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MICROBIAL MINERAL TRANSFORMATIONS AT THE Fe(II)/Fe(III) REDOX BOUNDARY FOR SOLID PHASE CAPTURE OF STRONTIUM AND OTHER METAL/RADIONUCLIDE CONTAMINANTS

Principal Investigator: F. Grant Ferris
Institution: University of Toronto
Co- Principal Investigator: Eric E. Roden
Instituion: University of Alabama

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Table of Contents

Executive Summary

The migration of ^{90}Sr in groundwater is a significant environmental concern at former nuclear weapons production sites in the U.S. and abroad (*10, 22 ,35*). Although retardation of ^{90}Sr transport relative to mean groundwater velocity is known to occur in contaminated aquifers (20), Sr^{2+} does not sorb as strongly to iron oxides and other mineral phases as do other metal-radionuclide contaminants. Thus, some potential exists for extensive ^{90}Sr migration from sources of contamination (*35, 44*).

Chemical or biological processes capable of retarding or immobilizing Sr^{2+} in groundwater environments are of interest from the standpoint of understanding controls on subsurface Sr^{2+} migration. In addition, it may be possible to exploit such processes for remediation subsurface Sr contamination. In this study we examined the potential for the solid phase sorption and incorporation of Sr^{2+} into carbonate minerals formed during microbial Fe(III) oxide reduction as a first step toward evaluating whether this process could be used to promote retardation of ^{90}Sr migration in anaerobic subsurface environments.

The presence of Sr^{2+} at concentrations up to 1.0 mM was found to have no detrimental effect on bacterial Fe(III)-reduction. Under non-growth conditions, and with dead cells, Sr^{2+} was immobilized by sorption to the bacteria and hydrous Fe(III)-oxide (HFO). Applying surface complexation theory in the data analyses yielded equilibrium $Sr2^{+}$ sorption constants for the bacteria, HFO, and bacteria-HFO composites that can be utilized in geochemical modeling of contaminant fate and behavior. The bacteria alone were found to have a higher affinity and sorption capacity for Sr^{2+} than HFO or the bacteria-HFO composites, but exhibited a greater sensitivity to ionic strength, which is indicative of outer-sphere complexation reactions.

In actively growing cultures, the solid phase capture of Sr^{2+} was enhanced considerably by the precipitation of siderite ($FeCO_3$). The efficiency of Sr^{2+} immobilization ranged from 99% at an initial dissolved Sr^{2+} concentration of 0.01 mM, to 90% at a dissolved Sr^{2+} concentration of 1.0 mM over a 30 day time period. In each case, the presence of high concentrations of Ca^{2+} (i.e., 10 mM) as a competing ion reduced the extent of Sr^{2+} solid phase capture to approximately 30% of the initial total dissolved Sr^{2+}. Rapid removal of dissolved Sr^{2+} in response to calcium carbonate precipitation (90% decrease in less than 1 day) was also measured in cultures of bacteria that hydrolyze urea.

Our demonstration of Sr^{2+} capture in carbonate mineral phases formed during bacterial HFO reduction and urea hydrolysis suggests that microbial carbonate mineral formation could contribute to Sr^{2+} retardation in groundwater environments. This process may also provide a mechanism for subsurface remediation of Sr^{2+} and other divalent metal contaminants that form insoluble carbonate precipitates.

1.0 Research Objectives

The behavior of strontium and other metal/radionuclides in natural multi-phase chemical systems is a critical concern at DOE sites where they exist as ubiquitous and mobile contaminants (*4, 10*). The concentrations and migration velocities of these contaminants depend on a number of linked physical, chemical and microbiological processes. Many of these processes, including immobilization of metal contaminants in authigenic mineral precipitates of microbial origin, are not well understood. This is particularly true of authigenic reactions involving bacterial reduction of Fe(III)-oxides in subsurface sediments (*27*). For example, although contaminant metals adsorbed by Fe(III)-oxides appear to be prone to release following reductive dissolution by bacteria (*3, 18, 24*), the same process will cause an increase in alkalinity that is conducive towards authigenic carbonate mineral precipitation (*7*). Moreover, the overall reaction involving dissimilatory iron reducing bacteria (with acetate or hydrogen as an electron donor),

$$FeOOH + 3HCO_3^- + 2Me^{2+} + e^- = FeCO_3 + 2MeCO_3 + 2H_2O$$

shows that carbonate mineral precipitation can contribute directly to solid phase immobilization of dissolved metals (e.g., Me^{2+} occuring as strontium). It is possible that this process could exceed the original capacity of Fe(III)-oxides to capture and retain metal contaminants. In a broader sense, microbial induced mineral precipitation represents an innovative immobilization concept relevant to development of new clean-up technologies aimed at solving DOE's complex environmental problems.

The potential to exploit bacterial Fe(III)-oxide reduction to induce carbonate mineral precipitation for the solid phase capture of strontium and other metal/radionuclide contaminants is inferred from two parallel lines of evidence. From microbiological investigations carried out over the last decade, it has become clear that diverse communities of microorganisms live in subsurface environments (*11, 17, 27, 36*). Iron-reducing bacteria are particularly common in anaerobic subsurface environments, and have been identified as protagonists of carbonate mineral precipitation (*7, 24, 28*). At the same time, geochemical analyses indicate that strontium, uranium, and other metals are commonly incoporated via adsorption and solid solution (i.e., coprecipitation) into carbonate minerals that precipitate from natural groundwaters in response to microbial activity (*16, 29*).

Microorganisms in subsurface environments are generally segregated according to their physiology into distinct redox zones along groundwater and contaminant plume flow paths (*27*). The zonation evolves in response to microbial consumption of dissolved oxygen via aerobic respiration with subsequent production of low molecular weight organic acids by fermentation (*33, 36*). A succession of anaerobic respiratory processes follow the declining redox potential (i.e., nitrate and/or Mn (IV) reduction), eventually leading to the onset of bacterial Fe(III)-oxide reduction (*34*).

The anaerobic physiology of Fe(III)-reducing bacteria is an important advantage for the design of in situ treatment processes. Since Fe(III)-reducers are anaerobes,

provision of oxygen is not a concern. Moreover, many subsurface sediments contain up to several weight percent of iron oxides which thus comprise a built-in solid phase electron sink for anaerobic respiration (*28, 34*). In addition, iron oxides are often much more abundant than nitrate or Mn(IV) oxides in the subsurface, so that iron reduction generally dominates organic matter metabolism in "suboxic" sedimentary environments (*24*). Iron-reducing bacteria effectively outcompete sulfate-reducing and methane-producing microorganisms (*6, 24*) because of their ability to maintain the concentration of common energy yielding substrates (e.g., hydrogen and acetate) at concentrations below what can be effectively utilized by sulfate-reducers and methane-producers (*5, 6, 8*). Thus Fe(III)-reducers are not expected to be adversly affected by competition with the other major groups of bacteria in iron oxide-rich anaerobic environments.

Most of the Fe(III) in subsurface sediments occurs in complex mixtures of different oxide minerals that vary greatly in their crystallinity, particle size, surface area, and reactivity. Studies with pure synthetic oxides have demonstrated that poorly cyrstalline iron oxides (e.g., hydrous ferric oxide or ferrihydrite) are most susceptible to bacterial reduction, whereas more cystalline oxides like goethite (a-FeOOH), hematite (Fe2O3), and akaganeite (b-FeOOH) support slower rates of Fe(III) reduction and are reduced to a much lesser extent (*24, 51*). However, recent studies suggest that a considerable fraction of the crystalline Fe(III) oxide content of subsoil and subsurface materials is subject to microbial reduction (*41*), which suggests that such oxides could support a considerable amount of Fe(III) reduction in situations where such they are abundant.

As bacterial Fe(III) reduction proceeds, concentrations of dissolved Fe(II) typically increase owing to sustained reductive dissolution of Fe(III)-oxides. This phenomenon is commonly observed over large areas in natural aquifers where distinct regions of anaerobic high-iron groundwater exist (*28*). In laboratory batch cultures, the accumulation of Fe(II) tends to attenutate rates of continued Fe(III) reduction. This effect has been attributed to a decrease in accessibility to Fe(III) imposed by sorption of Fe(II) onto Fe(III)-oxide surfaces (*41*), as well as the surfaces of Fe(III)-reducing bacterial cells themselves (*49*). Whether other metals, in particular strontium, have the same inhibitory affect is not known.

Microbial precipitation of carbonate minerals has been extensively documented in freshwater, marine, and groundwater systems (*16, 21, 47*). In many of these studies, phototrophic cyanobacteria are cited as causative agents of carbonate deposition; however, iron-reducing bacteria are also known to mediate carbonate mineral precipitation (*7, 24, 40*). Despite the involvement of a wide range of microorganisms, microbially precipitated carbonate phases commonly exhibit an extremely fine-grain size (i.e., often less than 1.0 μm in diameter) (*15, 43*). This is normally attributed to rapid precipitation from highly oversaturated solutions (*44*). In addition, the precipitates accumulate extracellularly, but often appear to originate on the external surfaces of cells as well as within their sheaths or capsules. When microorganims grow in epilithic biofilms on sand grains or rock surfaces, porous carbonate coatings develop (*15, 16*).

This pattern of mineralization reflects of the inherent ability of microbial cells to serve as templates for heterogenous nucleation and crystal growth (*14, 39, 43*).

The ability of microbial cells to serve as efficient nucleation sites for the precipitation of carbonate minerals arises from their capcity to adsorb and concentrate dissolved metallic ions (*13, 14, 43, 49*). This uptake of metallic ions tends to shift the chemical equilibrium in favor of carbonate precipitation by decreasing the degree of oversaturation needed for crystal nucleation on the cell surface. In free energy terms, this corresponds to a lowering of the activation energy barrier that normally retards spontaneous crystal nucleation and growth (*45*). The effect is apt to be quite pronounced in metastable solutions that are at or oversaturated with respect to carbonate minerals (*44*).

Alkalinity changes (i.e., an increase in dissolved inorganic carbon concentrations and/or pH) that accompany the growth of many carbonate precipitating microorganisms provide a good explanation for the onset of mineral precipitation (*23, 45, 46*). In terms of solution chemistry, a rise in alkalinity can induce carbonate precipitation by increasing the degree to which water is oversaturated (*15, 16, 46*). The following general reactions apply to Fe(III)-reducing bacteria using acetate (equation 1) or hydrogen (equation 2) with goethite as a terminal electron acceptor (*7*):

$$8FeOOH + CH_3COO^- + 3H_2O = 8Fe^{2+} + 2HCO_3^- + 15OH^- \quad (1)$$

$$8FeOOH + 4H_2 = 8Fe^{2+} + 16OH^- \quad (2)$$

These equations show that production of bicarbonate and hydroxyl ions is expected from bacterial Fe(III)-oxide reduction using acetate as the electron donor. Conversely, Fe(III) reduction coupled to hydrogen oxidation yields only hydroxyl ions. In both cases, alkalinity should increase to make carbonate precipitation more favorable (*16, 46*). A rise in pH and precipitation of siderite ($FeCO_3$) is known to occur during growth of Fe(III)-reducing bacteria in bicarbonate-buffered culture media (*25, 26, 40*). In addition, the accumulation of siderite in some marine sediments and groundwater systems is interpreted initiated by these processes (*1, 7*). Ultimately, however, the type of carbonate mineral phase that precipitates as a result of microbial activity will be controlled by the chemical composition of the aqueous environment in which the microorganisms are growing. For example, calcite precipitation proceeds in waters containing mostly Ca^{2+}, whereas huntite or magnesite precipitates from Mg^{2+}-rich waters (*15, 46*).

In fractured granitic bedrock, anaerobic oxidation of organic material by Fe(III)-reducing bacteria appears to be the main source of alkalinity contributing to ongoing low temperature precipitation of calcite (*29, 37, 38*). Studies of groundwater trace element interactions with fresh carbonate precipitates in these subsurface hydraulic systems indicate that significant amounts of strontium (100 to 9000 ppm), barium (100 to 1000 ppm), uranium (0.3 to 3.5 ppm), and other metals are commonly incorporated into calcite (*29*). High concentrations of strontium have also been measured in cyptocrystalline calcites precipitated by epilithic cyanobacteria growing in a groundwater discharge zone

(*16*). In each case, laboratory determined values for the adsorption of corresponding metal ions on calcite are small in comparison to the solid phase concentrations measured in the natural precipitates. The implication is that solid solution formation (i.e., coprecipitation) is an important mechanism for retention of trace metals by calcite (*31*).

Although strontium concentrations of up to 1.0 weight percent are common in biogenic aragonite (i.e., a CaCO3 polymorph that comprises the shells of higher organisms like molluscs), normal values for strontium in calcite tend to be much lower (*2, 9*). This is attributed to the availability of more space in the aragonite structure in comparison to that of calcite; however, high precipitation rates contribute to a more efficient partitioning of strontium into calcite (*31*). As too much of the larger strontium ion may destabilize the rhombohedral structure of calcite, the effect of small grain size on eliminating long range lattice effects in fresh precipitates is considered to be an important factor contributing to the stability of the strontium-calcite solid solutions (*2*). In a broader context this implies that solid solution formation involving trace metal species like strontium may actually be significantly enhanced in natural groundwater systems , through rapid microbial precipitation of fine-grained carbonate minerals (*16, 29*).

Taken together, these lines of microbiological and geochemical evidence suggest that an opportunity exists to employ microbial mineral transformations at the Fe(II)/Fe(III) redox boundary for solid phase capture of strontium and other metal/radionuclide contaminants. Specifically, the precipitation of authigenic carbonate minerals by Fe(III)-reducing bacteria has considerable potential to contribute directly to solid phase immobilization of dissolved metal ions like strontium. This novel immobilization concept is relevant to the development of new clean-up technologies aimed at solving DOE's complex environmental problems. In working towards the long-term needs of DOE Environmental Management, it will be important to assess geochemical relationships between bacterial Fe(III)-oxide reduction and carbonate mineral precipitation. Similarly, a better understanding of the extent to which microbial carbonate precipitation effects the solid and aqueous phase distribution of strontium is required. The research summarized in this report is thus focused on developing a better understanding of microbiological and geochemical controls on carbonate mineral precipitation reactions that are caused by bacterial reduction of Fe(III)-oxides, and on identifying the potential contribution of these processes to solid phase capture and immobilization of strontium in the subsurface.

2.0 Methods and Results

The impact of the Fe(III)-reducing bacteria *Shewanella alga* strain BrY on the solid phase partioning of dissolved Sr^{2+} was initially investigated in a series of experiments using live cells, dead cells (heat-treated at 80 ^{o}C), and isolated cell envelope fractions. Synthetic hydrous ferric oxide (HFO) used in the experiments was prepared in the laboratory by the gradual titration of $Fe(NO_3)_3 \cdot 9H_2O$ with 1 M NaOH to pH 7. The resulting HFO gel precipitate was centrifuged at 7000 rpm for 10 minutes, rinsed three times, and resuspended in sterile ultrapure water (UPW). Total Fe(III) content of the gel was measured by a phenanthroline spectrophotometric assay.

Dissolved Fe(II) accumulated in response to reductive dissolution of HFO by *S. alga* in experiments conducted with live cells. The presence of 1 mM Sr^{2+} did not adversely influence the production of dissolved Fe(II) by the bacteria. In all experimental systems, pH increased slightly owing presumably to a draw-down in the pCO_2 of the culture medium in response to equilibration with the N_2 atmosphere of the anaerobic chamber used in the investigation. However, in the presence of the live Fe(III)-reducing bacteria the pH increase was sufficient to bring about supersaturation with respect to siderite after approximately two days. The presence of siderite, as well as small amounts of vivianite, after 5 days of incubation were confirmed by X-ray diffraction.

A steady increase over time in the solid phase capture of Sr^{2+} was observed in all of the various experimental systems. The highest measured solid phase concentrations of Sr^{2+} always occurred in the live cell experiments, followed by the cell envelope, dead cell, and finally the abiotic HFO control. These variations extend directly from two different kinds of processes that contributed to the solid phase capture of Sr^{2+}. In the dead cell, cell envelope, and abiotic HFO systems, sorption reactions alone account entirely for the uptake and retention of Sr^{2+}. In the live cell experiments, the solid phase capture of Sr^{2+} occurred not only through sorption to the bacterial cells themselves, but also from incorporation into precipitating siderite, either through sorption or coprecipitation mechanisms. Overall, these results showed that non-viable *S. alga* cells and *S. alga* cell envelopes can sorb significantly greater quantities of Sr^{2+} compared to HFO alone, and that siderite preciptation in live Fe(III)-reducing *S. alga* cultures enhances the solid phase partioning of Sr^{2+}.

In order to quantify sorption reactions involving Sr^{2+} under Fe(III)-reducing conditions, bacteria (*S. alga* strain BrY and *Shewanella putrefaciens* strain CN-32)were grown overnight at 37^{o} C in Trypticase soy broth (TSB, media pH ~ 7). Prior to use in the sorption experiments, the bacteria were washed by centrifugation three times with, and resuspended in, sterile UPW. Bacteria cell concentrations used in the experiments were equivalent to an optical density of 0.4 at 600 nm (approx. $8x10^8$ cells/mL = 0.18 g/L; DAPI [4',6-diamidino-2-phenylindole dihydrochloride] epifluorescence counts on cell suspensions in pure water). Cell viability was evaluated by epifluorescence microscopy direct counting using the LIVE/DEAD Baclight™ Bacteria Viability Kit (Molecular Probe Inc. L-7007). Viability counts indicated that the majority (>90%) of the bacterial cells remained intact and viable throughout the sorption experiments. Fresh HFO was prepared synthetically, as described above, immediately prior to each set of experiments. An Fe concentration of 45 mM Fe(III) was used in the sorption assays.

To prepare Fe(III)-reducing bacterial cells coated with HFO, *S. alga* cell suspensions were washed and resuspended in UPW as explained above at a cell density equivalent to an $O.D._{600\ nm}$ of 0.4 (approximately $8x10^8$ cells/mL = 0.18 g/L). An $Fe(NO_3)_3 \cdot 9H_2O$ solution was then added to make a final total Fe concentration of 0.8 mM Fe(III). The solution of *S. alga* cells and 0.8 mM Fe(III) was then gently stirred for 1h at a pH of ~3 to promote Fe(III) sorption to the bacterial surface. This pH was chosen as it is below the critical pH with respect to supersaturation and precipitation of $Fe(OH)_{3(s)}$ for

these experimental conditions. After one hour the cell-Fe(III) solution was slowly titrated with 1 M NaOH at 0.5 pH unit intervals, with an equilibration time of 15 minutes at each interval, until a final pH of 7 was reached. The solution was then maintained at this pH and gently shaken for 12 hours to allow complete HFO precipitation. Fe(III) was not detected in solution after this period; i.e. all of the Fe(III) added to solution was precipitated as HFO. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) confirmed the presence of iron precipitates at the bacterial surface. X-Ray diffraction analyses were used to determine that the HFO precipitates used in these experiments were amorphous both in the presence and absence of bacteria.

Strontium sorption experiments were carried out using the four substrate systems (*S. alga*, *S. putrefaciens*, HFO, and *S. alga*-HFO composite) over a range of Sr^{2+} concentrations (10^{-2}-$10^{-5.5}$ M at $10^{-0.5}$ M increments) and pH (2.5-11 at 0.5 pH increments) under dilute aqueous, non-growth conditions. Experimental samples were gently shaken at room temperature for 2 hours. This relatively short equilibration time (determined through time course experiments) has been shown to maintain cell viability and to approach equilibrium conditions without measurable dissolution of HFO at lower pH values. After 2 hours, the solution pH was measured and used in the calculation of complexation constants, maximum binding capacities and the construction of pH sorption edge diagrams. Measured concentrations were thus assumed to be equal to activities (*12, 44*), and were subsequently used to quantify Sr^{2+} partitioning. Solid phase strontium concentrations (Sr_S) were determined by taking the difference between the total Sr^{2+} (Sr_T) and dissolved Sr^{2+} (Sr_D) concentrations. Samples for Sr_D were filtered through 0.22 µm sterile acrodisc filters with a Supor membrane, and measured using atomic absorption spectrophotometry (AAS), or inductively coupled plasma atomic emission spectroscopy (ICP-AES). The experimental solutions were found to be undersaturated with respect to Sr-carbonates and hydroxides over the pH range of the sorption assays (MINEQL+).

Significant Sr^{2+} sorption occurred at significantly lower pH values to the bacteria and *S. alga*-HFO composite (5.5 to 5.9) compared to HFO (7.6). Geochemical modeling using a generalized Langmuir equation showed that the bacteria sorb significantly greater quantities of Sr^{2+} (maximum sorptive capacity: BSr_{max} = 0.079 and 0.075 mmol.g^{-1} for *S. alga* and *S. putrefaciens*, respectively) than the HFO (0.001 mmol.g^{-1}). The observed BSr_{max} for the *S. alga*-HFO composite (0.034 mmol.g^{-1}) was less than the combined sorptive properties of its components (BSr_{max} = 0.041 mmol.g^{-1}), likely reflecting HFO masking of bacterial surface binding sites.

In sorption reactions involving bacteria and HFO, deprotonation of amphoteric surface functional groups provide specific sites for the complexation of metal ions (*12, 13*). The extent to which dissolved metals are retained is therefore strongly pH dependent (*45*). Surface charge develops also as a consequence of deprotonation and sorption reactions. In the simplest model for charged surfaces, this contributes to the formation of a diffuse electrochemical double layer (*13, 45*). The innermost layer extends from fixed charges at the solid surface, whereas the outer layer is formed by a diffuse swarm of ions that maintain the overall electroneutrality of the surface (*45*).

While deprotonation reactions and solid phase charge development are confined to the inner layer, sorbed ions can accumulate either as specific surface chemical (i.e., inner-sphere) complexes, or as electrostatically associated diffuse layer (i.e., outer-sphere) complexes (*13*, *45*). A convenient method to distinguish between inner-sphere and outer-sphere complexes is to assess the effect of ionic strength on sorption equilibria (*13*). A strong dependence on ionic strength is typical for an outer-sphere complex (*45*). Moreover outer-sphere complexes involve electrostatic bonding patterns that are less stable than convalently bonded inner-sphere complexes. Accordingly, the influence of ionic strength on Sr^{2+} sorption to bacteria, HFO, and bacterial-HFO composites was investigated.

The ionic strength sorption experiments were carried out with three substrate systems (*S. alga*, HFO, *S. alga*-HFO composite, as described above) over a pH range of 2.5-11 (at 0.5 pH increments), known concentrations of $NaNO_3$ (0.01 and 0.1 M), and at constant 10^{-5} M Sr^{2+}. Sorption of Sr^{2+} to *S. alga* exhibited a strong dependence on ionic strength with the maximum concentration of solid phase strontium (BSr_{max}) decreasing from 79 $\mu mol.g^{-1}$ under dilute aqueous conditions to 4 $\mu mol.g^{-1}$ at high ionic strength (0.1 M $NaNO_3$). Corresponding apparent surface complex formation (K^{S}_{Sr}) values for *S. alga* increased from $10^{-0.51}$ to $10^{-0.26}$ with increasing ionic strength, implying that only high affinity sites remain to bind Sr^{2+} under conditions of increased ionic strength. In contrast, Sr^{2+} sorption to HFO surfaces was independent of ionic strength with BSr_{max} and K^{S}_{Sr} remaining relatively constant (approximately 1 $\mu mol.g^{-1}$ and $10^{-2.1}$, respectively) under increasing ionic strength conditions. The ionic strength dependent sorptive behaviour exhibited by *S. alga* is consistent with electrostatic outer-sphere complexation reactions occurring in the diffuse layer, whereas inner-sphere complexation reactions account for the Sr^{2+} sorption behaviour of HFO. The bacteria-HFO composite solid exhibited moderate ionic strength dependence with maximum binding capacities decreasing from 34 $\mu mol.g^{-1}$ (dilute conditions) to 24 $\mu mol.g^{-1}$ (0.1 M $NaNO_3$). These results suggest that Fe^{3+} sorption and precipitation at the bacterial surface alters the electrochemical surface properties of the composite solid, buffering the effects of increased ionic strength on subsequent Sr^{2+} sorption.

The solid phase paritioning of Sr2+ under active Fe(III)-reducing conditions was investigated further with the groundwater dissimilatory Fe(III)-reducing bacterium *S. putrefaciens* strain CN32 in defined bicarbonate-buffered (30 mM $NaHCO_3$) medium containing amorphous hydrous ferric oxide (30-40 mmol Fe(III) L^{-1}) as a model Fe(III) oxide phase and lactate (20 mM) as carbon/energy source. Medium quartz sand (100 g L^{-1}) was added to provide surfaces for carbonate mineral nucleation, simulating a saturated groundwater system. $SrCl_2 \cdot 2H_2O$ was added from sterile, anaerobic stock solutions to achieve final concentrations of 1.0, 0.1 or 0.01 mM. In some experiments, $CaCl_2 \cdot 2H_2O$ was added to a final concentration of ca. 10 mM. A 1-2 hr equilibration period preceded inoculation of cultures with ca. 10^8 mL^{-1} of TSB-grown CN32 cells. Culture vessels were incubated statically at 30°C in the dark. Aqueous and solid-phase samples were collected periodically and analyzed for total (0.5M HCl extraction) and aqueous Fe(II) (Ferrozine), total and aqueous Sr and Ca (ICP), total (unfiltered) and aqueous inorganic carbon (headspace GC technique), and pH. The amount of Sr associated with carbonate phases

was determined via selective extraction with alkaline (pH 8.0) citrate-dithionite to remove residual oxide phases. Saturation indices for carbonate minerals were computed based on measured aqueous phase solute concentrations with correction for ionic strength effects using MINEQL+.

The bacterial Fe(III)-reduction experiments showed again that Sr at concentrations up to 1 mM had no detrimental effect on HFO reduction by strain CN32. Between 15% (0.1 and 0.01 mM Sr_T) and 40% (1 mM Sr_T) of added Sr was in solution at the start of the Ca-free HFO reduction experiments. Testing showed that essentially all of the initial solid-associated Sr was sorbed to HFO+CN32 cell surfaces. Based on previous studies of Sr sorption to HFO and CN32, sorption by bacterial surfaces was probably minor compared to HFO at their respective concentrations in the experiments.

Sr was released to solution in parallel with Fe(II) during the initial stages of HFO reduction in Ca-free cultures (Figs. 1-3, panels A&B). These results are consistent with previous studies indicating the potential for release of sorbed or coprecipitated metals during microbial Fe(III) oxide reduction (*24*). After the initial phase of aqueous Sr and Fe(II) accumulation, concentrations of these species declined in parallel with the accumulation of solid-phase inorganic carbon ($\Sigma MeCO_3$, Figs. 1-3, panel C). pH values increased from ca. 7.0 to ca. 8.0 during HFO reduction in Ca-free cultures. Greater than 90% of Sr_T was removed from solution at all three levels of Sr addition (Table 1). No Fe(II) production or changes in Sr speciation or pH occurred in uninoculated controls.

Table 1. Sr immobilization during microbial HFO reduction

Experiment	% Sr Immobilized	% Immobilized Sr in Carbonates
1 mM Sr_T, - Ca	90.4	38.2
0.1 mM Sr_T, - Ca	98.0	76.5
0.01 mM Sr_T, - Ca	99.0	100
1 mM Sr_T, + Ca	29.7	46.8
0.1 mM Sr_T, + Ca	35.4	76.5
0.01 mM Sr_T, + Ca	33.3	100

The addition of 10 mM $CaCl_2$ strongly influenced the behavior of Sr in our culture systems. Calcium competed with Sr for sorption sites on HFO+CN32 surfaces, such that only ca. 20-40% of added Sr was sorbed at the start of the experiments. Similar to Ca-free cultures, sorbed Sr was initially released during HFO reduction. However, the degree of subsequent Sr immobilization was reduced relative to Ca-free cultures, with only ca. 30% of Sr_T removed from solution after 30 d of incubation (Table 1). Major declines in aqueous Ca concentration occurred 5-10 d into the incubations (Figs. 1-3, panel B), indicating $CaCO_3$ precipitation. The onset of carbonate mineral precipitation was reflected in a sharp increase in $\Sigma MeCO_3$ and a parallel drop in culture pH (Figs. 1-3, panel C).

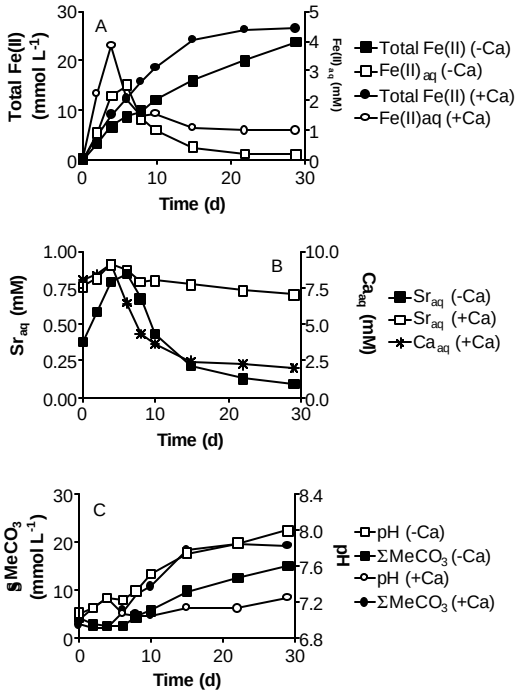

Fig. 1. HFO reduction (A), aqueous [Sr] & [Ca] (B), and pH & solid-phase inorganic carbon ($\Sigma MeCO_3$) concentration in 1 mM ΣSr cultures.

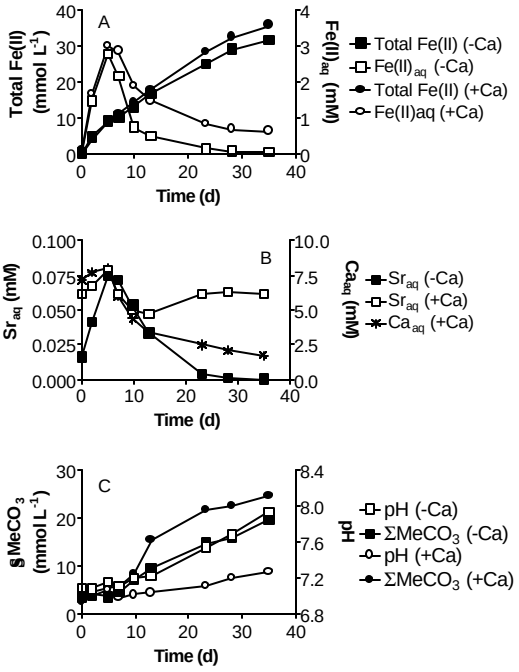

Fig. 2. HFO reduction (A), aqueous [Sr] & [Ca] (B), and pH & solid-phase inorganic carbon ($\Sigma MeCO_3$) concentration in 0.1 mM ΣSr cultures.

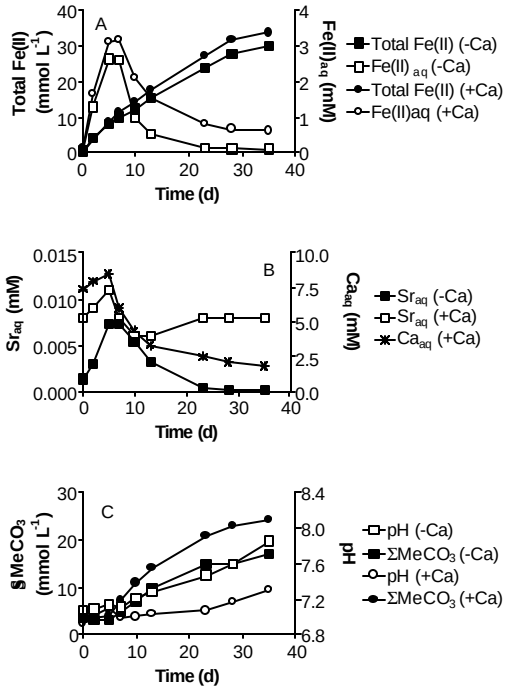

Fig. 3. HFO reduction (A), aqueous [Sr] & [Ca] (B), and pH & solid-phase inorganic carbon ($\Sigma MeCO_3$) concentration in 0.01 mM ΣSr cultures.

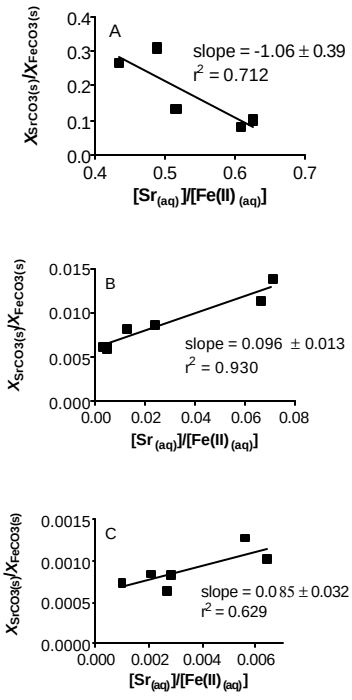

Fig. 4. Partitioning of Sr into carbonate minerals formed during HFO reduction in Ca-free 1 mM (A), 0.1 mM (B) and 0.01 mM (C) ΣSr cultures.

XRD and SEM/EDS analyses identified siderite as a major end product of HFO reduction in Ca-free culture systems; no other crystalline Fe(II)-bearing minerals were detected. XRD analysis suggested that ankerite, a co-precipitate of calcite and siderite (*30*), was the primary mineral formed in Ca-amended cultures. SEM analysis confirmed the typical rhombic crystal structure of calcite and siderite minerals. $SrCO_3$ could not be detected by XRD in any of the cultures due to the relatively low abundance of Sr compared to $FeCO_3$ and/or $CaCO_3$ phases. However, EDS analysis during SEM revealed the presence of Sr associated with carbonate phases in both Ca-free and Ca-amended cultures.

End-point samples from the experiments shown in Figs. 1-3 were treated with alkaline citrate-dithionite (CD) and filtered to separate carbonate from noncarbonate Sr-bearing mineral phases. Subsequent ICP and GC analyses of minerals captured on the filter demonstrated that the filterable Fe(II) and inorganic carbon retained after CD extraction were comparable to values determined by acidification of whole vs. filtered samples of the same culture. In the 1 mM Sr_T experiments, only 35-40% of the solid-phase Sr was retained on the filters after CD treatment (Table 1). A nonacid-extractable phase (low crystallinity goethite as indicated by XRD) formed over time in most of the HFO reduction cultures. We suspect that the CD extraction released Sr that was captured within this non-carbonate Fe(III) oxide phase. Alternatively, some Sr may have become trapped within amorphous Fe(II)-hydroxide phases that are likely to have formed in the cultures. A larger fraction (76-100%) of the Sr immobilized in 0.1 and 0.01 mM Sr_T cultures was recovered within carbonate mineral phases in both Ca-free and Ca-amended cultures (Table 1).

Saturation index calculations indicated that the 1 mM Sr_T systems were supersaturated with respect to $SrCO_3$ at the start of the experiments, although testing showed that no $SrCO_3$ formation occurred in uninoculated control cultures. Alkalinity production during HFO reduction was likely responsible for triggering precipitation of $SrCO_3$ in the Ca-free cultures. The inhibition of Sr immobilization in Ca-amended 1 mM Sr_T cultures can be attributed at least in part to consumption of DIC and alkalinity during $CaCO_3$ precipitation.

In contrast to the 1 mM Sr_T systems, the aqueous phase remained undersaturated with respect to $SrCO_3$ at all times in the 0.1 and 0.01 mM Sr_T cultures. These results suggest that some mechanism other than $SrCO_3$ precipitation was responsible for the extensive incorporation of Sr into carbonate phases in the Ca-free cultures. A strong linear correlation was observed between the mole fraction of Sr in carbonates and the aqueous-phase Sr:Fe(II) ratio in these experiments (Fig. 4B,C), which suggested that Sr partitioned into $FeCO_3$ phases produced during bacterial HFO reduction. These results differ from those obtained in the 1 mM Sr_T Ca-free systems, in which Sr partitioning into carbonate phases was not positively correlated with the aqueous-phase Sr:Fe(II) ratio (Fig. 4A). The potential for Sr to coprecipitate with $FeCO_3$ was verified in chemical (abiotic) precipitation experiments under pH and carbonate alkalinity conditions similar to those present in the HFO reduction cultures. These experiments yielded an apparent non-thermodynamic partition coefficient (*32*) of ca. 0.02. This D value is 4-5 times

lower than the slope of the linear regression lines in Fig. 4B,C, which suggests that Sr partitioning into $FeCO_3$ was enhanced by some mechanism during microbially-induced $FeCO_3$ formation. Although differences in the rate of major element precipitation could potentially account for these contrasting results (*32*), rates of $FeCO_3$ formation during the abiotic precipitation experiments were comparable to or greater than those observed during HFO reduction. Hence, the mechanism for biogenic enhancement of Sr incorporation into $FeCO_3$ remains unclear, but may be related to the unique geochemical conditions (e.g. elevated pH and/or [Sr] relative to bulk phase) at the cell-mineral interface.

Abiotic $FeCO_3$ precipitation experiments were conducted in the presence and absence of 1 mM $CaCl_2$ to assess the mechanism of Ca inhibition of Sr immobilization in the Ca-amended 0.1 and 0.01 mM Sr_T culture systems. The presence of 1 mM Ca dramatically decreased (ca. 10-fold) the apparent D value for Sr partitioning into $FeCO_3$. Interestingly, the apparent D value for Ca partitioning into $FeCO_3$ observed in these experiments (0.020) was quite similar to that obtained for Sr in Ca-free systems (0.023). These results suggest that competition between Ca and Sr for incorporation into biogenic $FeCO_3$ may explain the inhibitory effect of Ca on Sr immobilization in the 0.1 and 0.01 Sr_T systems.

Elevated solid phase concentrations of strontium (up to 2.0 weight percent) were also attained in less than one day by using bacterial urea hydrolysis to produce calcium carbonate (calcite and some vaterite, as determined by XRD) with decreases in dissolved strontium concentrations of up to 90 percent. Solid phase distribution coefficients approached four orders of magnitude (10^4). This discovery demonstrated that solid phase capture of strontium by microbial carbonate mineral precipitation is not only feasible, but can be highly effective and accomplished in a rapid fashion. At the same time, a diverse assemblage of carbonate mineral grains were produced that included spherical accretions, as well as dumbbell-shaped polymorphs. Scanning electron microscopy showed that bacteria were often embedded directly within the mineral grains, suggesting that the cells served as heterogeneous nucleation templates for carbonate precipitation.

3.0 Relevance, Impact and Technology Transfer

Demonstration of Sr^{2+} solid phase capture in response to microbial induced carbonate mineral precipitation, initiated by Fe(III)-reduction or urea-hydrolysis, suggests that considerable potential exists to adapt this novel immobilization concept to the development of new clean-up strategies for DOE sites where strontium and other metal/radionuclides exist as ubiquitous and often mobile contaminants. These studies have also fundamentally advanced our understanding of how microorganisms may influence contaminant fate and behavior in subsurface environments. Moreover, this investigation has enabled a new EMSP research initiative at INEEL under the direction of R.W. Smith (rqs@inel.gov) focused on calcite precipitation and trace metal partitioning in groundwater and the vadose zone of arid western environments.

4.0 Project Productivity

This project has accomplished the original goal of developing a better quantitative understanding of microbiological and geochemical controls on carbonate mineral precipitation reactions that are caused by bacterial reduction of Fe(III)-oxides, and has identified potential contributions and constraints of these processes for the solid phase capture and immobilization of strontium in the subsurface.

5.0 Personnel Supported

5.1 Graduate students

T.D. Small (M.S.)
J.R. Howell (M.S.)
V.K. Keith (M.S.)

5.2. Postdoctoral research associates

Dr. L.A. Warren
Dr. N. Parmar
Dr. M.R. Leonardo

6.0 Publications

6.1 Published in peer-reviewed journals

T.S. Small, L.A. Warren, E.E. Roden, and F.G. Ferris. 1999. Sorption of strontium by bacteria, Fe(III) oxide and bacteria-Fe(III) oxide composites. Environmental Science and Technology 33: 4465-4470.

Howell, J.R., R.J. Donahoe, E.E. Roden, and F.G. Ferris. 1998. Effects of microbial iron oxide reduction on pH and alkalinity in anaerobic bicarbonate-buffered media: implications for metal mobility. Mineralogical Magazine 62A: 657-658.

6.2 Published in proceedings

Warren, L.A. and F.G. Ferris. 1998. Solid phase partitioning of uranium and copper in the presence of hydrous ferric oxide and bacteria. Procedings of the 9th International Symposium on Water-Rock Interactions, G.B. Arehart and J.R. Hulston, editors, A.A. Balkema, Rotterdam, 115-117.

6.3 Accepted/submitted/in preparation for publication

Parmar, N., L.A. Warren, E.E. Roden, and F.G. Ferris. Solid phase capture of strontium by the iron reducing bacterium Shewanelle alga strain BrY. Chem. Geol. Accepted.

Small, T.D., L.A. Warren, and F.G. Ferris. Influence of ionic strength on strontium sorption to bacteria, Fe(III) oxide and bacteria-Fe(III) oxide surfaces. Submitted to Applied Geochemistry.

Small, T.D. and F.G. Ferris. Solid phase partitioning of Sr^{2+} by bacteria-Fe(III) oxide composites. In preparation for Geochim. Cosmochim. Acta.

Roden, E.E., M.R. Leonardo, and F.G. Ferris. Immobilization of strontium in carbonate minerals formed during microbial reduction of hydrous ferric oxide. In preparation for Geochim. Cosmochim. Acta.

Keith, V.K. and E.E. Roden. Immobilization of aqueous strontium during bacterial reduction of synthetic goethite. In preparation for Appl. Geochem.

6.4 Graduate student theses

Small, T.D. 2000. Sorption of strontium to bacteria, Fe(III) oxide, and bacteria-Fe(III) oxide composites in relation to contaminant fate. M.S. Thesis, Department of Geology, University of Toronto, Toronto, Ontario.

Howell, J.R. 1998. Effects of microbial Fe(III) oxide reduction on pH, DIC, and carbonate mineral formation: implications for metal mobility. M.S. Thesis, Department of Geology, University of Alabama, Tuscaloosa, Alabama.

Keith, V.K. 2000. Immobilization of aqueous strontium during bacterial reduction of synthetic goethite. M.S. Thesis, Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama.

7.0 Interactions

7.1 Participation at meetings

Roden, E.E. and F.G. Ferris. 2000. Immobilization of aqueous strontium during carbonate mineral formation coupled to microbial Fe(III) oxide reduction. American Chemical Society National Meeting (submitted).

Roden, E.E., M.R. Leonardo, V.K. Keith and F.G. Ferris. 1999. Immobilization of aqueous strontium during carbonate mineral formation coupled to microbial Fe(III) oxide reduction. International Symposium on Subsurface Microbiology.

Keith, V.K. and E.E. Roden. 1999. Immobilization of aqueous strontium during bacterial reduction of synthetic Fe(III) oxides. American Society for Microbiology Annual Meeting.

Leonardo, M.R., F.G. Ferris, and E.E. Roden. 1999. Sr2+ immobilization by authigenic carbonate precipitation under iron-reducing conditions. American Society for Microbiology Annual Meeting.

Parmar, N., L.A. Warren, and F.G. Ferris. 1999. Impact of Fe(III) reduction on Fe(II)/Fe(III) mineral transformation and solid phase capture of strontium. XIV International Symposium on Environmental Biogeochemisty.

Roden, E.E., M.R. Leonardo, and F.G. Ferris. 1999. Immobilization of strontium during carbonate mineral-formation coupled to microbial Fe(III) oxide reduction. XIV International Symposium on Environmental Biogeochemisty.

Small, T.D., L.A. Warren, E.E. Roden, and F.G. Ferris. 1999. Sorption of strontium by bacteria, Fe(III) oxide and bacterial-Fe(III) oxide composites. XIV International Symposium on Environmental Biogeochemisty.

Leonardo, M.R., F.G. Ferris, and E.E. Roden. 1998. Analysis of iron-carbonate mineral formation during microbial reduction of synthetic amorphous iron oxide. American Society for Microbiology General Meeting.

Warren, L.A., N. Parmar, and F.G. Ferris. 1998. Strontium, uranyl, and copper incorporation in bacterially mediated calcite precipitation. . Geological Socieity of America Annual Meeting, Toronto.

Parmar, N., L.A. Warren, and F.G. Ferris. 1998. Solid phase capture of strontium by the iron reducing bacteria Shewenella alga. . Geological Socieity of America Annual Meeting.

Small, T.D., L.A. Warren, and F.G. Ferris. 1998. Strontium sorption to bacterial and Fe oxide surfaces. . Geological Socieity of America Annual Meeting.

Maurice, P.A., L.A. Warren, F.G. Ferris. 1998. Calcite precipitation by B. pasteurei: AFM imaging of microbial-mineral interactions. Geological Socieity of America Annual Meeting.

Warren, L.A., F.G. Ferris and E.E. Roden. 1997. Strontium reactions at Shewenella and hydrous ferric oxide (HFO) surfaces. Geological Society of America Annual Meeting.

Howell, J.R., R.J. Donahoe, and E.E. Roden. 1997. Effects of microbial iron oxide reduction on pH and alkalinity in anaerobic bicarbonate-buffered media. American Geophysical Union Fall Meeting.

8.0 Transitions and Future Work

This investigation has enabled a new EMSP research initiative at INEEL under the direction of R.W. Smith (rqs@inel.gov) - Calcite precipitation and trace metal

partitioning in groundwater and the vadose zone: remediation of strontium-90 and other divalent metals and radionuclides in arid western environments.

9.0 Patents

None

10.0 Literature Cited

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