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REACTIVITY OF IRON-RICH CLAYS WITH URANIUM THROUGH REDOX TRANSITION ZONES

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ABSTRACT

This project performed thermodynamic, kinetic, and mineral structural studies on the reactivity of phyllosilicate Fe(II/III) with metal-reducing bacteria, and with two important poly-valent DOE contaminants (chromium and uranium) that show high mobility in their oxidized state. We focused on Fe-bearing phyllosilicates because these are important components of the reactive, fines fraction of Hanford, Oak Ridge, and Idaho National Laboratory sediments. Iron-bearing phyllosilicates strongly influence the redox state and mobility of Cr and U because of their limited hydraulic conductivity, high specific surface area, and redox reactivity. This was a collaborative project between Penn State (W.D. Burgos – PI), Miami University (H. Dong – Co-PI), and Argonne National Laboratory (K. Kemner and M. Boyanov – Co-PIs). Penn State and Miami University were funded together but separately from ANL. This report summarizes research findings and publciations produced by Penn State and Miami University.

We conducted a series of laboratory experiments that used varied combinations of specimen clay minerals, U or Cr or nitroaromatic compounds (NACs), and pure cultures of dissimilatory metal-reducing bacteria (DMRB). Wet chemical techniques were used to measure soluble and extractable concentrations of Fe and contaminants. Microscopic (TEM) and spectroscopic (Mössbauer) techniques were used to characterize reacted solids. Major findings from this research include:

1. Standard extraction procedures cannot be accurately applied for the determination of clay-Fe(II/III) and U(IV/VI) in clay mineral-U suspensions. We developed and validated a sequential extraction method for determination of clay-Fe(II/III) and U(IV/VI) in clay-U suspensions. In our so-called “H_3PO_4-HF-H_2SO_4 sequential extraction” method, H_3PO_4-H_2SO_4 is used first to solubilize and remove U, and the remaining clay pellet is subject to HF-H_2SO_4 digestion. Physical separation of U and clay eliminates valence cycling between U(IV/VI) and clay-Fe(II/III) that otherwise occurred in the extraction solutions and caused analytical discrepancies.

2. The final extent of bioreduction of iron-bearing nontronite (NAu-2; 4.1 mol Fe/g clay) and montmorillonite (SWy-2; 0.40 mol Fe/g clay) corresponded directly to the standard state reduction potentials of the clay minerals ($E_{H,clay}$). The final extent of bioreduction of iron-bearing phyllosilicates was consistent with measured values of mid-point potentials of respiratory enzymes of *Shewanella* species. The extent of contaminant reactivity (e.g., U(VI) reduction) was controlled by $E_{H,clay}$.

3. The rates of NAC oxidation by clay-Fe(II) were well predicted by linear free energy relationships (LFERs). Using a suite of NACs with clays chemically or biologically reduced to the same reduction potential ($E_{H,clay}$ = constant), we found that the logarithm of the NACs’ rate constants correlated with one-electron reduction potential values of the NACs ($E^1_{H,NAC}$). Using clays chemically or biologically reduced to the same reduction potential with a single NAC ($E^1_{H,NAC}$ = constant), we found that the logarithm of the NAC’s rate constants correlated to the reduction potential of the clay ($E_{H,clay}$).

4. The rate and extent of bioreduction of structural Fe(III) in clay minerals were positively correlated with the percent smectite in the S–I series (i.e., layer expandability). Fe(II) in the bioreduced clay minerals was reactive to reduce Tc(VII) to Tc(IV). Reduced NAu-2 showed the highest reactivity toward Tc(VII), and reduced illite exhibited the least. Crystal chemical parameters play important roles in controlling the rate and extent of bioreduction and the reactivity toward Tc(VII) reduction.

5. Smectite minerals such as nontronite and montmorillonite were most effective in reducing aqueous Cr(VI). Characterization of the Cr-clay reaction product with electron microscopy revealed that reduced chromium was possibly in the form of sub-nanometer Cr_2O_3 in association with residual clay minerals as micro-aggregates. This textural association was expected to minimize the chance of Cr(III) reoxidation upon exposure to oxidants.

6. Sulfate-reducing bacterium *Desulfovibrio vulgaris* was able to reduce structural Fe(III) in smectite-illite minerals, and AQDS enhanced the reduction rate and extent. In the presence of AQDS, sulfate had little effect on Fe(III) bioreduction. In the absence of AQDS, sulfate increased the reduction rate and capacity, suggesting that sulfide produced during sulfate reduction reacted with the phyllosilicate Fe(III).

PROJECT SUMMARY

This report summarizes research from the project titled 'Reactivity of iron-rich clays with uranium through redox transition zones' funded through the Subsurface Biogeochemical Research Program, Office of Science, United States Department of Energy. This was a collaborative project between Penn State University, Miami University (Ohio), and Argonne National Laboratory. This project summary expands on the major findings listed in the ABSTRACT and provides a list of all related peer-reviewed publications that acknowledged support from this grant.

Sequential extraction method for determination of Fe(II/III) and U(IV/VI)

Uranium contamination is a problem at many U.S. Department of Energy (DOE) sites and uranium ore-processing sites. One strategy for in situ remediation is to add electron donors to stimulate anaerobic conditions and reduce mobile uranyl (VI) to sparingly soluble uraninite ($U(IV)O_2(s)$). However, further studies have shown that uraninite can be reoxidized by nitrate, iron oxides, and iron(III)-bearing phyllosilicates, in addition to oxygen. The mass of iron associated with phyllosilicate minerals is higher than the mass of iron associated with oxide minerals at several DOE sites. Because phyllosilicates provide a large solid-phase reservoir of Fe(II/III) that can oxidize U(IV) or reduce U(VI) they may strongly influence the redox state and mobility of uranium.

Standard extraction procedures cannot be accurately applied for the determination of clay-Fe(II/III) and U(IV/VI) in clay mineral-U suspensions such that advanced spectroscopic techniques are required. Instead, we developed and validated a sequential extraction method for determination of clay-Fe(II/III) and U(IV/VI) in clay-U suspensions. In our so-called "H_3PO_4-HF-H_2SO_4 sequential extraction" method, H_3PO_4-H_2SO_4 is used first to solubilize and remove U, and the remaining clay pellet is subject to HF-H_2SO_4 digestion (Figure 1). Physical separation of U and clay eliminates valence cycling between U(IV/VI) and clay-Fe(II/III) that otherwise occurred in the extraction solutions and caused analytical discrepancies. We further developed an "automated anoxic KPA" method to measure soluble U(VI) and total U (calculate U(IV) by difference), and modified the conventional HF-H_2SO_4 digestion method to eliminate a series of time-consuming weighing steps. We measured the kinetics of uraninite oxidation by nontronite using this sequential extraction method and anoxic KPA method and measured a stoichiometric ratio of 2.19 ± 0.05 mol clay-Fe(II) produced per mol U(VI) produced (theoretical value of 2.0). We found that we were able to recover 98.0 – 98.5% of the clay Fe and 98.1 – 98.5% of the U through the sequential extractions. Compared to the theoretical stoichiometric ratio of 2.0, the parallel extractions of 0.5 M HCl for clay-Fe(II) and 1 M $NaHCO_3$ for U(VI) leached two-times more Fe(II) than U(VI). The parallel extractions of HF-H_2SO_4 for clay Fe(II) and 1 M $NaHCO_3$ for U(VI) leached six-times more Fe(II) than U(VI). Results from this study were published in Luan and Burgos (2012) Sequential extraction method for determination of Fe(II/III) and U(IV/VI) in suspensions of iron-bearing phyllosilicates and uranium. *Environmental Science & Technology.* 46, 11995-12002.

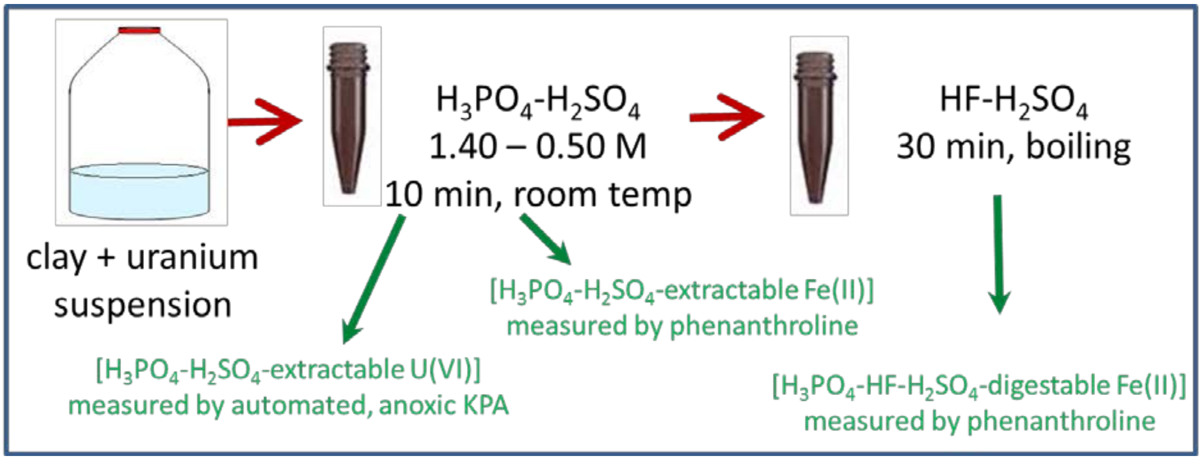

Figure 1. Schematic of sequential extraction procedure developed as part of this project.

Bioreduction of clay-Fe(III) minerals controlled by standard state reduction potentials

We examined the co-reduction of structural Fe(III) in the Fe-rich nontronite NAu-2 and U(VI) by *Shewenalla putrefaciens* CN32. In our first experiments, we examined if U played an active or passive role in the bioreduction of structural Fe(III) by tracking the rate and extent of reduction. We hypothesized that U would play an active role by serving as an electron shuttle for the bacteria, which would influence the kinetics, but not the extent, of reduction. Experiments were also conducted to determine what factors influence the extent of reduction; including the presence or absence of an electron shuttle, and the length of the incubation period. We also performed a series of abiotic experiments in which NAu-2 was exposed to biogenic uraninite (U(IV)), reduced AQDS (AH_2DS), or dithionite. Using reduction potential values for NAu-2 recently measured by our group, we found good agreement between calculated reduction potential values of structural Fe(II)/Fe(III) in NAu-2, U(IV)/U(VI), and AQDS/AH_2DS, suggesting that thermodynamics likely controls the extent of bioreduction of clay minerals in natural systems.

The bioreduction of soluble U(VI) to sparingly soluble U(IV) can promote the reduction of clay-Fe(III) through valence cycling. The reductive precipitation of U(VI) to uraninite was previously reported to occur only after a substantial percentage of clay-Fe(III) had been reduced. Using improved analytical techniques we show that concomitant bioreduction of both U(VI) and clay-Fe(III) by *Shewanella putrefaciens* CN32 can occur. Soluble electron shuttles were previously shown to enhance both the rate and extent of clay-Fe(III) bioreduction. Using extended incubation periods we show that electron shuttles enhance only the rate of reduction (overcoming a kinetic limitation) and not the final extent of reduction (a thermodynamic limitation). The first 20% of clay-Fe(III) in nontronite NAu-2 was relatively “easy” (i.e., rapid) to bioreduce while the next 15% of clay-Fe(III) was “harder” (i.e., kinetically-limited) to bioreduce and the remaining 65% of clay-Fe(III) was effectively biologically un-reducible. In abiotic experiments with NAu-2 and biogenic uraninite, 16.4% of clay-Fe(III) was reduced in the presence of excess uraninite. In abiotic experiments with NAu-2 and AH_2DS, 18.5 to 19.1% of clay-Fe(III) was reduced in the presence of excess and variable concentrations of AH_2DS. A thermodynamic model based on published values of the non-standard state reduction potentials at pH 7.0 (E'_H) showed that the abiotic reactions between NAu-2 and uraninite had attained apparent equilibrium (Figure 2). This model also showed that the abiotic reactions between NAu-2 and AH_2DS had attained apparent equilibrium. The final extent of clay-Fe(III) reduction was well correlated to the standard state reduction potential at pH 7.0 ($E^{0\prime}_H$) of all of the reductants used in these experiments (AH_2DS, CN32, dithionite, uraninite). Results from this study were published in Luan et al. (2014) Thermodynamic controls on the microbial reduction of iron-bearing nontronite and uranium. *Environmental Science & Technology*. 48, 2750-2758.

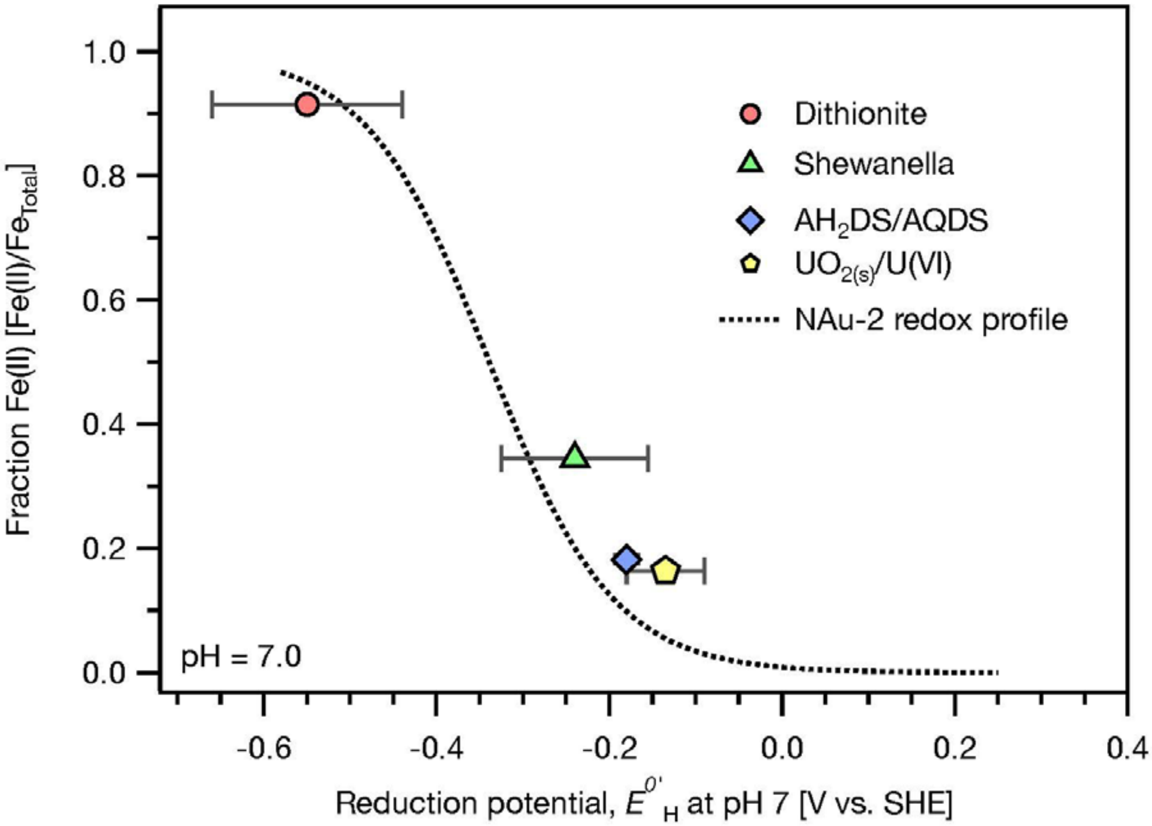

Figure 2. Measured fractions of Fe(II) vs. reported standard state reduction potential values at pH 7.0 ($E^{0\prime}_H$) for four reductants. The dashed line is the fitted $E^{0\prime}_H$ profile for clay-Fe(II/III) in nontronite NAu-2 as determined by mediated electrochemical reduction.

Linear Free Energy Relationships (LFERs) between clay-Fe(II) and contaminants

Prior studies have provided important mechanistic insights into redox reactions that occur between iron-bearing clay minerals and contaminants. In some cases, researchers found that contaminant reduction potentials relate to reduction rates. What remained unclear from these studies was if and how the reduction potential of structural Fe(II) in the clay mineral ($E_{H,clay}$) also influenced contaminant reduction rates. Answering this question has been historically challenging due to difficulties in measuring $E_{H,clay}$. Recently, however, our group developed a mediated electrochemical technique to measure reduction potential values for structural Fe in clay minerals as a function of Fe(II)/$Total_{Fe}$. These measurements provided the first redox profile distributions to relate the reduction extent of structural Fe(II) to $E_{H,clay}$, which now makes correlations between $E_{H,clay}$ and contaminant reduction rates possible.

Nitroaromatic compounds (NACs) are ubiquitous environmental contaminants that are susceptible to biological and abiotic reduction. Prior works have found that for the abiotic reduction of NACs, the logarithm of the NACs' rate constants correlate with one-electron reduction potential values of the NACs ($E^1_{H,NAC}$) according to linear free energy relationships (LFERs). Here we extend the application of LFERs to the bioreduction of NACs and to the abiotic reduction of NACs by bioreduced (and pasteurized) iron-bearing clay minerals. A linear correlation (R^2=0.96) was found between the NACs' bioreduction rate constants (k_{obs}) and $E^1_{H,NAC}$ values. The LFER slope of log k_{obs} versus $E^1_{H,NAC}/(2.303RT/F)$ was close to one (0.97), which implied that the first electron transfer to the NAC was the rate-limiting step of bioreduction. LFERs were also established between NAC abiotic reduction rate constants by bioreduced iron-bearing clay minerals (montmorillonite SWy-2 and nontronite NAu-2) (Figure 3). The second-order NAC reduction rate constants (k) by bioreduced SWy-2 and NAu-2 were well correlated to $E^1_{H,NAC}$ (R^2 = 0.97 for both minerals), consistent with bioreduction results. However, the LFER slopes of log k versus $E^1_{H,NAC}/(2.303RT/F)$ were significantly less than one (0.48-0.50) for both minerals, indicating that the first electron transfer to the NAC was not the rate-limiting step of abiotic reduction.

The second-order rate constants were found to be relatively similar for Sy-2 and NAu-2, varying by at most a factor of 1.5. In comparison, previous studies using chemically-reduced clays varied by a factor of ~10,000. The differences in these studies point to the importance of the reduction potential of the clay minerals (Figure 3). In our experiments, we bioreduced or chemically reduced clay minerals to the same reduction potential. In previous studies, clays were fully reduced to final potential that differed by over 250 mV and the more reduced clays reacted with the NACs at much faster rates. Because most contaminated aquifers become reduced via biological activity, the reactivity of biogenic clay-Fe(II) towards reducible contaminants is particularly important.

Finally, we demonstrated that the rate of 4-acetylnitrobenzene reduction by bioreduced SWy-2 and NAu-2 correlated to the reduction potential of the clay ($E_{H,clay}$, R^2 = 0.95 for both minerals), indicating that the clay reduction potential also influences its reactivity (Figure 4). Results from these studies were published in Luan et al. (2015a) Iron(III)-Bearing Clay Minerals Enhance Bioreduction of Nitrobenzene by *Shewanella Putrefaciens* CN32. *Environmental Science & Technology*. 49, 1418−1426, and Luan et al. (2015b) Linear Free Energy Relationships for the Biotic and Abiotic Reduction of Nitroaromatic Compounds. *Environmental Science & Technology*. 49, 3557-3565.

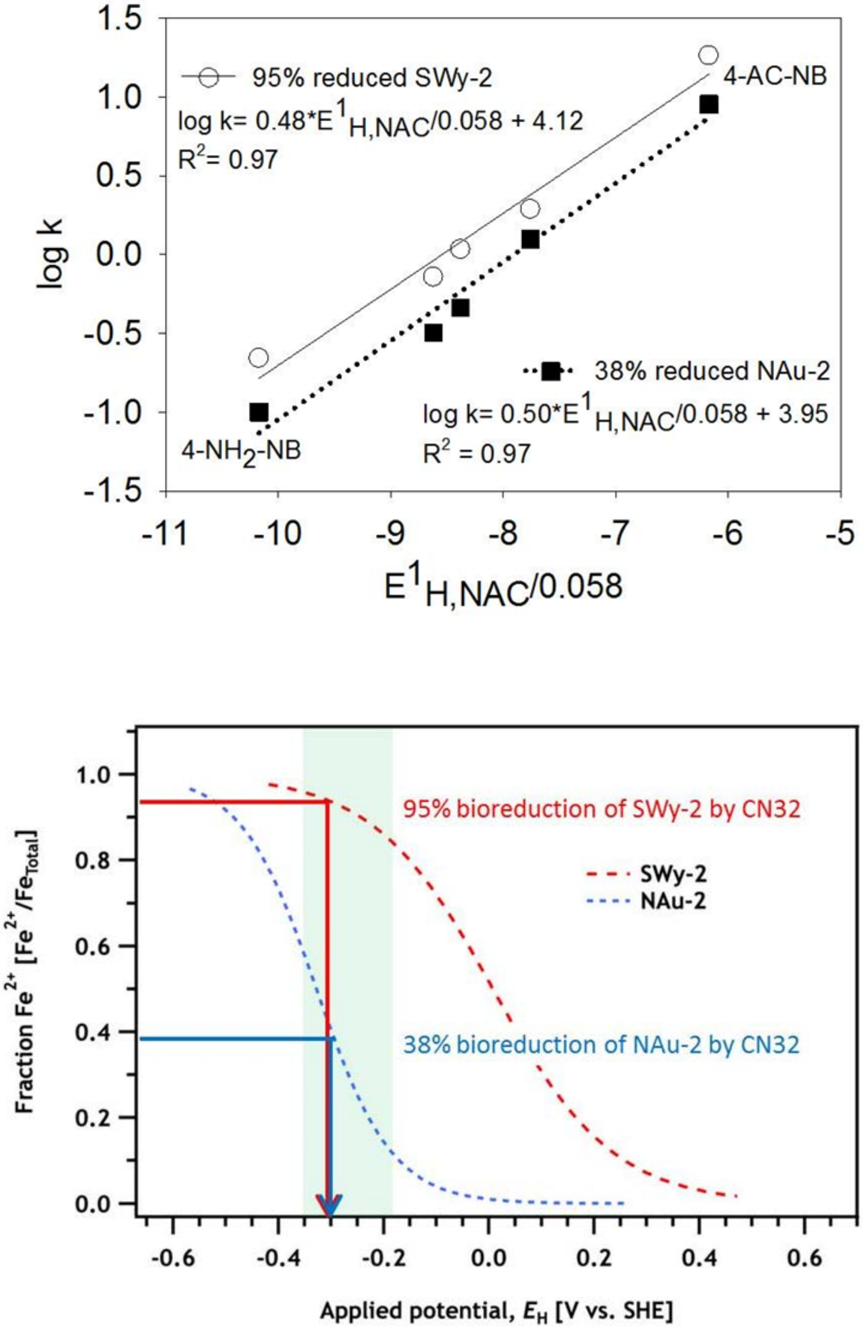

Figure 3. Linear free energy relationships for second-order log k versus $E^1_{H,NAC}/0.058$ (at 20°C) for the abiotic reduction of five NACs by bioreduced clay minerals (top). Redox profiles ($E_{H,clay}$) of montmorillonite SWy-2 and nontronite NAu-2 as determined by mediated electrochemical reduction (bottom). The shaded region in the bottom panel denotes the range of reduction potentials reported for respiratory enzymes of *Shewanella* species.

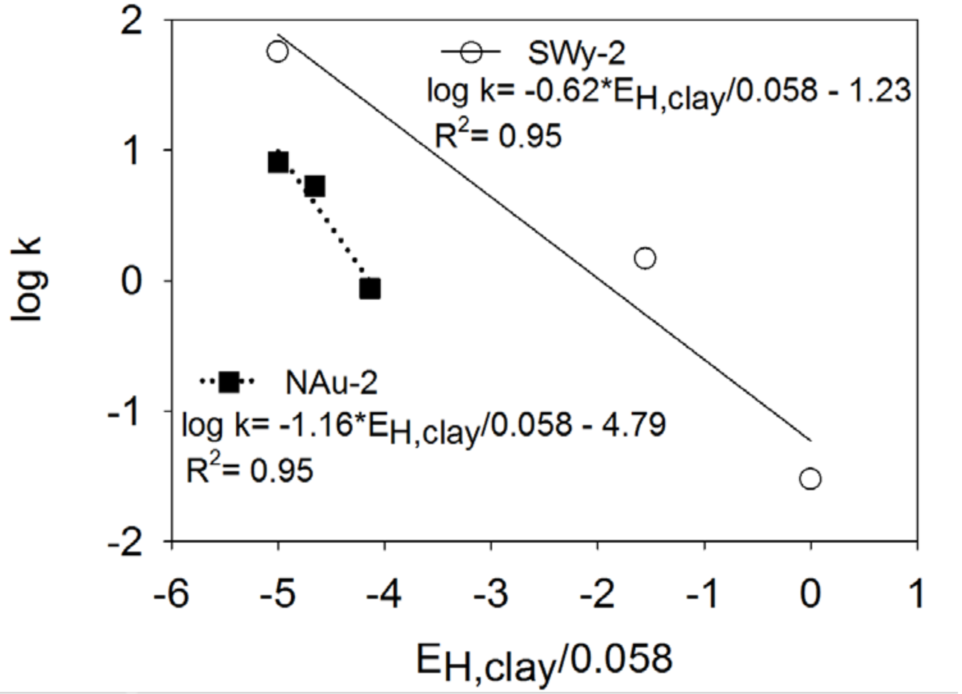

Figure 4. Abiotic reduction of 4-acetyl-nitrobenzene by clay-Fe(II) in clay minerals bioreduced to variable extents. Linear free energy relationship for log k versus $E_{H,clay}$/0.058 (at 20°C) for the abiotic reduction of 4-AC-NB by bioreduced clay minerals.

Reactivity of reduced smectites towards technetium(VII) and chromium(VI)

The extent and initial rate of bioreduction of structural Fe(III) in clay minerals were positively correlated with the percent smectite in the S–I series (i.e., layer expandability). Fe(II) in the bioreduced clay minerals was reactive to reduce Tc(VII) to Tc(IV). Reduced NAu-2 showed the highest reactivity toward Tc(VII), and reduced illite exhibited the least. The initial rate of Tc(VII) reduction was positively correlated with the percent smectite in the S–I series. Fe(II) in chlorite and palygorskite was also reactive toward Tc(VII) reduction. These data demonstrate that crystal chemical parameters (layer expandability, Fe and Fe(II) contents, and surface area, etc.) play important roles in controlling the extent and rate of bioreduction and the reactivity toward Tc(VII) reduction. Reduced Tc(IV) resides within clay mineral matrix, and this association could minimize any potential of reoxidation over long term.

Smectite minerals such as nontronite and montmorillonite were most effective in reducing aqueous Cr 6+. For all the clay minerals, the ratio of total Fe(II) oxidized to Cr 6+ reduced was close to the expected stoichiometric value of 3. Characterization of the Cr-clay reaction product with electron microscopy revealed that reduced chromium was possibly in the form of sub-nanometer Cr2O3 in association with residual clay minerals as micro-aggregates. This textural association was expected to minimize the chance of Cr(III) reoxidation upon exposure to oxidants. These results are important for our understanding of how various clay minerals may be used to reductively immobilize the heavy metal contaminant Cr in the environment.

Microbial reduction of clay-Fe(III) and metal contaminants

Thermophilic methanogen *M. thermautotrophicus* is capable of reducing reduce Cr(VI) to toxic Cr(III) and Co(III) to Co(II) with potential application in metal bioremediation, especially at high temperature subsurface radioactive waste disposal sites.

Sulfate-reducing bacterium *Desulfovibrio vulgaris* was able to reduce structural Fe(III) in smectite-illite minerals, and AQDS enhanced the reduction rate and extent. In the presence of AQDS, sulfate had little effect on Fe(III) bioreduction. In the absence ofAQDS, sulfate increased the reduction rate and capacity, suggesting that sulfide produced during sulfate reduction reacted with the phyllosilicate Fe(III). The extent of bioreduction of structural Fe(III) in the clay minerals was positively correlated with the percentage of smectite and mineral surface area of these minerals. X-ray diffraction, and scanning and transmission electron microscopy results confirmed formation of illite after bioreduction. These data collectively showed that *D. vulgaris* could promote smectite illitization through reduction of structural Fe(III) in clay minerals.

RESEARCH PRODUCTS:

Luan, F., Liu, Y., Griffin, A.M., Gorski, C.A., and Burgos, W.D. (2015a) Iron(III)-Bearing Clay Minerals Enhance Bioreduction of Nitrobenzene by *Shewanella Putrefaciens* CN32. *Environmental Science & Technology*. 49, 1418−1426.

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