High-Resolution Mineralogical Characterization and Biogeochemical Modeling of Uranium Reduction Pathways at the NABIR Field-Research Center

Final Technical Report

Natural and Accelerated Bioremediation Research Program

Program Element: Biogeochemistry

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Abstract of Technical Final Report

High-Resolution Mineralogical Characterization and Biogeochemical Modeling of Uranium Reduction Pathways at the NABIR Field-Research Center

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We have successfully completed a proof-of-concept, one-year grant on a three-year proposal from the former NABIR program, and here we seek additional two-year funding to complete and publish the research. Using a state-of-the-art 300-kV, atomic resolution, Field Emission Gun Transmission Electron Microscope (TEM), we have successfully identified three categories of mineral hosts for uranium in contaminated soils: (1) iron oxides; (2) mixed manganese-iron oxides; and (3) uranium phosphates. Method development using parallel electron energy loss spectroscopy (EELS) associated with the TEM shows great promise for characterizing the valence states of immobilized U during bioremediation. We have also collected 27 groundwater samples from two push-pull field biostimulation tests, which form two time series from zero to approximately 600 hours. The temporal evolution in major cations, anions, trace elements, and the stable isotopes ^{34}S, ^{18}O in sulfate, ^{15}N in nitrate, and ^{13}C in dissolved inorganic carbon (DIC) clearly show that biostimulation resulted in reduction of nitrate, Mn(IV), Fe(III), U(VI), sulfate, and Tc(VII), and these reduction reactions were intimately coupled with a complex network of inorganic reactions evident from alkalinity, pH, Na, K, Mg, and Ca concentrations. From these temporal trends, apparent zero-order rates were regressed. However, our extensive suite of chemical and isotopic data sets, perhaps the first and only comprehensive data set available at the FRC, show that the derived rates from these field biostimulation experiments are composite and lump-sum rates. There were several reactions that were occurring at the same time but were masked by these pseudo-zero-order rates. A reaction-path model comprising a total of nine redox couples (NO_3^-/NH_4^+, $MnO_2(s)/Mn^{2+}$, $Fe(OH)_3(s)$ $/Fe^{2+}$, $TcO_4^-/TcO_2(s)$, $UO_2^{2+}/UO_2(s)$, SO_4^{2-}/HS^-, CO_2/CH_4, ethanol/acetate, and H^+/H_2.) is used to simulate the temporal biogeochemical evolution observed in the field tests. Preliminary results show that the models based on thermodynamics and more complex rate laws can generate the apparent zero-order rates when several concurrent or competing reactions occur. Professor Alex Halliday of Oxford University, UK, and his postdoctoral associates are measuring the uranium isotopes in our groundwater samples. Newly developed state-of-the-art analytical techniques in measuring variability in $^{235}U/^{238}U$ offer the potential to distinguish biotic and abiotic uranium reductive mechanisms.

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SUMMARY RESULTS FROM ONE YEAR OF DOE SUPPORT

Award DE-FG02-04ER63740: "High-resolution mineralogical characterization and biogeochemical modeling of uranium reaction pathways at the FRC." PI: Chen Zhu; co-PI: David Veblen; Amount: $99,325 to Indiana University; 2/1/04-1/31/06.

Award DE-FG02-04ER63764: same title. PI: David R. Veblen; co-PI: Chen Zhu; Amount: $30,000 to Johns Hopkins University; 2/1/04-1/31/06.

We received a one-year, Proof-of-Principle grant for the project from the former NABIR program. Here, we report the substantial progress from this single year of funding, and we seek two additional years of funding to complete and publish the research that we have started.

Publications and Presentations that Acknowledged the Award

Refereed Journal Articles

Stubbs, J. E., Elbert, D. C., Veblen, D. R., Zhu, C. (2006) Electron microbeam investigation of uranium-contaminated soils from Oak Ridge, TN, USA. ***Environmental Science & Technology*** 40, 2108-2113.

Manuscripts in preparation

Zheng, Reeder, Zhu, and others, In situ reaction rates from field bio-stimulation experiments. 80% completed. To be submitted to ***Geochimica et Cosmochimica Acta***

Zheng, Zhu, and others, Geochemical modeling of biogeochemical reactions during field bio-stimulation experiments. 50% completed. To be submitted to ***Geochimica et Cosmochimica Acta***

Presentations at National and International Conferences

Zheng, Z., M.D. Reeder, Zhu, C., (2006) In situ reaction rates from a field biostimulation experiment. Geochimica et Cosmochimica Acta, Proceedings of 16th Annual Goldschmidt Conference, the Annual Meeting of the Geochemical Society, Melbourne, Australia, August 27-September 1, 2006.

Stubbs, J. E., Elbert, D. C., Veblen, D. R (2006) Mineral Hosts for Uranium in Oak Ridge Soils (Invited Talk), ***EOS Trans. AGU***, 87, Joint Assembly Supplement Abstract V23A-02.

Reeder, M., Zhu, C., (2005) Determination of in situ reaction rates as a result of biostimulation at the Field Research Center, Oak Ridge, TN. Geological Society of America Abstr. with Programs, v. 37, no. 7, p. 381.

Presentations at DOE PI Meetings and Workshops

Reeder, M., Zhu, C. and Veblen, D. R. (2005) Sequential Biogeochemical Redox Reactions as a Result of Biostimulation at the Oak Ridge Field Research Center. NABIR Principal Investigator workshop, March 18 – 20, 2005, Warrenton, Virginia.

D. R. Veblen, C. Zhu, L. Krumholz, C. Sterling, E. Potter, and A. N. Halliday (2004) High-Resolution Mineralogical Characterization and Biogeochemical Modeling of Uranium Reduction Pathways at the NABIR Field-Research Center. Presentation at the NABIR PI meeting, March 14-17, 2004, Warrenton, Virginia.

Stubbs, J.E., Elbert, D.C., Veblen, D.R., and Zhu, C. (2004). Preliminary Electron Microscopic Characterization of Uranium Contaminated Sediment (Poster). Oak Ridge Field Research Center Workshop, Oak Ridge, TN.

Invited lectures, symposia, and seminars

Chen Zhu has given fifteen 15 invited seminars during 2004-2006.

Education Accomplishments

Mathew Reeder: 9/2004-06, M.S. 2006, Indiana University; Thesis Title: "Geochemical evolution and in situ reaction rates during a field biostimulation experiment at the Field research Center"

Joanne Stubbs (9/2004-06, M.S. in Environmental Science & Policy, 2004, Johns Hopkins University; Thesis Title: "Analysis of uranium contaminated regolith using electron microscopic techniques;" M.A. in Earth & Planetary Sciences, 2006.

1. Biogeochemical Reaction Network

1a) Field Sampling and Laboratory Analyses

We successfully collected groundwater samples in April, 2004, with the generous assistance of the FRC personnel and the Istok team. Twenty-seven groundwater samples from two push-pull field biostimulation tests in Area 2 constitute two time-series from zero to approximately 600 hours. With these samples, we have successfully analyzed major and trace elements, as well as stable isotopes of ^{34}S, ^{18}O in sulfate, ^{15}N in nitrate, and ^{13}C in dissolved inorganic carbon (DIC). We have also won the interests of Professor Alex Halliday (University of Oxford), and he and his post-doctoral associates have agreed to measure the uranium isotope and Fe isotope fractionation during the biostimulation experiments. Mr. David Watson, the site manager for the Oak Ridge FRC, has already sent one set of groundwater samples to Professor Halliday. This collaboration and synergetic activity brings new state-of-the-art analytical techniques to the ERSP program.

Together with U and Tc analyses by the Istok team, we have obtained a very comprehensive chemical and isotopic data set regarding sequential/multiple redox reactions induced by microbial activity. Data sets from previous studies of redox reactions are not as nearly complete as ours. Bioremediation of radionuclides like U and Tc is challenging because the radionuclides are typically in very low but hazardous concentrations, while concentrations of potential competing electron acceptors are overwhelmingly higher. Successful field-scale bioremediation must be accomplished in the presence of these competing terminal electron acceptors in the groundwater systems. To unravel the complex network of reactions as a result of biostimulation, it is essential to have comprehensive data on elemental concentrations of cations, anions, trace metals, and stable isotopes.

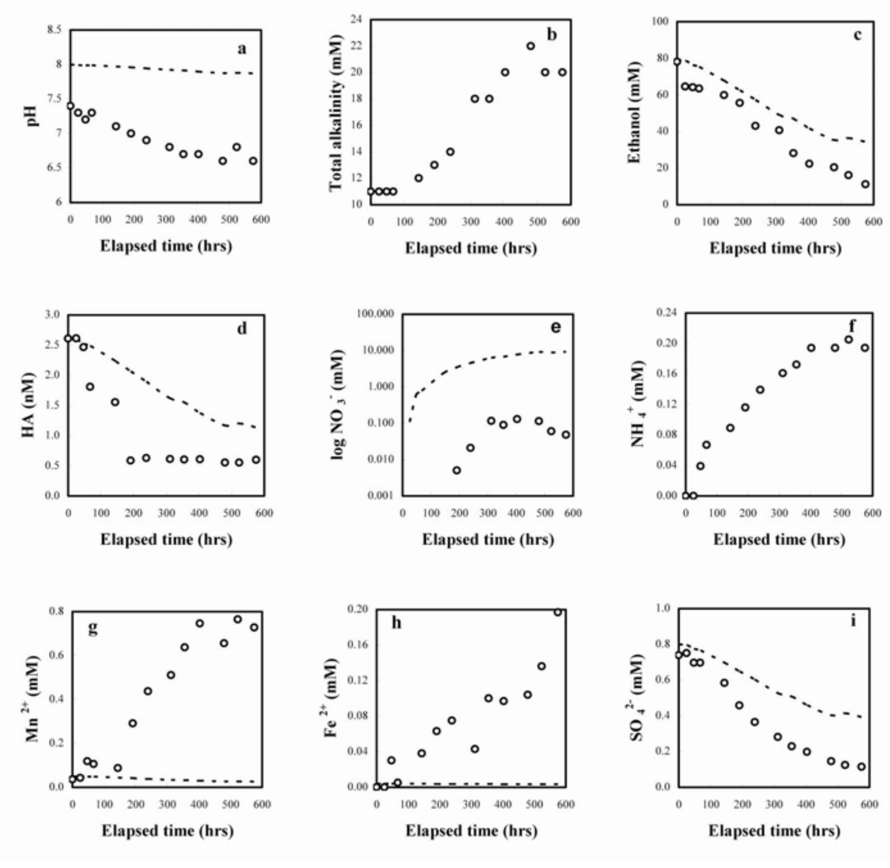

Fig. 1. Temporal changes in measured chemical concentrations in the time series groundwater samples. HA stands for humic and fulvic acids. The dashed lines represent the hypothetical concentrations resulting from mixing if no biogeochemical reactions have occurred. The departure of measured concentrations (symbols) from the dashed lines indicate that biogeochemical reactions have occurred if the system can be approximate as a mixed reactor.

1b) Analytical Results

Our aqueous geochemistry and isotopic data have delineated temporal trends of C, N, S, Fe, U, Tc, and Mn (Figs. 1, 2). The trends in these diagrams generally reflect two dominant processes: mixing of the injected groundwater (amended G835 groundwater) with background groundwater (DP-06) due to advection and dispersion and biogeochemical reactions in response to stimulated microbial activity. With a number of simplifying assumptions of the hydraulic system of interest (Haggerty et al., 1998; Snodgrass and Kitanidis, 1998), we calculated the mixing contributions from the three conservative tracers Br^-, Cl^-, and DCBA.

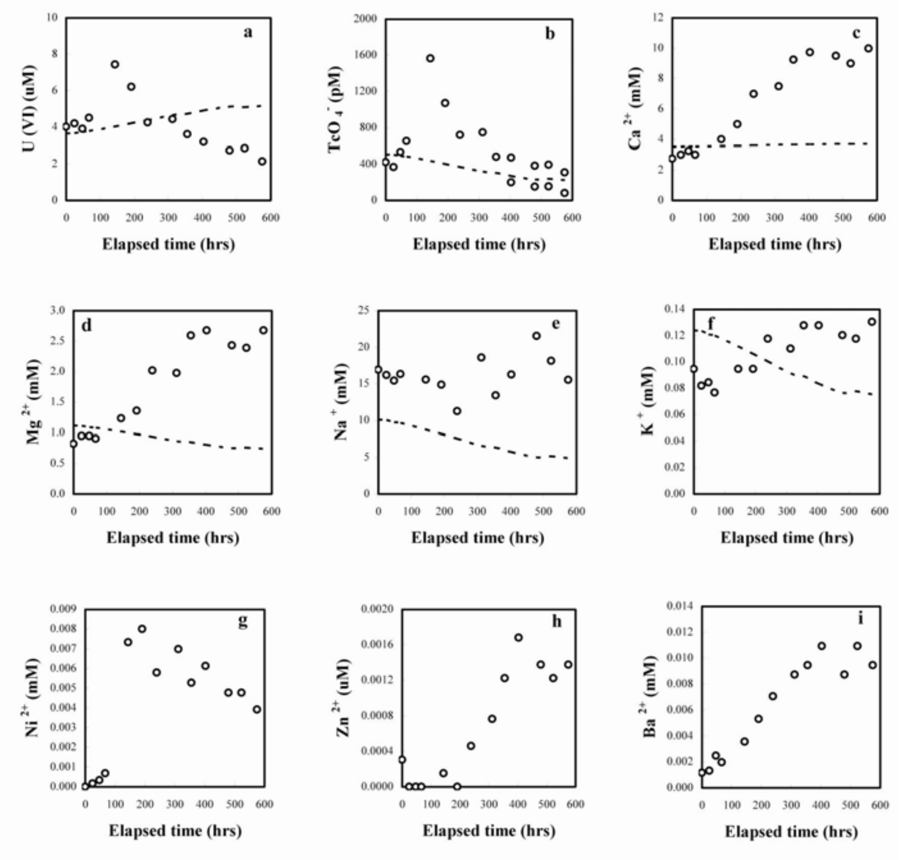

Fig. 2. Changes in cation concentrations as a function of elapsed time. The dashed lines represent the hypothetical concentrations resulting from mixing if no biogeochemical reactions have occurred. The departure of measured concentrations (symbols) from the dashed lines indicate that biogeochemical reactions have occurred if the system can be approximate as a mixed reactor.

Figures 1 and 2 above show that the measured concentrations for cations and anions (symbols) depart significantly from the calculated mixing concentrations (dashed lines). These departures indicate that (1) biostimulation resulted in nitrate, Mn(IV), Fe(III), U(VI), sulfate, and Tc(VII) reduction; and (2) these reduction reactions were intimately coupled with a complex network of inorganic reactions, evident from the discrepancy between measured and calculated mixing concentrations for alkalinity, pH, Na, K, Mg, Ca, and trace metals (Fig. 2).

By subtracting the mixing contribution, we calculated the reacted concentrations (RC on Fig. 3, with a method modified from Haggerty et al., 1998; Snodgrass and Kitanidis, 1998). Figure 3 shows that a linear trend forms for most chemicals. From these trends, apparent zero-order rates were regressed (Table 1 on p.5). These in-situ reaction rates are generally comparable to the rates reported in other shallow aquifer systems, but one to five orders of magnitude lower than the rates obtained under laboratory conditions.

1c) Characteristics of the Field in-situ Rates

The calculated rates from these field biostimulation experiments are composite, bulk, or lump-sum rates. They may even reflect the hydraulic structure of the particular experiments if the assumption of a completely mixed reactor is not valid (such as our case after 400 hours). There may be several reactions that were occurring at the same time but are masked by these pseudo-zero-order rate laws. Our comprehensive chemical and isotopic data, perhaps the first comprehensive data set available, illustrate vividly the composite nature of the field–derived reaction rates.

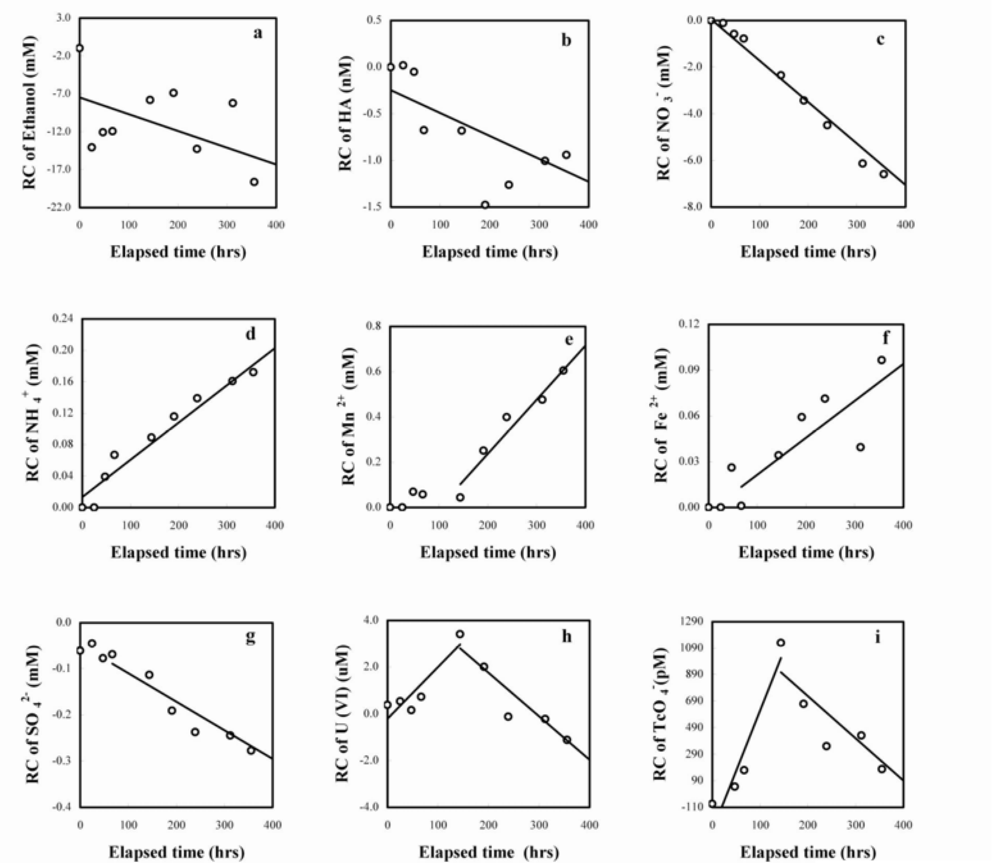

Fig. 3. The calculated reacted concentrations for organic compounds and main redox compounds as a function of time elapsed. The linear trends indicate an apparent zero–order rate law. The solid lines were obtained from linear regression. HA stands for humic and fulvic acids. Ethanol concentrations do not form a linear trend but correlate with ^{13}C data.

A case in point is uranium. U(VI) concentrations increased sharply from ~ 4 μM to 8 μM in the first 140 hours, and then decreased to 2 μM by the end of the experiment (Fig. 2a). An increase of uranium above the mixing level indicates mass transfer from the aquifer solid matrix to the groundwater. Below, we explain that TEM observations indicate that there are at least three distinct U(VI) hosts in the contaminated sediment at the site: (1) iron oxides; (2) uranium phosphates; and (3) mixed Mn-Fe oxides (Stubbs et al., 2006a). Desorption from Fe or Fe-Mn oxyhydroxides could have occurred before uranium reduction took place, perhaps due to the increase of bicarbonate concentrations. The increase of uranium could also have resulted from dissolution of the uranium-phosphate phase. However, the decrease of U(VI) with time after 140 hours did not reflect a simple uranium reduction reaction as prescribed by an apparent zero-order rate law. Figure 4 shows that the uranium concentration decrease coincided with an increase in Fe^{2+} and Mn^{2+} concentrations, reflecting reductive dissolution of Fe and Mn oxyhydroxides that could release sorbed U. This network of reactions means that the observed decrease in uranium concentration as a function of time could be a compound effect of both uranium reduction and desorption from dissolving iron oxides.

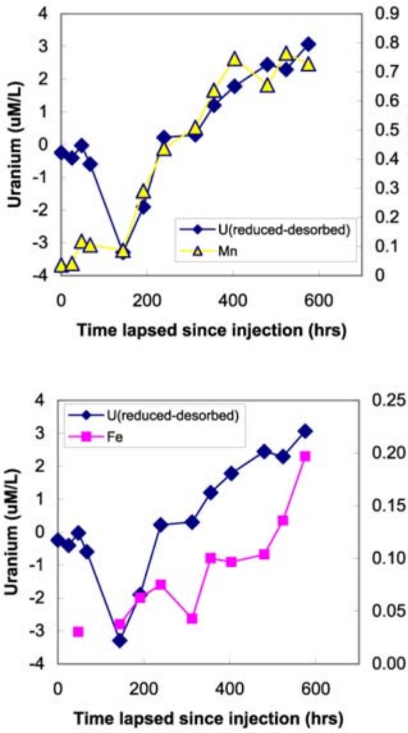

Fig. 4. Comparison of reacted uranium as a function time with Fe and Mn

Thus, uranium reduction rates calculated from time-series data are apparently lower than the actual reduction rates, which underscores the significance of a comprehensive geochemistry study measuring not only the contaminant of concern but also a comprehensive suite of cations, anions, and isotopes, and all integrated solid phases.

Our isotopic data have captured distinct trends of fractionation. $\delta^{15}N$ decreased linearly as a function of time. $\delta^{34}S$ increased 5 per mil in the first 100 hours, but decreased slightly with time. The $\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{18}O\text{-}SO_4^{2-}$ data form a distinct trend. The $\delta^{13}C$ data show a fluctuation with time, but the fluctuation correlates well with the concentrations of consumed ethanol. These stable isotope data added new information to the FRC studies.

Table1. In–situ reaction zero–order rates estimated and reported in literature (mol $L^{-1}s^{-1}$)

Reaction	This study	Literature	Sources
Ethanol consumption	$-6.11\times10^{-9} \sim -3.92\times10^{-8}$	$-5.56\times10^{-9} \sim 6.94\times10^{-8}$	Istok et al. (2004)
HA consumption	$-6.94\times10^{-13} \sim -1.11\times10^{-12}$		
Nitrate reduction	-4.94×10^{-9}	-3.06×10^{-9}	Istok et al. (2004)
		$-1.8\times10^{-9} \sim -3.6\times10^{-9}$	Dhakar and Burdige (1996)
		$-6.1\times10^{-12} \sim -1.1\times10^{-9}$	Ludvigsen et al. (1998)
UO_2^{2+} desorption	6.11×10^{-12}	6.94×10^{-13}	Senko et al. (2002)*
		8.33×10^{-12}	Istok et al. (2004)*
		2.08×10^{-11}	Finneran et al. (2002)*
TcO_4^- desorption	2.52×10^{-15}		
UO_2^{2+} reduction	-5.17×10^{-12}	-6.67×10^{-12}	Istok et al. (2004)
		$-2.31\times10^{-13} \sim -5.79\times10^{-13}$	Long et al. (2005)
		-1.0×10^{-10} (lab)	Lovley and Phillips (1992)
		-6.58×10^{-10} (lab)	Truex et al. (1997)
		-5.0×10^{-10} (lab)	Spear et al. (1999)
		$-6.39\times10^{-11} \sim -9.17\times10^{-11}$ (lab)	Liu et al. (2002)
TcO_4^- reduction	-8.83×10^{-16}	$-2.78\times10^{-16} \sim -2.97\times10^{-14}$	Istok et al. (2004)
		$-5.56\times10^{-11} \sim -5.56\times10^{-9}$ (lab)	Fredrickson et al. (2004)
		-2.78×10^{-9} (lab)	Liu et al. (2002)
		-2.03×10^{-9} (lab)	Wildung et al. (2000)
		-8.33×10^{-10} (lab)	Lloyd et al.(2000)
SO_4^{2-} reduction	-2.78×10^{-10}	$-9.83\times10^{-12} \sim -2.95\times10^{-11}$	Jakobsen and Postma (1999)
		-2.9×10^{-9} (lab)	Roychoudhury et al. (1998)
Fe^{2+} production	5.56×10^{-11}	$1.65\times10^{-11} \sim 4.85\times10^{-11}$	Jakobsen and Postma (1999)
		$1.5\times10^{-10} \sim 5.8\times10^{-10}$	Ludvigsen et al. (1998)
		2.78×10^{-9} (lab)	Burgos et al. (2002)
		4.17×10^{-9} (lab)	Fredrickson et al.(2004)
Mn^{2+} production	6.67×10^{-10}	7.4×10^{-11}	Ludvigsen et al. (1998)
		4.17×10^{-9} (lab)	Fredrickson et al. (2004)
		3.6×10^{-9} (lab)	Schwarzenbach et al. (1993)
NH_4^+ production	1.39×10^{-10}		
Ba^{2+} production	8.33×10^{-12}		

All reaction rates refer in-situ reactions, unless otherwise indicated with term "lab" following the number.

* In situ rates for U oxidation by NO_3^-.

Thus, this very comprehensive dataset of aqueous geochemistry and isotopes provides an excellent opportunity to decipher the biogeochemical reaction pathways in a complex remediation system, as well as contributing to the fundamental understanding of redox geochemistry. In addition, the U and Fe isotopes that Prof. Halliday and associates are currently analyzing will add a new dimension to this project. Together with the high-resolution mineralogical data we have collected and propose to collect here, this is a very valuable data set.

1d) Geochemical modeling

We used the groundwater chemical and mineralogical data we collected to perform speciation and solubility modeling. Thermodynamic data are from Grenthe et al. (1992), the database compiled by Dr. Jim Johnson at Lawrence Livermore National Laboratory for Eq3/6 (Wolery, 1992), and data compiled by Brooks (2001) and Fredrickson et al. (2000, 2002), Liu et al. (2002), and other sources. Previous studies have successfully used geochemical modeling as a tool for interpreting bioreduction of uranium and technetium (Abdelouas et al., 1998b, 1999; Wildung et al., 2000). Baseline uranium geochemistry at the FRC has been modeled recently by Brooks (2001). Following Abdelouas et al. (1998a) and Fredrickson et al. (2000, 2002), we used speciation-solubility models to calculate the "effective redox potentials" for redox reactions under field experimental conditions (Fig. 5).

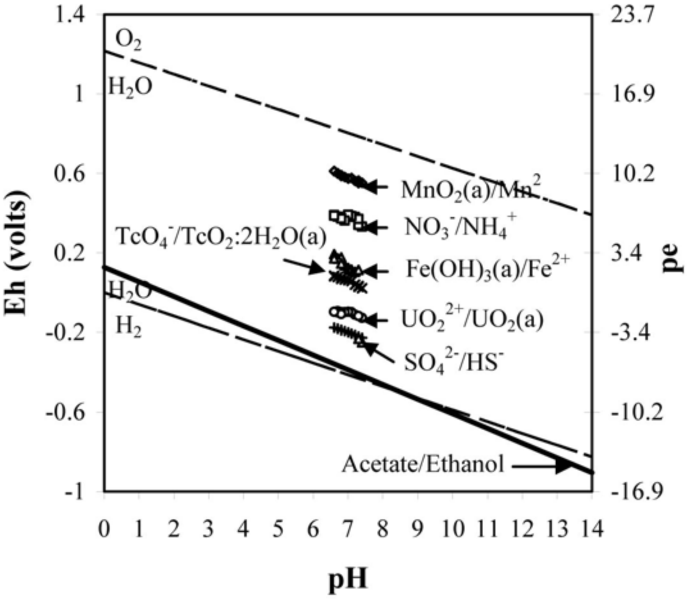

Fig. 5. pH-Eh diagram showing the calculated redox couples based on the chemical concentrations in the extracted water. The ratios of acetate to ethanol and sulfate to sulfide are assumed to be 1.0. MnO_2 refers Birnessite and Fe $(OH)_3$ (a) refers to amorphous ferric iron oxide. UO_2^{2+} denotes UO_2 $(CO_3)_3^{4-}$ species, which is the dominant species in the pH range of 7.4-6.6 in our samples. The standard E^o values for different redox couples are mostly calculated based on data in the WATEQ4f database.

We are using forward kinetic reaction-path models to simulate the temporal biogeochemical evolution observed in the field tests. Dilution by background groundwater is modeled as a mixing process. Our chemical model is a mixed kinetics and equilibrium model. Aqueous speciation and complexation and surface complexation reactions are simulated as local and partial equilibria, and microbially mediated redox reactions are modeled using the rate law of Jin and Bethke (2005). Dissolution and precipitation reactions are modeled using the formulation of Lasaga (1981). A total of 9 redox couples are included: NO_3^-/NH_4^+, $MnO_2(s)/Mn^{2+}$, $Fe(OH)_3(s)$ $/Fe^{2+}$, $TcO_4^-/TcO_2(s)$, $UO_2^{2+}/UO_2(s)$, SO_4^{2-}/HS^-, CO_2/CH_4, ethanol/acetate, and H^+/H_2. Additional aqueous components include Br^-, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, and Ba^{2+}. Although the modeling work is not completed, preliminary results show that the more mechanistic models based on thermodynamics and kinetic theories can generate the apparent zero-order rate laws when several concurrent or competing reactions occur.

2. High-resolution Electron Microscopy

2a) Laboratory Work

In our proof-of-concept work, we have extensively studied two samples from Area 3 (FWB103-00-40E, 39.5 ft depth, and FWB103-00-42D, 42 ft) using a electron microprobe analysis (EMPA) and variety of transmission electron microscopy (TEM) methods: conventional TEM amplitude-contrast imaging, high-resolution (HRTEM imaging), selected-area electron diffraction (SAED), convergent-beam electron diffraction (CBED), energy-dispersive X-ray emission spectroscopy (EDS), electron energy-loss spectroscopy (EELS), and chemical mapping by energy-filtered TEM (EFTEM). Since we have already published this work in the premier journal *Environmental Science and Technology* (Stubbs et al., 2006), only a brief summary of this work will be given here.

The primary conclusion of our study was that there are at least three, distinct mineralogical hosts for uranium in the Area 3 core samples we studied: (1) iron oxides (including oxyhydroxides), (2) mixed Mn–Fe oxides; and (3) a discrete uranium phosphate mineral having U:P ≅ 1:1 (consistent with minerals in the autunite and meta-autunite groups). As shown in Figures 6 and 7, the Fe oxides occur as two texturally different forms: pseudomorphs after pyrite (FeS_2) within weathered shale and free-floating particles in the very fine-grained

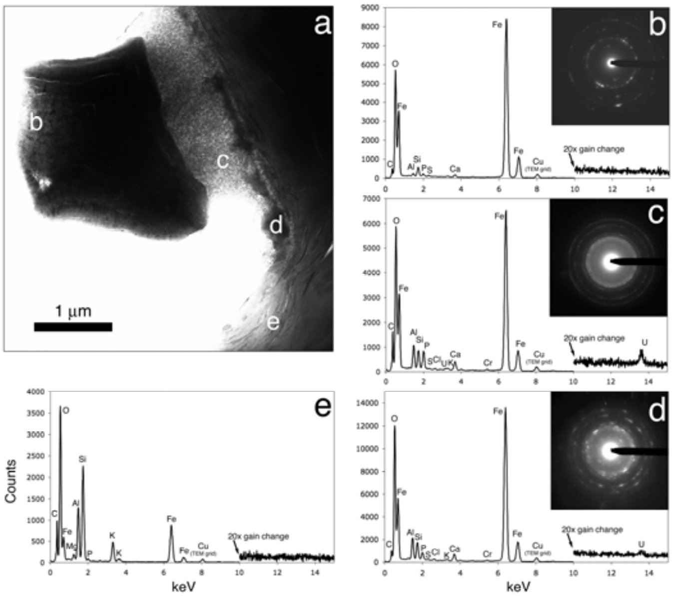

Figure 6. (a) Bright-field TEM image of iron oxide pseudomorph occurring within shale. (b-e) SAED and EDS collected from (b) hematite core with no detectable U; (c) ferrihydrite rim, which contains appreciable U and P, as well as Cr; (d) goethite halo containing lesser amounts of U and P, as well as Cr; and (e) surrounding clay matrix with no detectable U.

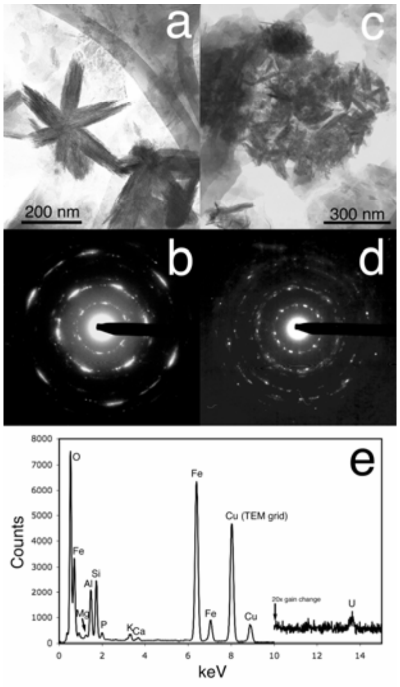

Figure 7. BFTEM image and SAED from (a) and (b) star-shaped goethite twin, and (c) and (d) mass of uranium-bearing goethite. Centermost ring in each SAED (~4.5 Å) is contributed by clay. (e) EDS from center of goethite star shows these twins host U, P and possibly Ca. EDS also shows Al, Si, Mg and K from clay. EDS from goethite mass (not shown) is similar.

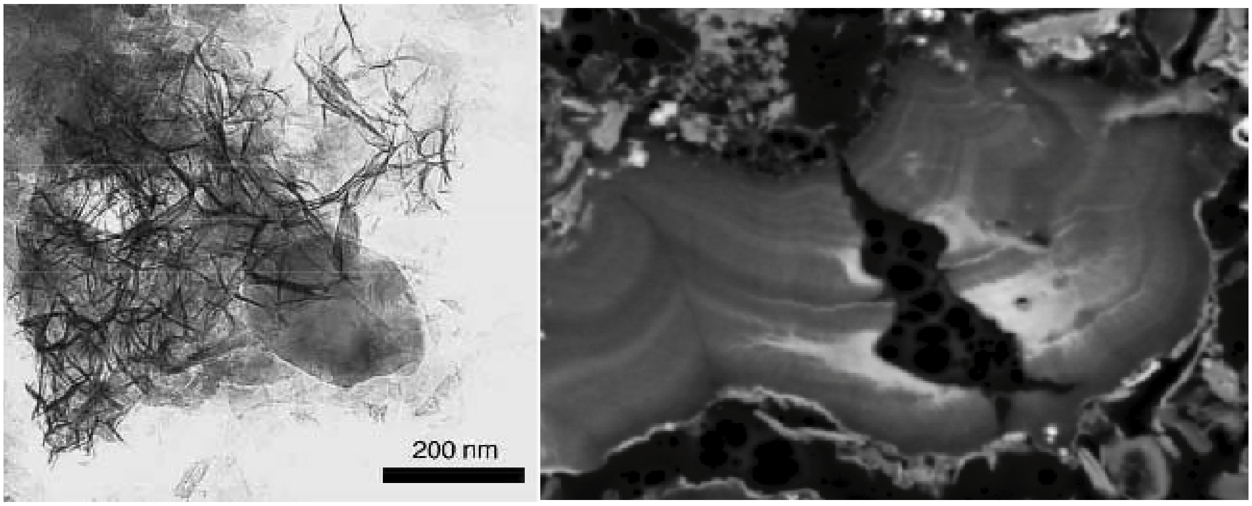

Figure 8. Uranium-containing Mn-Fe oxides. Left: Bright-field TEM image of a small particle from fines, containing U, Ba, and Pb. Right: Backscattered electron image of botryoidal, vein-filling morphology in coherent shale, containing U and Ba.

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unconsolidated portions of the samples. Similarly, the mixed Mn–Fe oxides occur as both free particles and vein fillings in coherent shale (Fig. 8). Morphologically, most of the loose particles are consistent with a phyllomanganate, whereas the vein-filling materials are probably chain and tunnel structured oxides. Perhaps the most interesting mineral host for uranium is the discrete U-phosphate mineral that

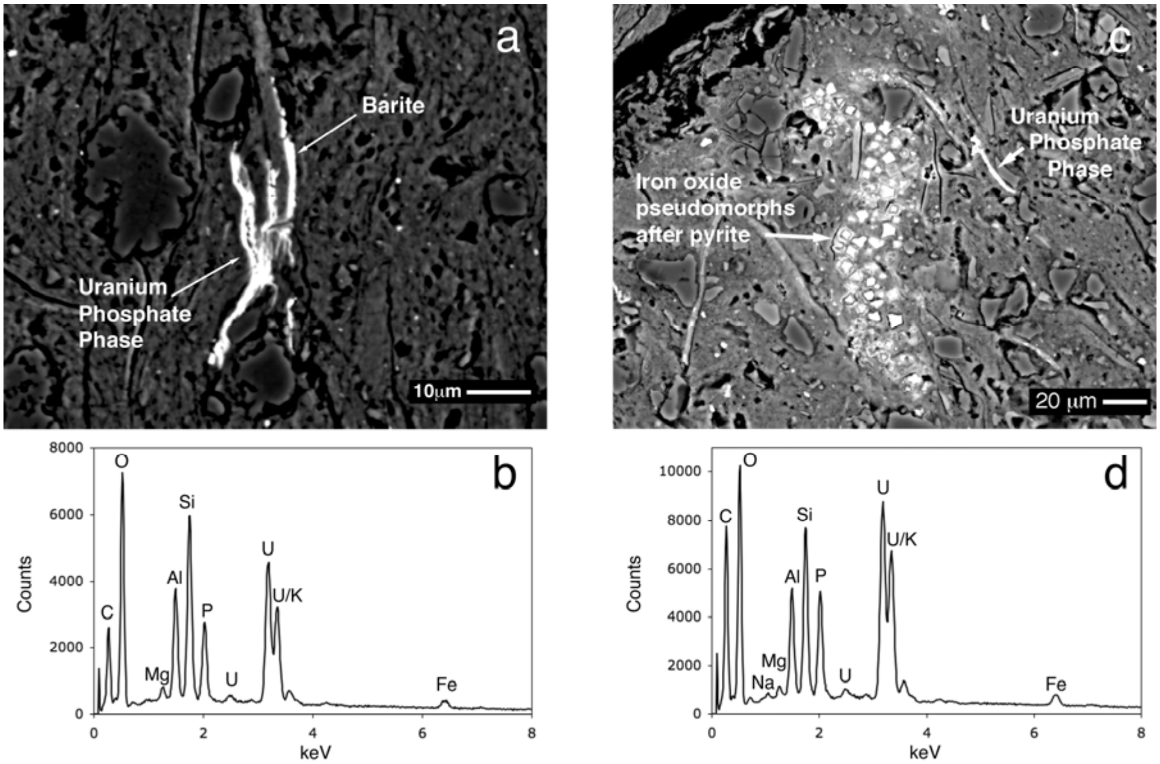

Figure 9. (a) and (c) BSE showing uranium phosphates. (b) and (d) EDS of uranium phosphates. Other peaks in spectra may result from difficulty in isolating phases under the beam.

occurs within coherent pieces of shale (Fig. 9). As noted above, its chemical composition is consistent with a mineral of the autunite or meta-autunite groups, and it's morphology is also consistent with these uranyl phosphate layer minerals (Suzuki et al., 2005).

It is interesting to note, and perhaps very significant, that in all of these different hosts uranium is always associated with phosphate. Below we propose to pursue this association through a combination of geochemical modeling and TEM studies.

3. Summary of the First Year's work and Suggestions for following-up work

As described above, we have performed a substantial amount of work for the single year of Proof-of-Concept funding. We have collected a valuable, and perhaps the most comprehensive, chemical and isotopic data set for the aqueous phase in Area 2, and we have obtained excellent results with electron microbeam methods, identifying the mineral hosts for uranium in contaminated soils from Area 3. We have also successfully recruited and trained graduate students and post-doctoral associates. Both PIs on the project have been highly productive, which should lend confidence that we shall overcome any difficulties and achieve the goals described in this proposal. After the one year's work, we are poised to make contributions to the ERSP program with our unique expertise and data sets.

We plan to continue analyzing, interpreting, and publishing the above-mentioned data. We also propose to pursue the tasks outlined in the original proposal that is partially given below, as well as adjustment in light of one year's work:

1. **U isotope fractionation as another potential indicator of the mechanisms of uranium reduction**

Professor Alex Halliday (now at University of Oxford, UK) and his former postdoctoral associates are analyzing the U isotopes on the set of groundwater samples Mr. David Watson sent to them. Experimental protocols for the precise measurement of $^{235}U/^{238}U$ by multiple-collector ICPMS (MC-ICPMS) have been developed. Using a Nu Instruments NuPlasma MC-ICPMS, we are able to resolve variations in $^{235}U/^{238}U$ at the 40 ppm level ($2\sigma_M$; 1 ppm = 1 part in 10^6) on sample sizes comprising 35 ng of uranium. Isotopic fractionation between ^{235}U and ^{238}U has been observed in low-temperature environments (Stirling et al., Fall Meeting of the AGU, 2003). Variability in $^{235}U/^{238}U$ therefore offers the potential to differentiate biotic abiotic uranium reductive mechanisms.

2. **Significance of the association of phosphate with uranium**

Immobilzation of uranium has previously been associated with phosphate (Jerden and Sinha, 2003; Jerden et al., 2003). In a recent study, Cheng et al. (2004) investigated effects of phosphate on U adsorption to goethite-coated sand through (1) competition with U for subsurface sites on iron oxides, which will decrease U adsorption; (2) competition with surface sites for coordination of U by forming aqueous U-P complexes; (3) formation of ternary surface complexes involving U and P; (4) precipitation of U-P solids.

Both the primary waste and sludge in the S-3 pond contained phosphate. Phosphate have also been measured in both contaminated soils and groundwater (Brooks, 2001). We intend to pursue this connection with an integrated mineralogical and aqueous geochemistry approach.

3. **Stable isotopes**

We have not had sufficient time or funds to interpret our isotope data. If the data analysis indicates the need for a second set of isotopic data, we have the capability of furthering the stable isotope study. Our experience with the first data set will help us to plan and execute field sampling, and we will be able to do the laboratory work at a higher level of sophistication. Indiana University houses one of the best stable isotope facilities in the country.

The facility is located in the Indiana University Biogeochemical Laboratories. A wide range of modern analytical equipment for biogeochemical research is available. Our new ThermoFinnigan Delta Plus XP mass-spectrometer is connected on-line with gas chromatographs (both oxidative and reductive interfaces), elemental analyzers (oxidative and reductive TC/EA), a Gas Bench, and a Hydrogen Device. We further have Finnigan MAT 252 mass spectrometers that are coupled with gas chromatographs (GC-MS) and on-line elemental analyzer/laser ablation units. We routinely determine carbon, sulfur, hydrogen, nitrogen, and oxygen isotopic ratios of individual compounds on-line in small samples. Our Delta Plus XP and the MAT 252 mass-spectrometers may also operate in dual-inlet mode for C, H, O, N, S isotopes.

4. Responses to Reviewer's Comments

Following the instructions of program managers, we have carefully considered the review comments during our one year research (from all three reviewers):

(a) Difficulty for TEM work to focus on the results of biostimulation of the NABIR FRC.
In developing EELS methods, we are using uranium minerals as standards in method development; our recent results (Stubbs et al., 2006a) indicate that we can, in fact work with these materials.

(b) definition of strategies for selection of grains for SEM will be essential but was not discussed in any details in proposal
These strategies have been developed in our microprobe and SEM studies; elemental mapping and BSE imaging have proved very useful in identifying phases of interest. We are also evaluating autoradiaography and fission-track mapping as possible approaches for locating U-rich sample regions.

(c) Valuable to compare the results of the detailed investigation with bulk compositions to confirm that the major precipitates have been examined
NABIR workshop/PI meetings have facilitated exchange of information and establishing collaboration with other NABIR PIs. We have met with Drs. Shelly Kelly and Ken Kemner of Argonne National Laboratory, and compared our electron microscopic results with their EXAFS data. These data are in agreement in principle. As we stated in our proposal, SEM is economic and will help safeguard that the atomics scale TEM observation are representative. We also will be working with Dr. Tim Scheibe on biostimulated materials to ensure our results are reasonable.

(d) poor resolution of U at low concentrations
We have already detected U in multiple mineral hosts with TEM (Stubbs et al., 2006; see above)

(e) impossible for EELS to differentiate valences of U
There now have been numerous studies where U(VI)/U(IV) has been determined with EELS, as referenced below.

(f) redox state change during sample preparation (TEM)
This is a very real problem, probably not Fe and Mn, but certainly for U. It is addressed below in section on sample preparation.

(g) Funding is not requested for Veblen, Veblen committed to the project? Current proposal not listed under Veblen's Current and Pending Support forms.
This was a misunderstanding. A separate budget was submitted from Johns Hopkins University for Professor Veblen, but the budget sheets were separated from the proposal when sent out for review. We believe that the current program managers are aware of Professor Veblen's involvement and commitment.