The Role of Biogenic Solids in the Reductive Stabilization of Metal Contaminants: Influences on Microbial Versus Chemical Pathways and Reaction Products

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PI: Scott Fendorf
Dept. of Geological and Environmental Sciences
Stanford University
Stanford, CA 94305
Phone: 650)723-5238
fendorf@stanford.edu
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INTRODUCTION

In situ stabilization of toxic metals and radionuclides such as chromium and uranium is an attractive approach for remediating many contaminated DOE sites. To enhance in situ remediation, microbiological reductive stabilization of contaminant metals has been, and continues to be, actively explored. It is likely that surface and subsurface microbial activity can alter the redox state of toxic metals and radionuclides so they are rendered immobile. The reaction products and their stability will depend on the specific mechanism by which reduction takes place—the focus of this work.

Uranium and Cr exist in more than one oxidation state in the surface- and near-surface environment; different oxidation states of these elements have markedly different properties. The fully oxidized species of Cr and U, both hexavalent forms, have a high solubility in soils and groundwater, and consequently they tend to be mobile in the environment. Chromate is also subject to biological uptake, a factor that contributes to its toxicity (Turner and Rust, 1971; Venitt and Levy, 1974). In contrast, the reduced form of chromium, Cr(III), has a limited hydroxide solubility and forms strong complexes with soil minerals; it is therefore less mobile and has a lower bioavailability than Cr(VI) (Sass and Rai, 1987; Fendorf et al., 1992). Similarly, the oxidized forms of U have a greater solubility (Smith and Martell, 1982) and hence exhibit greater mobility in soils and subsurface environments than either U(IV) or U(V) (Duff et al., 1999). We may conclude that oxidized forms of these radionuclides and metals are subject to enhanced migration through surface and subsurface environments. Reductive stabilization is therefore a desirable pathway for these elements.

Importance of Iron in Contaminant Fate

Microbially and chemically induced mineral transformations within soils have a profound influence on the mobility and toxicity of contaminants. Iron minerals in particular often control the cycling of trace metals and thus have a profound impact on the migration and bioavailability of contaminants such as chromium and uranium. The
cycling of iron in surface and subsurface environments is controlled by microbial processes and the geochemical environment, which itself is in part a manifestation of microbial activity. Accordingly, we have invested a large effort in defining the evolution of iron phases in a transition from an aerobic to an anaerobic state—a transition that will be invoked by carbon addition, whether it be natural or anthropogenically induced. Previously studies have described possible products derived from batch experiments. Here we conduct experiments under advective flow and provide quantitative results (rates and products) on the biomineralization of iron. As a consequence of our findings over the past two years, one can make an accurate projection on the interaction of uranium and chromium in surface or subsurface materials undergoing active iron reduction.

The importance of microorganisms in the biogeochemical cycling of Fe is well-recognized. Dissimilatory iron reducing bacteria (DIRB), which are ubiquitous in aquatic soils and aquifers, couple the oxidation of organic matter or $\text{H}_2$ to the reduction of various Fe(III) oxide phases in order to obtain energy for growth and function. Although iron-reducing bacteria can utilize crystalline iron oxides as well as poorly crystalline phases, the latter are more bioavailable and hence considered the primary terminal electron acceptor for DIRB (Roden et al., 2000). This phenomena is thought to be responsible for the persistence of more crystalline iron oxides in the environment (Lovley and Phillips, 1986).

Ferrous iron solid phases observed in batch (closed) systems following microbial respiration of ferrihydrite under varying environmental conditions include siderite, magnetite, vivianite, and green rust (Fredrickson et al., 1998). The chemical environment in which microbial iron reduction occurs determines the rate and extent of iron reduction and the nature of the reduced solids. Factors dictating the specific biomineralization pathway may include pH, redox potential, carbonate concentration and, mostly, respiration-driven biogenic Fe(II) supply rate and magnitude.

Microbial reduction of crystalline and poorly crystalline iron oxides can be limited in static flow systems by passivation of the oxide and cell surface by Fe(II) (Roden et al., 1996), a factor limited under advective-flow (Roden et al., 2000). We have therefore investigated prolonged microbial reduction of short-range ordered Fe(III)
oxides under advective flow using *Shewanella putrefaciens* (CN32) and 2-line ferricydrite-coated sand in an artificial groundwater and bicarbonate buffered media. We further investigate the solid-phases produced following dissimilatory iron reduction emphasizing the evolution of the secondary precipitates and their relationship with Fe(II) concentration throughout the column. The progression of Fe phase transformations is monitored both temporally and spatially (throughout the column). Ultimately, we use the information gleaned from this study to propose mechanisms of Fe biomineralization and the role of microorganisms in solid-phase transformations of ferricydrite.

**EXPERIMENTAL ATTRIBUTES**

We examined microbiologically-induced Fe mineral transformations using *Shewanella putrefaciens* strain CN32 (hereinafter referred to as CN32) in an artificial groundwater media under advective flow. Experiments were conducted using columns having a 3.8 cm diameter and a 25 cm length that were packed with bacterially inoculated ferricydrite-coated sand. Flow velocities through the column were maintained at 3 pore volumes per day (water velocity of 0.6 m/d). Our experiments provide a unique combination of biological, chemical, and hydrologic processes that are examined with state-of-the-art spectroscopic and microscopic techniques.

**IRON BIOMINERALIZATION**

Effluent lactate concentrations decrease to markedly within the first 12 h and then rapidly increased by 24 h after reaction initiation. From day 2 to 15, lactate concentrations gradually increase toward the influent concentration. Correspondingly, Fe and acetate concentrations in the column effluent mirror those of lactate, peaking at ~1 d, both concentrations nearly 1 mM.

Spatial trends in acetate and lactate concentration along the column mirror each other as well. Acetate concentrations decrease over the course of the experiment. While acetate concentrations increase almost linearly upgradient, Fe concentrations increase
markedly at 7 cm from the inflow over the course of the experiment. Additionally, while the shape of acetate and lactate profiles are similar over time, Fe profiles throughout the column change substantially. Of considerable interest is the low Fe(II) concentrations at the bottom of the column -- ferrous iron concentrations average 0.02 mM within the bottom portion (3 cm) of the column throughout time.

Microbial numbers decrease several orders of magnitude within the columns over the first 16 d of reaction. After 2 d, the microbial community is uniformly distributed throughout the column having ~10^8 colony forming units (CFU) g^-1. Two days later, the microbial numbers decrease an order of magnitude throughout the column, except for the bottom section, which decreases nearly 3 orders of magnitude. Following 16 d, a more heterogeneous microbial distribution is observed ranging from 10^3 to 10^4 CFU g^-1. However, the bottom section of the column does not significantly decrease in microbial numbers between 4 and 16 d.

Solid-phase extractions indicate a relatively homogeneous distribution of Fe. The predominant solid-phase products are goethite and magnetite. After 2 d of reduction, goethite concentrations range from 15-21% and magnetite from 0-15%. Omitting the initial 2 cm of the column, magnetite and goethite proportions appear to mirror each other. Following additional reduction, the proportion of goethite remains relatively constant, ranging from 13-23% at 4 d and 8-23% after 16 d. Magnetite, however, continues to increase through time, peaking at 59% following 16 d. Between 4 and 16 d, goethite formation ceases and magnetite increases with the proportions of magnetite mirroring those of ferrihydrite. Goethite is predominantly associated with the surface of ferrihydrite with minor amounts associated with the microbial cell surface. Magnetite crystals average ca. 50 nm and display both cubic and botryoidal crystal habits and are associated with the ferrihydrite surface and not the cell envelope.

IMPLICATION ON IRON CYCLING AND CONTAMINANT FATE
Temporal changes in effluent acetate and Fe concentrations suggest an initial period of rapid bacterial reduction (first 10 days), followed by a period of much slower reduction (10 to 48 days). Stoichiometric amounts of acetate were produced with lactate consumed,
suggesting that the primary organic carbon-consuming reaction is the incomplete oxidation of lactate to acetate and CO₂.

If the reaction products were conservative, the effluent would contain 4 moles of Fe(II) for every mole of acetate. For the first 10 days of the experiment, the molar ratio of dissolved Fe(II) to acetate was <1:1, indicating that during this period of rapid bacterial reduction most of the produced Fe(II) accumulated in the column. The initial reaction period corresponds to the greatest visual change in the column, with a shift in color from orange to black. Indeed, the dominant sink for Fe(II) appears to be magnetite. At the effluent end of the column magnetite is the dominant secondary product while at the influent end goethite formation is observed. Pore-water concentrations suggest a driving force for solid-phase conversion: at a given pH and alkalinity, aqueous Fe(II) concentrations dictate the resulting solid. At low iron concentrations, ferrihydrite converts to goethite while higher concentrations lead to the development of magnetite.

The accumulation of magnetite and goethite within the column corresponds with a 10-fold decrease in the rate of reduction despite abundant electron donor (lactate). Although goethite and magnetite are metabolized by iron reducing bacteria, the effective rate of reduction is much slower. Reduction rates of the remaining ferrihydrite diminish as well. Using the final observed rate of acetate production as an estimate of Fe(III) consumption, the predicted time to consume all of the remaining Fe(III) is >500 days. This value is 50 times longer than the 10 days required to consume the first 20% of the original Fe(III) present in the columns. Interestingly, despite the active reduction of ferrihydrite and associated mineralogical transformations, Fe remains predominantly in the ferric form—at the termination of these experiments only 20% of the original Fe had been lost to the pore water and, of the fraction remaining, >80% remains oxidized.

Our observations reveal the importance of coupled pore-water chemistry and advective flow on the products of microbially induced ferrihydrite reduction. They also illustrate the potential complexity of secondary mineral phase formation; three different mineral phases (the original substrate and 2 secondary products) were observed at appreciable levels in these 15 cm long column experiments. The formation of goethite and magnetite clearly alters the reactivity of the solid matrix toward reductive dissolution, contaminant retention, and redox reactivity.
Alterations in iron mineralogy will have profound consequences on contaminant fate. For elements strongly retained by ferric oxides, such as arsenic and phosphorus, reductive biomineralization will lead to a release of toxins into the dissolved phase. For example, phosphate retention decreases nearly 50-fold, on a per mass basis, upon conversion of ferrihydrite to magnetite. In contrast, biomineralization will lead to facile reductants potentially with the capacity to stabilize uranium and chromium. Indeed, simulations (which we will expanded upon and detail later) using initial reduction rates indicate that the fate of chromate will be defined entirely by dissolved and adsorbed Fe(II) along with green rust in biogenic iron systems. The fate of uranium, in contrast, is defined by microbial (enzymatic) reduction in combination with geochemical sorption to reduced minerals.

LITERATURE CITED


RESULTING PUBLICATIONS


