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Elucidating Bioreductive Transformations within Physically Complex Media: Impact on the Fate and Transport of Uranium and Chromium

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SUMMARY

Uranium and chromium are two elements of particular concern within the DOE complex that, owing to their abundance and toxicity, appear well suited for biologically mediated reductive stabilization. Subsurface microbial activity can alter the redox state of toxic metals and radionuclides, rendering them immobile. Furthermore, anaerobic bacterial metabolic products will help to buffer pulses of oxidation, typically from fluxes of nitrate or molecular oxygen, and thus may stabilize reduced contaminants from oxidative mobilization. Imparting an important criterion on the probability that contaminants will undergo reductive stabilization, however, is the physical nature along with the chemical and physical heterogeneity of the media. In our study we have been investigating the impact of chemical/mineralogical heterogeneity on uranium reduction, with an emphasis on iron transformations and resulting impacts on contaminant retention. We have, in particular, emphasized considering chemical/mineralogical and physical complexity on bioreduction of metals. Over the past year we have also made appreciable advances on discerning geochemical constraints on microbially mediated reduction of U(VI) and on means to discern spatial heterogeneity in operative biogeochemical reactions within soils and sediments.

RESEARCH OBJECTIVES

The goal of this research is to quantify bioreductive transformations controlling uranium and chromium sequestration, with a specific emphasis on variations in reactive components, inclusive of bacteria. Our work has sought to answer specific questions relevant to the success of the NABIR mission. Can uranium and chromium be stabilized with respect to transport through reductive stimulation within natural media? What spatial variation in degree and mechanisms/pathways of reductive stabilization exist? Our specific objectives of this research are to determine: (1) the impact of geochemical heterogeneity on metal reduction, (2) alterations in mineral reactivity with changes in surface composition, (3) the stability of reaction products, and (4) a comprehensive kinetic reaction model for uranium and chromium reduction.

RESEARCH PROGRESS AND IMPLICATIONS

The fate and transport of radionuclides such as uranium and heavy metals such as chromium are dictated in part by biogeochemical transformations of the contaminant and phases comprising the environmental matrix. Ferric (hydr)oxides, for example, are ubiquitous components of surface and subsurface environments that have critical controls on biogeochemical cycles of carbon and most trace elements, and thus biogeochemical transformations of iron (hydr)oxides have direct impacts on contaminant cycling. We have therefore been investigating the biogeochemical controls on the fate and transport of uranium in

chromium with a particular emphasis on transformation of iron phases within subsurface environment. Our studies encompass increasing complexity within the chemical/mineralogical, biological, and physical realms while continuing to seek molecular-level detail on operating biogeochemical processes. Specifically, we have conducted a series of experiments ranging from static-flow systems with a single mineral phase to hydrodynamic column experiments having the full suite of biological and chemical heterogeneity observed within subsurface environments. In sum, our studies provide revealing details on the biogeochemical factors exerting dominant impacts on contaminant fate.

Iron (Hydr)oxide Transformation

Owing to its high surface area and intrinsic reactivity, ferrihydrite, a short-range order ferric hydroxide common to soils and sediments, serves as a dominant sink for numerous metals in subsurface environments and is considered the primary Fe-phase for microbial metabolism (Fredrickson et al., 1998). However, ferrihydrite may be a short-lived intermediate within such systems and its fleeting presence will have profound impacts on metal biogeochemical cycles (Benner et al., 2002; Hansel et al., 2003; Zachara et al., 2002). Introduction of Fe(II) by reductive dissolution of Fe(III) minerals, for example, converts ferrihydrite to Fe phases varying in their retention and reducing capacity (Fendorf et al., 2000). Thus, the fate of numerous (in)organic ions, along with the decomposition of organic matter, is dictated by the Fe(II)-induced secondary mineralization of ferrihydrite. While Fe(II) concentration is the master variable dictating secondary mineralization pathways following reductive dissolution of ferrihydrite, our studies reveal that the kinetics of conversion and ultimate mineral assemblage are a function of competing mineralization pathways influenced by pH and stabilizing ligands.

Natural environments often include numerous organic and inorganic constituents that may appreciably alter the reactivity of iron (hydr)oxides relative to pristine synthetic analogs, which are frequently used in laboratory experiment. Many oxyanions (e.g. phosphate and arsenate) interact strongly with iron (hydr)oxides via surface complexation and may therefore affect Fe(III) reducibility, stability, and mineralization pathways. A systematic study examining modifications in surface composition on microbially mediated iron transformations was lacking. Accordingly, we investigated the impact of surface-associated oxyanions at varying coverages, using phosphate as a model constituent, on the extent of ferrihydrite reduction and resulting biomineralization pathway under static- and dynamic-flow conditions.

We conducted batch and column studies examining ferrihydrite bioreductive transformation with a systematic variation in phosphate surface coverage. Ferrihydrite was reacted with PO_4^{3-} at concentrations from 10 μM to 2000 μM in synthetic groundwater medium for three days. Phosphate adsorption was well described by the Langmuir equation, and the monolayer sorption (Γ) was 0.28 g P Kg^{-1} solids (or 797 mmole P Kg^{-1} ferrihydrite). Batch reactions were performed with synthetic groundwater medium and ferrihydrite-coated sand. Phosphate was coated to various degrees (50 and 100 % of the adsorption maximum, Γ_{max}) on the ferrihydrite-coated sand. The role of phosphate on secondary mineralization was investigated by inoculation with *S. putrefaciens* to a cell density of $\sim 10^8 \text{ mL}^{-1}$ (biotic) or by reacting 0.2 or 2 mM ferrous sulfate (abiotic) with the (P-)Fe-coated sand for 7 d. Flow experiments were conducted in columns (3.8 cm diameter (ID)) with 4 sampling ports located at 1.7, 5, 8.3, and 11.7 cm along the flow-path. Ferrihydrite-coated sand was coated with phosphate to Γ_{max} , washed once in synthetic groundwater medium, and then inoculated with *S. putrefaciens*

to a cell density of $\sim 8 \times 10^8 \text{ g}^{-1}$. The flow rate was maintained at 373 mL d^{-1} (2.9 pore volumes) upward through the column.

An inverse correlation between ferrous iron production and surface-associated phosphate illustrates how strongly sorbing oxyanions stabilize the structure of ferrihydrite toward dissimilatory Fe reduction. Additionally, the extent of ferrihydrite reduction being modified, the transformation pathway is also vastly different in the presence of phosphate. A four-fold decrease in the extent of ferrihydrite transformation is observed in the presence of phosphate at maximum surface coverage, correlating well with the corresponding decrease in Fe(II) production. The most notable difference in the secondary iron phases is the apparent inhibition of goethite. Magnetite and green rust formation, albeit not the proportion, is in concurrence with previous studies performed in a minimal growth medium (Glasauer et al., 2003) but differs from the secondary phases formed (i.e. vivianite and siderite) under condition where the growth medium is optimized to achieve more rapid rates of reduction (Fredrickson et al., 1998).

In contrast to the extent of ferrihydrite mineralization (44 and 59 % transformed) observed in the absence of phosphate, only 10 to 20 % of the ferrihydrite is mineralized in the presence of phosphate. This result supports the premise that phosphate stabilizes the structure of ferrihydrite by inhibiting reduction, poisons the formation of goethite, and promotes the formation of green rust and vivianite—the latter which forms only at high ferrous iron and phosphate concentrations. The formation of carbonate green rust under biotic conditions in the absence of phosphate is likely a result of the microbial respiration which results in the formation and accumulation of bicarbonate under batch conditions. .

Bioreduction and mineralization pathways of iron (hydr)oxides are influenced by strongly sorbing oxyanions such as phosphate. Our studies illustrate slow respiration on a presumably ‘bioavailable’ form of Fe(III), even when supplied with an electron donor (lactate) concentration of 3 mM; ‘leaner’ conditions common to most natural environments would likely exaggerate the limited reduction of phosphated ferrihydrite. Additionally, the absence of goethite and typical lower fraction of crystalline Fe phases in phosphatic soils results from a stabilization of ferrihydrite. Thus, when considering the reactivity of iron phases, it is important to appreciate modifications induced by surface composition such as that noted here for phosphate.

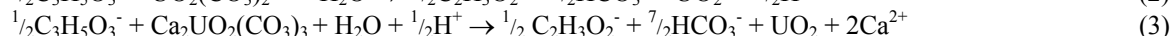
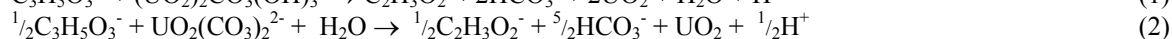
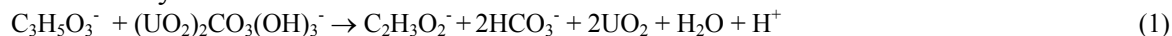
Geochemical Constraints on Uranium Reduction

In soils and sediments, the oxidized state of uranium (U(VI)) is typically mobile, particularly under conditions conducive to the formation of uranyl-carbonate complexes. While in contrast, the reduced state (U(IV)) forms phases with limited solubility such as uraninite. Like other toxic metals and radionuclides (e.g. Cr, Tc), the fate and transport of uranium is in part dictated by microbial mediated reduction—which, as noted above, yields the subsequent precipitation U(IV) phases such as uraninite. While biological or biologically mediated reduction of uranium is well demonstrated within isolated and constrained laboratory experiments, factors confounding uranium reduction within natural environments remain unresolved. We have therefore conducted a series of experiments that explore the impact of completing electron acceptors and uranyl species on reductive stabilization of uranium.

A critical factor affecting the mobility and reducibility of uranium is the aqueous speciation of uranyl. Uranyl-carbonate complexes have classically been considered the dominant aqueous species of U(VI); however, recently $\text{Ca-UO}_2\text{-CO}_3$ ($\text{CaUO}_2(\text{CO}_3)_3^{2-}$, $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$) complexes have been described. We initiated our experiments examining the influence of calcium on uranyl reduction within complex mineralogical assemblages containing

iron coated sands under dynamic flow conditions. Parallel flow systems of ferrihydrite coated sands inoculated with *Shewanella putrefaciens* were investigated, one containing a feed solution 4 mM Ca and the other having no Ca. The presence of dissolved calcium in combination with Fe(III) dramatically limits the extent of dissimilatory uranium reduction. No reduction, and minimal retention, transpires in the presence of Ca while reductive precipitation is so extensive in the absence of Ca that U(VI) never transports more than 5-cm within the flow-field and results in nearly 4000 mg Kg⁻¹ of uranium over a 54 d reaction period (and uranyl reduction shows no signs of diminishing). Here, uranium is sequestered near the inlet-flow area via reduction of U(VI) to a less soluble U(IV) precipitate that resides both in periplasmic space and on the outer membrane.

Although a multitude of U(VI) species reside under the conditions evaluated within this study, there are two dominant species, Ca₂UO₂(CO₃)₃, (UO₂)₂CO₃(OH)₃⁻, under the geochemical conditions of this study; a third species, UO₂(CO₃)₂²⁻, resides at appreciable fractions of the total uranium. Considering these three species, reduction reactions coupled with lactate oxidation are described by the stoichiometries of reactions 1 to 3.



Rate data for the various systems conform reasonably well to a pseudo-first order kinetic expression dependent on the total U(VI) concentration and having an observed (or overall) rate coefficient representing various factors.

$$- \frac{d[U(VI)]}{dt} = k_{obs}[U(VI)]$$

The observed rate coefficient, k_{obs} , for U(VI) reduction in the absence of Ca is the fraction of total uranium residing in the carbonato complex. With the exception of systems with ferrihydrite, which acts as a competing electron acceptor, the averaged k_1 value ($k_1 = k_{obs}/K_p$, where K_p is the proportion of carbonato species and is equal to 1 in the absence of Ca) is $1.18 \times 10^{-2} \text{ h}^{-1}$, giving the following operative rate expression.

$$- \frac{d[U(VI)]}{dt} = k_1[(UO_2)_x(CO_3)_y]$$

In Ca-bearing solutions, the rate expression becomes more convoluted and would be described by an observed rate coefficient encompassing the initial proportion of the U-carbonato complex, its rate of regeneration, and the rate of Ca ternary complex reduction. The rate expression for the reduction of U(VI) is therefore

$$- \frac{d[U(VI)]}{dt} = k_1[(UO_2)_x(CO_3)_y] + k_2 [Ca_2UO_2(CO_3)_3] + k_1k_3[Ca_2UO_2(CO_3)_3]$$

The decrease in rate coefficient with increased Ca concentrations (Figure 4) indicates that $k_1 \gg k_3$, leading to the simplification

$$- \frac{d[U(VI)]}{dt} = (k_2 + k_3) [Ca_2UO_2(CO_3)_3]$$

Decreasing rate coefficients with increased Ca concentrations described in Table 4 thus represent the combined contributions of k_2 and k_3 and increased proportions (effective concentration) of the Ca ternary complex. The observed rate coefficient would be proportional to $k_{2,3}$ (where $k_{2,3} = k_2 + k_3$), with the proportionality coefficient (K_p) again being the fraction of total uranium residing in the ternary complex ($K_p = -1.15[Ca] + 0.97$) and can be described as $\ln k_{2,3} = 30.5[Ca_2UO_2(CO_3)_3] - 7.18$.

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