacterial cells were determined using S k-edge XANES spectroscopy. Hg(II) was found to complex entirely with cell bound thiols at low Hg:biomass ratios. For *Bacillus subtilis* and *Shewanella oneidensis* MR-1 cells, the Hg-S stoichiometry changed from

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*bacterium, Orenia metallireducens* strain Z6, was isolated from briny groundwater extracted from a 2.02 km-deep borehole in the Illinois Basin, IL. This organism shared 96% 16S rRNA gene similarity with *Orenia marismortui* but demonstrated physiological properties previously unknown for this genus. In addition to exhibiting a fermentative metabolism typical of the genus *Orenia*, strain Z6 reduces various metal oxides [Fe(III), Mn(IV), Co(III), and Cr(VI)], using H2 as the electron donor. Strain Z6 actively reduced ferrihydrite over broad ranges of pH (6 to 9.6), salinity (0.4 to 3.5 M NaCl), and temperature (20 to 60°C). At pH 6.5, strain Z6 also reduced more crystalline iron oxides, such as lepidocrocite (γ-FeOOH), goethite (α-FeOOH), and hematite (α-Fe2O3). Analysis of X-ray absorption fine structure (XAFS) following Fe(III) reduction by strain Z6 revealed spectra from ferrous secondary mineral phases consistent with the precipitation of vivianite [Fe4(PO4)3] and siderite (FeCO3). The draft genome assembled for strain Z6 is 3.47 Mb in size and contains 3,269 protein-coding genes. Unlike the well-understood iron-reducing *Shewanella* and *Geobacter* species, this organism lacks the c-type cytochromes for typical Fe(III) reduction. Strain Z6 represents the first bacterial species in the genus *Orenia* (order Halanaerobiales) reported to reduce ferric iron minerals and other metal oxides. This microbe expands both the phylogenetic and physiological scopes of iron-reducing microorganisms known to inhabit the deep subsurface and suggests new mechanisms for microbial iron reduction. These distinctions from other *Orenia* spp. support the designation of strain Z6 as a new species, *Orenia metallireducens* sp. nov.

*Tepidibacillus decaturensis* sp. nov., a microaerophilic, moderately thermophilic iron-reducing bacterium isolated from 1.7 km depth groundwater. Dong Y, Sanford RA, Boyanov MI, Kenmer KM, Flynn TM, O’Loughlin EJ, Locke RA, Weber JR, Egan SM, Fouke BW (2016) *International Journal of Systematic and Evolutionary Microbiology*, Vol. 66(10), p. 3964–3971. A Gram-stain-negative, microaerophilic rod-shaped organism designated as strain Z9T was isolated from groundwater of 1.7 km depth from the Mt. Simon Sandstone of the Illinois Basin, Illinois, USA. Cells of strain Z9T were rod shaped with dimensions of 0.3×(1–10) μm and stained Gram-negative. Strain Z9T grew within the temperature range 20–60 °C (optimum at 30–40 °C), between pH 5 and 8 (optimum 5.2–5.8) and under salt concentrations of 1–5 % (w/v) NaCl (optimum 2.5 % NaCl). In addition to growth by fermentation and nitrate reduction, this strain was able to reduce Fe(III), Mn(IV), Co(III) and Cr(VI) when H2 or organic carbon was available as the electron donor, but did not actively reduce oxidized sulfur compounds (e.g. sulfate, thiosulfate or S8). The G+C content of the DNA from strain Z9T was 36.1 mol%. Phylogenetic analysis of the 16S rRNA gene from strain Z9T showed that it belongs to the class *Bacilli* and shares 97 % sequence similarity with the only currently characterized member of the genus *Tepidibacillus*, *T. fermentans*. Based on the physiological distinctness and phylogenetic information, strain Z9T represents a novel species within the genus *Tepidibacillus*, for which the name *Tepidibacillus decaturensis* sp. nov. is proposed.

Stoichiometry of Hg-thiol complexes on bacterial cell envelopes. Mishra B, Shoenfelt E, Yu Q, Yee N, Fein JB, and Myneni SCB (2017) *Chemical Geology*, Vol. 464, p. 137–146. We have examined the speciation of Hg(II) complexed with intact cell suspensions (1013 cells L–1) of *Bacillus subtilis*, a common gram-positive soil bacterium, *Shewanella oneidensis* MR-1, a facultative gram-negative aquatic organism, and *Geobacter sulfurreducens*, a gram-negative anaerobic bacterium capable of Hg-methylation at Hg(II) loadings spanning four orders of magnitude (120 nM to 350 μM) at pH 5.5 (± 0.2). The coordination environments of Hg on bacterial cells were analyzed using synchrotron based XANES and EXAFS spectroscopy at the Hg L3 edge. The abundance of thiols on intact cells was determined by a fluorescence-spectroscopy based method using a soluble bromobimane, monobromo(trimethyl-ammonio)bimane (qBBr) to block thiol sites, and potentiometric titrations of biomass with and without qBBr treatment. The chemical forms of S on intact bacterial cells were determined using S k-edge XANES spectroscopy. Hg(II) was found to complex entirely with cell bound thiols at low Hg:biomass ratios. For *Bacillus subtilis* and *Shewanella oneidensis* MR-1 cells, the Hg-S stoichiometry changed from
Hg-S₃ to Hg-S₂ and Hg-S (where ‘S’ represents a thiol site such as is present on cysteine) progressively as the Hg(II) loading increased on the cells. However, *Geobacter sulfurreducens* did not form Hg-S₃ complexes. Because the abundance of thiol was highest for *Geobacter sulfurreducens* (75 μM/g wet weight) followed by *Shewanella oneidensis* MR-1 (50 μM/g wet weight) and *Bacillus subtilis* (25 μM/g wet weight), the inability of Hg(II) to form Hg-S₃ complexes on *Geobacter sulfurreducens* suggests that the density and reactivity of S-amino acid containing cell membrane proteins on *Geobacter sulfurreducens* are different from those of *Bacillus subtilis* and *Shewanella oneidensis* MR-1. Upon saturation of the high affinity thiol sites at higher Hg:biomass ratios, Hg(II) was found to form a chelate with α-hydroxy carboxylate anion. The stoichiometry of cell envelope bound Hg-thiol complexes and the associated abundance of thiols on the cell envelopes provide important insights for understanding the differences in the rate and extent of uptake and redox transformations of Hg in the environment.

**b. Future scientific goals**

**FY2018**

- Investigate Hg⁰ oxidation by Mn⁰Ⅲ,Ⅳ oxides; prepare manuscript (KG1.5).
- Complete MBR studies of redox cycling of clays and sulfur species: incubate Fe-rich clays with sulfate and iron- or sulfate-reducing bacteria; characterize the changes in Fe/S speciation while cycling oxidation conditions; analyze data from years 2 and 3; prepare manuscript. (KG2.2).
- Investigate the reduction of U⁷⁺ by biogenic Mn⁰ species; prepare manuscript. (KG2.4).
- Complete experiments and x-ray measurements to determine the speciation of Fe, S, and contaminants along the column; refine reactive transport model. Prepare manuscript describing results from years 1-3 (KG3.1).
- Complete column studies of U⁷⁺ remobilization under oxic conditions; compare columns with uraninite and non-uraninite U⁷⁺ phases, explore the effects of carbonate and phosphate; characterize the spatiotemporal species profiles along the column by using x-ray techniques; characterize the effluents; prepare manuscript (KG3.2).
- Prepare manuscript on results of experiments examining the effects of electron shuttles on the microbial oxidation of Fe²⁺ in clays and microbial community development in systems inoculated with native microbial communities in soils/sediments (KG4.1).
- Expand bioinformatics to include analysis of whole-genome shotgun sequence data (KG4.2).
- Prepare manuscript on evolution of initially diverse microbial communities over the course of a biostimulation experiment with Fe³⁺, S, and electron donor (KG4.2).
- Expand the repertoire of redox conditions; characterize microbial community steady states via rRNA targeted surveys and metagenomic-enabled approaches (KG4.3).

**c. Scientific results that may shift current research focus and/or identify new knowledge gaps**

Results obtained to this point are on track with anticipated outcome and, in some cases, ahead of schedule.

We foresee new opportunities to apply synchrotron x-ray radiation produced at the APS to investigate the chemical and spatial characteristics of carbon within sediments and soils. Specifically, we have investigated the utility of non-resonant inelastic x-ray scattering (often referred to as x-ray Raman) for determining the chemical bonds associated with carbon atoms in soils. Results from the measurement of organic and inorganic standards, Alaskan Permafrost material, and soils from the Spruce Site indicate that this technique is very promising. We have also performed experiments at the APS to determine the feasibility of developing these capabilities at the MRCAT/EnviroCAT. Developing these capabilities at the
MRCAT/EnviroCAT will provide the necessary access to this technique that we will need to address biogeochemical studies related to carbon chemistry within soils and sediments. We are requesting support in FY2018 from DOE BER to establish these capabilities at the MRCAT/EnviroCAT insertion device beam line.

We have begun internal discussions at Argonne to determine potential new research directions for the Argonne SBR SFA. Specifically, we foresee opportunities to either align our molecular to core scale research with PNNL SFA (bringing additional U Biogeochemical research to the field site) and/or ORNL SFA field scale (bringing additional Hg Biogeochemical research to the field site) activities or to create a separate focus on wetland biogeochemistry. We plan to engage SBR Program Officers (Hirsch, Bayer, and Lesmes) as we further develop these concepts in the coming months.

d. Collaborative research activities with external researchers in pursuit of program objectives

Integration of scientific and technical expertise, plus communication among all of the researchers who are part of the Argonne Subsurface Biogeochemical Research (SBR) SFA, is critical for the success of the Argonne SBR SFA science program. Active participation by external collaborators with the Argonne staff provides complementary expertise essential to the productivity of the research program. The following activities between Argonne and external SFA researchers since June 2016 are worthy of note:

- Beginning in June 2016, a graduate student (Jackie Mejia) from the lab of Dr. Eric Roden at the University of Wisconsin, Madison is spent 7 months with members of the Argonne SBR SFA to integrate biogeochemical and ‘omics approaches into her research on Fe and C biogeochemistry.
- Although presently not funded by the Argonne SBR SFA, collaborative research efforts with Drew Latta and Michelle Scherer from the University of Iowa in the study of the interactions of U with clays is continuing. Dr. Latta has participated in the preparation of samples for characterization at the Advanced Photon Source.
- Dr. Kemner met with Dr. Scott Brooks (ORNL) at the SBR PI meeting in 2017 to discuss the use of x-ray microspectroscopic imaging to determine the spatial distribution of the Hg valence state in biofilms collected from Hg contaminated streams on the Oak Ridge site. Scott has stated that they will contact us when the samples are available that are appropriate for synchrotron measurements.
- Dr. Kemner met with Dr. Tim Scheibe (PNNL) to discuss participation in the yearly PNNL SBR SFA All Hands Meeting. We expect that some members of the Argonne SBR SFA will attend the PNNL All Hands Meeting to help determine future research directions for the Argonne SBR SFA.
- Dr. Rob Sanford (University of Illinois, Urbana-Champaign (UIUC) has been overseeing a researcher (Dr. Yiran Dong) at UIUC who is collaborating with the SFA team. She is testing the metabolic potential of deep subsurface metal-reducing bacteria isolated from groundwater collected from the Illinois Basin and their impact on the formation of secondary iron minerals. In addition to the two manuscripts that have been published since June 2016, we expect an additional manuscript to result from this work that will be submitted for publication.
- Dr. Man-Jae Kwon, a research scientist at the Korean Institute of Science and Technology (KIST) Gangneun, has continued an active no-cost collaboration with the Argonne SBR SFA. He works closely with Drs. O’Loughlin, Boyanov, and Kemner on projects of mutual interest involving Fe and S biogeochemistry.
- Drs. O’Loughlin, Boyanov, Mishra, and Kemner worked closely with Dr. Sen Yan, an Assistant Professor in the Department of Geochemistry at the China University of Geosciences-Wuhan. As a visiting scholar at Argonne in 2015, Dr. Yan worked with the Argonne SBR SFA team to investigate UVI interactions with biotic and abiotic green rusts and is currently working with the SFA team on a manuscript to be submitted in FY 2018.
- Dr. Renata Rodrigues (Universidade Federal de Alfenas), a professor at Universidade Federal in Brazil, will spend a year on sabbatical working in the Argonne Biogeochemistry labs to investigate effects of pH and sulfate concentration on heavy metal valence states in samples from bioreactor
experiments that simulate bioremediation treatment procedures. Biostimulation via ethanol additions and investigations of methane production and consumption will also be part of these studies. Members of the Argonne SBR SFA team will work closely with Dr. Rodrigues and make optimal use of synergies between her work and the Argonne SBR SFA efforts.

The role each external collaborator plays in the ANL SFA is shown below. Their contributions to specific milestones within the Argonne SBR SFA since June 2016 were noted in the “Progress on Milestones” discussion above.

Maxim Boyanov: Physicist (stationed at the Bulgarian Academy of Science); Experimental design and synchrotron data collection and analysis related to biogeochemical transformations of S, Fe, and U.

Bhoopesh Mishra: Physicist (Illinois Institute of Technology and University of Leeds); Since the early part of FY 2017 Dr. Mishra was no longer a local Argonne member of the Argonne SBR SFA; synchrotron data collection and analysis related to abiotic transformations of Fe, Mn, S, and Hg and development of x-ray Raman capabilities for measurement of carbon chemistry in soils.

Tim Scheibe: Modeler (PNNL); Consult on experimental design for material from the Columbia River-groundwater interface.

Scott Brooks: Environmental Engineer (ORNL); Collaborate on experimental design related to spatial distribution of Hg chemistry within biofilms.

Carlo Segre: MRCAT/EnviroCAT Associate Director (Illinois Institute of Technology); Operates MRCAT/EnviroCAT at the Advanced Photon Source.

Rob Sanford: Microbiologist (University of Illinois, Urbana-Champaign); Test the metabolic potential of deep subsurface metal-reducing bacteria isolated from groundwater collected from the Illinois Basin and their impact on the formation of secondary iron minerals.
5) Staffing and budget summary

a/b. Allocation by program tasks and to support external collaborations

The allocation of FY2017 funds is shown below.

<table>
<thead>
<tr>
<th>Institution</th>
<th>FY2017 Funding ($K)</th>
<th>Status FY2018 ($K)</th>
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<tbody>
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<td>Kemner</td>
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<tr>
<td>Antonopoulos</td>
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<td>University of Illinois</td>
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<tr>
<td>Boyanov (Bulgarian Academy of Science)</td>
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| Illinois Institute of Technology | 150                 | Active (100)^

The subcontract to IIT will be decreased to $100K to cover beam line support at a reduced level relative to FY 2017. Dr. B. Mishra’s efforts are longer supported.

c. Personnel actions and procedures

Argonne SBR SFA staffing changes that occurred or will occur in FY2017 include the following: Dr. Bhoopesh Mishra primarily is stationed at the University of Leeds instead of the Illinois Institute of Technology and will no longer be supported by Argonne SBR SFA programmatic funds. Although not supported financially in FY2018, it is expected that Dr. Mishra will finish development and submission of manuscripts related to electron transfer reactions between Hg and Mn. We have investigated the potential to replace Dr. Mishra’s efforts. However, with the funding originally provided for Dr. Mishra’s efforts now available for other efforts, we can only partially support a postdoc to continue that line of research. In light of that, we are increasing the level of support for Dr. O’Loughlin so that he can continue some of the efforts originally planned for Dr. Mishra.

Argonne encourages new/young investigators to pursue innovative research ideas through LDRD support as well as proposals to funding agencies. Dr. Flynn (previous Argonne Director’s Fellow) has been supported by Argonne LDRD funds to support his efforts after the expiration of his Director’s Fellowship at the end of September 2013. Our new and young investigators are also provided with the opportunity to present their work at local, national, and international scientific conferences. Dr. Flynn’s effort on the Argonne SBR SFA has been supported at no cost to the SFA. He presented the results of his work at the 24th International Goldschmidt Conference during the summer of FY2014 and at many other conferences since then (see appendix). The Laboratory views attendance and presentations at such meetings as providing both continuing education and networking opportunities to these staff members, which strengthens the SFA effort.

d. National Laboratory investment in the program

Argonne’s Director’s Post-doctoral Fellowship program provided support for Dr. Flynn’s position in FY2012 and FY2013. This program is a highly competitive national program (http://www.dep.anl.gov/postdocs/directorpostdoc.htm) that requires an Argonne sponsor who will work together with the candidate to propose a project as the justification for the requested position. Dr. Kemner recruited Dr. Flynn from the University of Illinois and, together, they produced a successful proposal that
was also relevant to topics being addressed by the Argonne SBR SFA. Joint Argonne and University of Chicago funds were used to support Dr. Flynn in FY2014 and FY2015. Since the beginning of FY2016, Dr. Flynn has been supported as an Argonne Strategic Hire with LDRD funds. The LDRD support for Dr. Flynn will end in the later part of FY 2018.

Argonne provided LDRD funds (~$225K/year) in FY2013-FY2015 to a project entitled “Identifying novel pathways for anaerobic microbial oxidation of methane to carbon dioxide” that included support for Dr. Ed O’Loughlin (Lead PI), Dr. Dion Antonopoulos, and Dr. Kemner. Argonne provided $50K of support in FY2016 to a project entitled “Developing Remote Automated Sensors to Direct Sampling of Aerobic-Anaerobic Switching in Floodplain Ecosystems” that included support for Dr. O’Loughlin. Argonne also provided $85K/year of LDRD support in FY2013-2015 to a project entitled “Imaging “ecological engineers”: a novel quantum dots approach to map microbes in complex soil structures with x-rays” that included support for Drs. Kemner and O’Loughlin. This project sought to develop x-ray tomographic methods to image the three-dimensional distribution of microbial cells and their metabolic state in microenvironments within opaque soil aggregates and is expected to be helpful for research related to the biogeochemical cycling of carbon as well as iron and sulfur. This work is now supported by DOE BER BSSD under the Argonne Small Worlds Project.

Argonne provided LDRD funds ($300K) in FY2014 to Dr. Kemner to investigate the feasibility of developing a scanning transmission x-ray microscope at a bending magnet beamline at the APS to image the spatial heterogeneities of carbon with ~50 nm spatial resolution within soils and sediments. Argonne is presently providing LDRD funds ($250K) in FY 2017 to Drs. Kemner and O’Loughlin on a project entitled “Integrating Atmospheric, Ecological, and Biogeochemical Monitoring in Wetlands.” Work on this project is enabling Drs. O’Loughlin and Kemner to explore potential opportunities enabled by changing some of the focus of the Argonne SBR SFA to the investigation of wetlands.

**Capital Equipment needs (FY2018)**

$260K is requested for the additional beam line components and effort required to develop an x-ray Raman spectrometer at the MRCAT/EnviroCAT insertion device beam line (sector 10) at the APS for investigations of carbon chemistries in bulk environmental samples. Besides providing a unique capability for the Argonne SBR SFA and the research community in general, this spectrometer will also enable higher throughput of x-ray Raman measurements for a project recently funded by DOE BER TES entitled “Resolving conflicting physical and biochemical feedbacks to climate in response to long-term warming” that includes the efforts of two of the members of the Argonne SBR SFA (Drs. Mishra and Kemner).
Appendix A. Manuscripts published, in press, or in review, since June 2016

(Authors in bold are Argonne SFA members)

Peer-reviewed Publications:


Manuscripts in Review:


Appendix B. Scientific Presentations since June 2016

Invited Presentations: (Presenting author is underlined)


5. **Mishra B** “Towards a molecular scale mechanistic understanding of mercury-microbe-mineral interactions”, Department Seminar, Department of Environmental Geosciences, University of Vienna, Austria. March 2017.


Contributed Presentations: (Presenting author is underlined)


