FINAL REPORT

Subsurface Uranium Fate and Transport: Integrated Experiments and Modeling of Coupled Biogeochemical Mechanisms of Nanocrystalline Uraninite Oxidation by Fe(III)-(hydr)oxides

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A Collaborative Research Project between Montana State University, University of California Davis, South Dakota School of Mines and Technology, and the Lawrence Berkeley National Engineering Laboratory

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Final Report - Coupled Biogeochemical Mechanisms of Nanocrystalline Uraninite
Oxidation by Fe(III)-(hydr)oxides

1. Executive Summary

Subsurface bacteria including sulfate reducing bacteria (SRB) reduce soluble U(VI) to insoluble U(IV) with subsequent precipitation of UO_2. We have shown that SRB reduce U(VI) to nanometer-sized UO_2 particles (1-5 nm) which are both intra- and extracellular, with UO_2 inside the cell likely physically shielded from subsequent oxidation processes. We evaluated the UO_2 nanoparticles produced by *Desulfovibrio desulfuricans* G20 under growth and non-growth conditions in the presence of lactate or pyruvate and sulfate, thiosulfate, or fumarate, using ultrafiltration and HR-TEM. Results showed that a significant mass fraction of bioreduced U (35-60%) existed as a mobile phase when the initial concentration of U(VI) was 160 µM. Further experiments with different initial U(VI) concentrations (25 - 900 µM) in MTM with PIPES or bicarbonate buffers indicated that aggregation of uraninite depended on the initial concentrations of U(VI) and type of buffer.

It is known that under some conditions SRB-mediated UO_2 nanocrystals can be reoxidized (and thus remobilized) by Fe(III)-(hydr)oxides, common constituents of soils and sediments. To elucidate the mechanism of UO_2 reoxidation by Fe(III) (hydr)oxides, we studied the impact of Fe and U chelating compounds (citrate, NTA, and EDTA) on reoxidation rates. Experiments were conducted in anaerobic batch systems in PIPES buffer. Results showed EDTA significantly accelerated UO_2 reoxidation with an initial rate of 9.5µM day^{-1} for ferrihydrite. In all cases, bicarbonate increased the rate and extent of UO_2 reoxidation with ferrihydrite. The highest rate of UO_2 reoxidation occurred when the chelator promoted UO_2 *and* Fe(III) (hydr)oxide dissolution as demonstrated with EDTA. When UO_2 dissolution did not occur, UO_2 reoxidation likely proceeded through an aqueous Fe(III) intermediate as observed for both NTA and citrate. To complement to these laboratory studies, we collected U-bearing samples from a surface seep at the Rifle field site and have measured elevated U concentrations in oxic iron-rich sediments.

To translate experimental results into numerical analysis of U fate and transport, a reaction network was developed based on Sani et al. (2004) to simulate U(VI) bioreduction with concomitant UO_2 reoxidation in the presence of hematite or ferrihydrite. The reduction phase considers SRB reduction (using lactate) with the reductive dissolution of Fe(III) solids, which is set to be microbially mediated as well as abiotically driven by sulfide. Model results show the oxidation of HS^- by Fe(III) directly competes with UO_2 reoxidation as Fe(III) oxidizes HS^- preferentially over UO_2. The majority of Fe reduction is predicted to be abiotic, with ferrihydrite becoming fully consumed by reaction with sulfide. Predicted total dissolved carbonate concentrations from the degradation of lactate are elevated ($\log(p_{CO2}) \sim -1$) and, in the hematite system, yield close to two orders-of-magnitude higher U(VI) concentrations than under initial carbonate concentrations of 3 mM. Modeling of U(VI) bioreduction with concomitant reoxidation of UO_2 in the presence of ferrihydrite was also extended to a two-dimensional field-scale groundwater flow and biogeochemically reactive transport model for the South Oyster site in eastern Virginia. This model was developed to simulate the field-scale immobilization and subsequent reoxidation of U by a biologically mediated reaction network.

2.0 Background

Project Objectives: U is a priority pollutant for the DOE. *In situ* immobilization of U
may be an attractive option at many sites for removal of U from the aqueous phase
because of low cost and minimal secondary waste generation. Understanding the stability
of reduced U is critical to the integration of biological U immobilization in the
remediation decision-making process at DOE sites. The goal of the hypothesis-driven
research was to improve the overall understanding of U mobility in the subsurface by
focusing on coupled biogeochemical mechanisms of oxidation (and thus remobilization)
of reduced U by Fe(III)-(hydr)oxides.

Project Description: The first task was targeted at understanding the fundamental
reactive nature of reduced nanocrystalline U and quantifying the coupled biogeochemical
mechanisms that lead to oxidation by Fe(III)-(hydr)oxides. Task 2 focused on
characterizing the resulting form, stability, and distribution of the Fe and U mineral
phases before, during, and after the oxidation of nanocrystalline uraninite. Task 3
developed a mechanistic numerical biogeochemical model to integrate experimental data
to allow scaling and predictions of biological and geological interactions between Fe and
U in natural subsurface environments.

Potential Impact: Over 130 sites in 31 states, under the jurisdiction of the U.S.
Department of Energy (DOE), comprise over 2 million acres of total contaminated area
and approximately 6.4 billion m^3 of contaminated media. Responsibility for management,
remediation or monitored natural attenuation, and long-term stewardship of these sites
falls on the DOE. The integrated tasks were targeted at understanding the fundamental
reactive nature of reduced nanocrystalline U and quantifying the coupled biogeochemical
mechanisms that lead to oxidation by Fe(III)-(hydr)oxides. By characterizing the
resulting Fe and U mineral phases before, during and after the oxidation of
nanocrystalline uraninite, experimental results obtained in Tasks 1 and 2 can be better
integrated into the development of a mechanistic numerical biogeochemical models. This
model can be used for scaling and predictions of biological and geological interactions
between Fe and U in natural subsurface environments. Understanding the stability of
reduced U is critical to remediation decision-making processes at DOE sites.

The results of the project have been published in a number of peer reviewed journal
articles. The abstracts and citations to those articles, given in section 3.0 below, make up
the bulk of our final report.

3.0 Publications

3.1 Publication 1

3.1.1. Citation:
Sani, R.K., B.M. Peyton, and A.Dohnalkova. Comparison of uranium(VI) removal by
Shewanella oneidensis MR-1 in flow and batch reactors. *Water Research*, 42(12): 2993-
3002, 2008.

3.1.2. Abstract:
To better understand the interactions among metal contaminants, nutrients, and
microorganisms in subsurface fracture-flow systems, biofilms of pure culture of
Shewanella oneidensis MR-1 were grown in six fracture-flow reactors (FFRs) of different
geometries. The spatial and temporal distribution of uranium and bacteria were examined
using a tracer dye (brilliant blue FCF) and microscopy. The results showed that plugging
by bacterial cells was dependent on the geometry of the reactor and that biofilms grown
in FFRs had a limited U(VI)-reduction capacity. To quantify the U(VI)-reduction
capacity of biofilms, batch experiments for U(VI) reduction were performed with
repetitive U(VI) additions. U(VI)-reduction rates of stationary phase cultures decreased
after each U(VI) addition. After the fourth U(VI) addition, stationary phase cultures
treated with U(VI) with and without spent medium yielded gray and black precipitates,
respectively. These gray and black U precipitates were analyzed using high-resolution
transmission electron microscopy, energy-dispersive X-ray spectroscopy, and X-ray
diffraction. Data for randomly selected areas of black precipitates showed that reduced U
particles (3–6 nm) were crystalline, whereas gray precipitates were a mixture of
crystalline and amorphous solids. Results obtained in this study, including a dramatic
limitation of *S. oneidensis* MR-1 and its biofilms to reduce U(VI) and plugging of FFRs,
suggest that alternative organisms should be targeted for stimulation for metal
immobilization in subsurface fracture-flow systems.

3.1.3. Summary of Major Results:
- Fracture plugging by bacterial cells was dependent on the geometry of the reactor
  and fracture system.
- Biofilms grown in FFRs had a limited U(VI)-reduction capacity, and U(VI)-
  reduction rates of stationary phase cultures decreased after each U(VI) addition.

3.2 Publication 2

3.2.1. Citation:
Sengor, SS, Barua S, Gikas P, Ginn TR, Peyton BM, Sani R, Spycher N., Influence of Heavy Metals on Microbial Growth Kinetics Including Lag Time: Mathematical Modeling and Experimental Verification, *Environmental Toxicology and Chemistry*, 28(10):2020-2029, 2009.

3.2.2. Abstract:
Heavy metals can significantly affect the kinetics of substrate biodegradation and microbial growth, including lag times and specific growth rates. A model to describe microbial metabolic lag as a function of the history of substrate concentration has been previously described by Wood et al. (Water Resour Res 31:553-563) and Ginn (Water Resour Res 35:1395-1408). In the present study, this model is extended by including the effect of heavy metals on metabolic lag by developing an inhibitor-dependent functional to account for the metabolic state of the microorganisms. The concentration of the inhibiting metal is explicitly incorporated into the functional. The validity of the model is tested against experimental data on the effects of zinc on Pseudomonas species isolated from Lake Coeur d'Alene sediments, Idaho, USA, as well as the effects of nickel or cobalt on a mixed microbial culture collected from the aeration tank of a wastewater treatment plant in Athens, Greece. The simulations demonstrate the ability to incorporate the effect of metals on metabolism through lag, yield coefficient, and specific growth rates. The model includes growth limitation due to insufficient transfer of oxygen into the growth medium.

3.2.3. Summary of Major Results:
- A heavy metal toxicity model is extended by including the effect of heavy metals on metabolic lag, yield coefficient, and specific growth rates.
- An inhibitor-dependent function was used to account for the metabolic state of the microorganisms including the culture history.

3.3 Publication 3

3.3.1. Citation:
Ginn, T.R., Generalization of the Multi-rate Basis for Time-Convolution to Unequal
Forward and Reverse Rates and Connection to Reactions with Memory, *Water Resources
Research*, 45, Article Number: W12419, 2009.

3.3.2. Abstract:

The convolution form used to express mass transfer between mobile and immobile
aqueous domains, and often associated with multirate mass transfer representations of
matrix diffusion processes, is generalized to the case of unequal forward and reverse rates
for each of the multiple rates of the multirate mass transfer and is shown to represent also
linear but non-Markovian reactions that kinetically partition mass between mobile and
immobile phases with rate of return to mobile phase dependent on contiguous time spent
in the immobile phase. In the case where a multirate model refers to multiple sites with
distributed mobilization or release rates but single-valued immobilization rate, an
equivalent formulation is found using single-site mobilization-immobilization with non-
Markovian mobilization rate dependent on contiguous time spent immobilized.

3.3.3. Summary of Major Results:
- Establishes that a multirate mass transfer characterized by an immobilization rate
  that is single valued and by a remobilization rate α arbitrarily distributed
  according to a density function $b(\alpha)$, has an equivalent representation as a single-
  rate immobilization-remobilization model.
- The remobilization rate is the ratio of the Laplace transform of $\alpha b(\alpha)$ to the
  Laplace transform of $b(\alpha)$.

3.4 Publication 4

3.4.1. Citation:
Sani, RK, Rastogi G, Moberly JG, Dohnalkova A, Ginn TR, Spycher N, Shende RV, and   Peyton BM. The toxicity of lead to *Desulfovibrio desulfuricans* G20 in the presence of goethite and quartz. J. Basic Microbiol., 50: 1–11, 2010

*3.4.2. Abstract***:**
An aqueous mixture of goethite, quartz, and lead chloride ($PbCl_2$) was treated with the sulfate-reducing bacterium, *Desulfovibrio desulfuricans* G20 (*D. desulfuricans* G20), in a medium specifically designed to assess metal toxicity. In the presence of 26 μM of soluble Pb, together with the goethite and quartz, *D. desulfuricans* G20 grew after a lag time of 5 days compared to 2 days in Pb-, goethite-, and quartz-free treatments. In the absence of goethite and quartz, however, with 26 μM soluble Pb, no measurable growth was observed. Results showed that *D. desulfuricans* G20 first removed Pb from solutions then growth began resulting in black precipitates of Pb and iron sulfides. Transmission electron microscopic analyses of thin sections of *D. desulfuricans* G20 treated with 10 μM $PbCl_2$ in goethite- and quartz-free treatment showed the presence of a dense deposit of lead sulfide precipitates both in the periplasm and cytoplasm. However, thin sections of *D. desulfuricans* G20 treated with goethite, quartz, and $PbCl_2$ (26 μM soluble Pb) showed the presence of a dense deposit of iron sulfide precipitates both in the periplasm and cytoplasm. Energy-dispersive X-ray spectroscopy, selected area electron diffraction patterns, or X-ray diffraction analyses confirmed the structure of precipitated Pb inside the cell as galena (PbS) in goethite- and quartz-free treatments, and iron sulfides in treatments with goethite, quartz, and $PbCl_2$. Overall results suggest that even at the same soluble Pb concentration (26 μM), in the presence of goethite and quartz, apparent Pb toxicity to *D. desulfuricans* G20 decreased significantly. Further, accumulation of lead/iron sulfides inside *D. desulfuricans* G20 cells depended on the presence of goethite and quartz.

*3.4.3. Summary of Major Results***:**
- Even at the same soluble Pb concentration (26 μM), in the presence of goethite and quartz, apparent Pb toxicity to *D. desulfuricans* G20 decreased significantly, indicating that lead toxicity *in situ,* might be significantly lower than that measured in aqueous systems.
- Accumulation of lead/iron sulfides *inside D. desulfuricans* G20 cells depended on the presence of goethite and quartz.

3.5 Publication 5

3.5.1. Citation:
Massoudieh, A., Bombardelli, FA, Ginn, TR, A biogeochemical model of contaminant
fate and transport in river waters and Sediments, *J Contaminant Hydrology*, 112(1-
4),103-117, 2010.

3.5.2. Abstract:
A quasi-two-dimensional model is presented for simulating transport and transformation
of contaminant species in river waters and sediments, taking into account the effect of
both biotic and abiotic geochemical reactions on the contaminant fate and mobility. The
model considers the downstream transport of dissolved and sediment-associated species,
and the mass transfer with bed sediments due to erosion and resuspension, using linked
advection–dispersion–reaction equations. The model also couples both equations to the
reactive transport within bed sediment phases. This is done by the use of a set of vertical
one-dimensional columns representing sediment layers that take into account the reactive
transport of chemicals, burial, sorption/desorption to/from the solid phase, and the
diffusive transport of aqueous species. Kinetically-controlled reversible solid-water mass
exchange models are adopted to simulate interactions between suspended sediments and
bulk water, as well as the mass exchange between bed sediments and pore water. An
innovative multi-time step approach is used to model the fully kinetic nonlinear reaction
terms using a non-iterative explicit method. This approach enables the model to handle
fast and near-equilibrium reactions without a significant increase in computational
burden. At the end, two demonstration cases are simulated using the model, including
transport of a sorbing, non-reactive trace metal and nitrogen cycling, both in the Colusa
Basin Drain in the Central Valley of California.

3.5.3. Summary of Major Results:

- This model takes into account the effect of both biotic and abiotic geochemical
  reactions on the contaminant fate and mobility, considering the downstream
  transport of dissolved and sediment-associated species.
- The mass transfer with bed sediments due to erosion and resuspension is
  accounted for using linked advection–dispersion–reaction equations.
- This approach enables the model to handle fast and near-equilibrium reactions
  without a significant increase in computational burden.

3.6 Publication 6

3.6.1. Citation:
Rastogi, G, S Osman, PA Vaishampayan, GL Andersen, LD Stetler, RK Sani. Microbial Diversity in Uranium Mining-Impacted Soils as Revealed by High-Density 16S Microarray and Clone Library. Microbial Ecology, 59(1), p. 94-108

3.6.2. Abstract:
Microbial diversity was characterized in mining-impacted soils collected from two abandoned uranium mine sites, the Edgemont and the North Cave Hills, South Dakota, using a high-density 16S microarray (PhyloChip) and clone libraries. Characterization of the elemental compositions of soils by X-ray fluorescence spectroscopy revealed higher metal contamination including uranium at the Edgemont than at the North Cave Hills mine site. Microarray data demonstrated extensive phylogenetic diversity in soils and confirmed nearly all clone-detected taxonomic levels. Additionally, the microarray exhibited greater diversity than clone libraries at each taxonomic level at both the mine sites. Interestingly, the PhyloChip detected the largest number of taxa in *Proteobacteria* phylum for both the mine sites. However, clone libraries detected *Acidobacteria* and *Bacteroidetes* as the most numerically abundant phyla in the Edgemont and North Cave Hills mine sites, respectively. Several 16S rDNA signatures found in both the microarrays and clone libraries displayed sequence similarities with yet-uncultured bacteria representing a hitherto unidentified diversity. Results from this study demonstrated that highly diverse microbial populations were present in these uranium mine sites. Diversity indices indicated that microbial communities at the North Cave Hills mine site were much more diverse than those at the Edgemont mine site.

3.6.3. Summary of Major Results:
- Microarray data demonstrated extensive phylogenetic diversity in soils and confirmed nearly all clone-detected taxonomic levels, and the microarray exhibited greater diversity than clone libraries at each taxonomic level at both mine sites.
- Clone libraries detected Acidobacteria and Bacteroidetes as the most numerically abundant phyla in the mine sites, but demonstrate that highly diverse microbial populations were present in these uranium mine sites.

3.7 Publication 7

3.7.1. Citation:
Spycher, NF, M Issarangkun, BD Stewart, SS Sengor, E Belding, TR Ginn, BM. Peyton, RK Sani. Biogenic uraninite precipitation and its reoxidation by iron(III) (hydr)oxides: A reaction modeling approach to reoxidation by iron(III) (hydr)oxides. *Geochim. Cosmochim. Acta,* 75(16):4426 – 4440, 2011, DOI:10.1016/j.gca.2011.05.008, 2011.

3.7.2. Abstract:
One option for immobilizing uranium present in subsurface contaminated groundwater is *in situ* bioremediation, whereby dissimilatory metal-reducing bacteria and/or sulfate-reducing bacteria are stimulated to catalyze the reduction of soluble U(VI) and precipitate it as uraninite (UO_2). This is typically accomplished by amending groundwater with an organic electron donor. It has been shown, however, that once the electron donor is entirely consumed, Fe(III) (hydr)oxides can reoxidize biogenically produced UO_2, thus potentially impeding cleanup efforts. On the basis of published experiments showing that such reoxidation takes place even under highly reducing conditions (e.g., sulfate-reducing conditions), thermodynamic and kinetic constraints affecting this reoxidation are examined using multicomponent biogeochemical simulations, with particular focus on the role of sulfide and Fe(II) in solution. The solubility of UO_2 and Fe(III) (hydr)oxides are presented, and the effect of nanoscale particle size on stability is discussed. Thermodynamically, sulfide is preferentially oxidized by Fe(III) (hydr)oxides, compared to biogenic UO_2, and for this reason the relative rates of sulfide and UO_2 oxidation play a key role on whether or not UO_2 reoxidizes. The amount of Fe(II) in solution is another important factor, with the precipitation of Fe(II) minerals lowering the Fe^{+2} activity in solution and increasing the potential for both sulfide and UO_2 reoxidation. The greater (and unintuitive) UO_2 reoxidation by hematite compared to ferrihydrite previously reported in some experiments can be explained by the exhaustion of this mineral from reaction with sulfide. Simulations also confirm previous studies suggesting that carbonate produced by the degradation of organic electron donors used for bioreduction may significantly increase the potential for UO_2 reoxidation through formation of uranyl carbonate aqueous complexes.

3.7.3. Summary of Major Results:
- The greater (and unintuitive) UO_2 reoxidation by hematite compared to ferrihydrite can be explained by the exhaustion of hematite from reaction with sulfide.
- Simulations confirm previous studies suggesting that microbial carbonate produced by the degradation of organic electron donors may significantly increase the potential for UO_2 reoxidation through formation of uranyl carbonate aqueous complexes.

3.8 Publication 8

3.8.1. Citation:
Sengör, S.S., Gikas, P., Moberly, J., Peyton, B., Ginn, T.R., Comparison of single and joint effects of Zn and Cu in continuous flow and batch reactors, *Journal of Chemical Technology & Biotechnology*, 87(3):374-330, 10.1002/jctb.2730, 2012.

*3.8.2. Abstract***:**
Microbial behavior in batch reactors may be different from that in continuous flow reactors, which is expected to affect microbial response to heavy metal exposure. Four parallel continuous flow reactors and batch growth tests were used to investigate the single and joint toxicity of Zn and Cu to *Arthrobacter* sp. JM018. The results indicated that Cu is more toxic than Zn under all conditions. In the batch reactors, all Zn concentrations showed a stimulatory effect on microbial growth. However in the continuous system, 125 μmol L^{-1} Zn exposure produced inhibition. In the case of mixed Zn and Cu exposures in the batch system, the presence of Zn reduced the severity of Cu inhibition, with a net impact of reduced growth in all cases, whereas in the continuous system microbial growth and substrate utilization rates sharply decreased and ceased. The results clearly showed that growth in batch reactors underestimated significantly the heavy metal inhibition, compared with the continuous system. Therefore, the results of batch reactor tests should not be used directly when heavy metal inhibition is to be interpreted for continuous flow systems.

*3.8.3. Summary of Major Results***:**
- Cu was more toxic than Zn under all conditions, however in the continuous system, 125 μmol L^{-1} Zn exposure produced inhibition.
- Results clearly showed that growth in batch reactors underestimated significantly the heavy metal inhibition, compared with the continuous system.
- Results of batch reactor tests should not be used directly when heavy metal inhibition is to be interpreted for continuous flow systems.

3.9 Publication 9

3.9.1. Citation:
Vogt, S, B Stewart, J Seymour, BM Peyton, S Codd. "Detection of Biological Uranium Reduction using Magnetic Resonance". *Biotechnol. Bioeng.*, 109(4):877-883   DOI: 10.1002/bit.24369, 2012.

3.9.2. Abstract:
The conversion of soluble uranyl ions (UO_2^{2+}) by bacterial reduction to sparingly soluble uraninite ($UO_{2(s)}$) is being studied as a way of immobilizing subsurface uranium contamination. Under anaerobic conditions, several known types of bacteria including iron and sulfate reducing bacteria have been shown to reduce U (VI) to U (IV). Experiments using a suspension of uraninite ($UO_{2(s)}$) particles produced by *Shewanella putrefaciens* CN32 bacteria show a dependence of both longitudinal (T_1) and transverse (T_2) magnetic resonance (MR) relaxation times on the oxidation state and solubility of the uranium. Gradient echo and spin echo MR images were compared to quantify the effect caused by the magnetic field fluctuations (T_2^*) of the uraninite particles and soluble uranyl ions. Since the precipitate studied was suspended in liquid water, the effects of concentration and particle aggregation were explored. A suspension of uraninite particles was injected into a polysaccharide gel, which simulates the precipitation environment of uraninite in the extracellular biofilm matrix. A reduction in the T_2 of the gel surrounding the particles was observed. Tests done in situ using three bioreactors under different mixing conditions, continuously stirred, intermittently stirred, and not stirred, showed a quantifiable T_2 magnetic relaxation effect over the extent of the reaction.

3.9.3. Summary of Major Results:
- A suspension of uraninite ($UO_{2(s)}$) particles produced by *Shewanella putrefaciens* CN32 bacteria show a dependence of both longitudinal (T_1) and transverse (T_2) magnetic resonance (MR) relaxation times on the oxidation state and solubility of the uranium.
- Three bioreactors under different mixing conditions, continuously stirred, intermittently stirred, and not stirred, showed a quantifiable T_2 magnetic relaxation effect over the extent of the reaction.

3.10 Publication 10

3.10.1. Citation:

Stewart, BD, C Girardot, N Spycher, RK Sani, BM Peyton. Influence of Chelating Agents on Biogenic Uraninite Reoxidation by Fe(III) (Hydr)oxides. *Environ. Sci. Technol.*, 47(1) p. 364-371   DOI: 10.1021/es303022p, 2013.

3.10.2. Abstract:

Microbially mediated reduction of soluble U(VI) to U(IV) with subsequent precipitation of uraninite, $UO_{2(S)}$, has been proposed as a method for limiting uranium (U) migration. However, microbially reduced UO_2 may be susceptible to reoxidation by environmental factors, with Fe(III) (hydr)oxides playing a significant role. Little is known about the role that organic compounds such as Fe(III) chelators play in the stability of reduced U. Here, we investigate the impact of citrate, DFB, EDTA, and NTA on biogenic UO_2 reoxidation with ferrihydrite, goethite, and hematite. Experiments were conducted in anaerobic batch systems in PIPES buffer (10 mM, pH 7) with bicarbonate for approximately 80 days. Results showed EDTA accelerated UO_2 reoxidation the most at an initial rate of 9.5 μM day^{-1} with ferrihydrite, 8.6 μM day^{-1} with goethite, and 8.8 μM day^{-1} with hematite. NTA accelerated UO_2 reoxidation with ferrihydrite at a rate of 4.8 μM day^{-1}; rates were less with goethite and hematite (0.66 and 0.71 μM day^{-1}, respectively). Citrate increased UO_2 reoxidation with ferrihydrite at a rate of 1.8 μM day^{-1}, but did not increase the extent of reaction with goethite or hematite, with no reoxidation in this case. In all cases, bicarbonate increased the rate and extent of UO_2 reoxidation with ferrihydrite in the presence and absence of chelators. The highest rate of UO_2 reoxidation occurred when the chelator promoted both UO_2 and Fe(III) (hydr)oxide dissolution as demonstrated with EDTA. When UO_2 dissolution did not occur, UO_2 reoxidation likely proceeded through an aqueous Fe(III) intermediate with lower reoxidation rates observed. Reaction modeling suggests that strong Fe(II) chelators promote reoxidation whereas strong Fe(III) chelators impede it. These results indicate that chelators found in U contaminated sites may play a significant role in mobilizing U, potentially affecting bioremediation efforts.

3.10.3. Summary of Major Results:

- EDTA accelerated UO_2 reoxidation with ferrihydrite, goethite, and hematite, and to a greater extent than NTA.
- The highest rate of UO_2 reoxidation occurred when the chelator promoted both UO_2 and Fe(III) (hydr)oxide dissolution, as demonstrated with EDTA.
- Reaction modeling suggests that strong Fe(II) chelators promote reoxidation, whereas strong Fe(III) chelators impede it.

3.11 Publication 11

3.11.1. Citation:
Singh G, Şengör SS, De J, Bhalla A, Kumar S, Stewart B, Spycher N, Ginn T, Peyton B, and Sani RK. 2013. Reoxidation of bioreduced uranium: Recent developments to elucidate the mechanisms. *Critical Reviews in Environmental Science and Technology* (In Press) . ID: 728522 DOI:10.1080/10643389.2012.728522

3.11.2. Abstract:

Uraninite is the most desirable end product of bioreduction, and has low solubility under reducing conditions. For effective long-term immobilization of uranium, there should be no biotic or abiotic reoxidation of the insoluble bioreduced U(IV). It has now been established that after in situ bioremediation of U(VI) using nutrient addition in the subsurface, a variety of combinations of physical, chemical, and biological factors control the rate and extent of oxidation of U(IV). This review describes those factors controlling U-reoxidation and addresses the research used to identify specific factors for U(IV) oxidation and possible mechanisms of reoxidation of various species of reduced U.

3.11.3. Summary of Major Results:
- Detailed molecular mechanisms of uraninite oxidation especially under reducing conditions remain unclear.
- Data suggest that elevated bicarbonate concentrations resulting from organic carbon stimulated microbial respiration favor U(IV) reoxidation/remobilization, even under reducing conditions.
- Reduced U can be oxidized through siderophores, humic substances, and organic ligands present in subsurface sites.

3.12 Publication 12

3.12.1. Citation:
Stewart, BD; PS Nico; C Cisasmu; BM Peyton. Uranium Interaction with Rifle Surface Seep Sediments. *In preparation.*

3.12.2. Abstract:
Owing to ecosystem and human health consequences, understanding uranium's potential for mobility in environmental settings is important. In anaerobic soils and sediments, oxidized U(VI) may be reduced through biological or chemical pathways to U(IV), forming sparingly soluble solids. However, formation of uranyl-calcium-carbonato complexes may limit reduction and authogenic UO_2 is susceptible to reoxidation. This is critical in settings near or at redox boundaries where ecosystems may be subjected to fluctuating redox conditions. We examined the nature and association of solid phase uranium in both oxidized and reduced sediments from a hillside surface seep in Rifle, Colorado where uranium is found naturally attenuated in the solid phase. Reduced sediments occur at the surface of the seep adjacent to pockets of freshly precipitated iron (III) phases, indicative of a redox boundary. Uranium release and solid-phase association was measured in systems containing sediment and 3 Fe(II) that were maintained under reducing conditions for 15d followed by 5d of oxidation to simulate a redox cycle. The freshly precipitated iron phase was also incubated with Fe(II) (no U) to investigate the transformation to more crystalline iron phases and contrast the natural material's reactivity to that of synthetic ferrihydrite. Results demonstrated that systems containing 30 mM Fe(II) incubated with the natural sediment for up to 14 weeks did not show any major changes in mineralogy, indicating that this material is much more recalcitrant in the environment than what would be predicted. It follows that uranium(VI) was not incorporated into the mineral structure of this material as has been observed in several studies with synthetic iron oxides.

3.12.3. Summary of Major Results:
- While natural red material found at site exhibits many ferrihydrite-like properties, it transforms in the presence of Fe(II) much less extensively and more slowly than what is predicted based on results from studies with pure ferrihydrite.
- The natural red material does not appear to incorporate uranium into its structure as has been observed with pure Fe(III) (hydr)oxides.
- Uranium(VI) adsorption capacity of the natural red material is significantly less than that of pure ferrihydrite phases, owing at least in part to surface area differences between the two materials.

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Oxidation by Fe(III)-(hydr)oxides

Major Project Participants: (PI) Dr. Brent M. Peyton, Montana State University (point of contact and coordinator for the combined research activity); (Co-PI) Dr. Timothy Ginn, University of California at Davis; (Co-PI) Dr. Rajesh K. Sani, South Dakota School of Mines and Technology; (Co-PI) Dr. Nicolas Spycher, Lawrence Berkeley Laboratory.