Bacterial Mineral Transformations for Solid Phase Capture of the Radionuclides: Strontium and Uranium

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1. Solid Phase Sr Partitioning with *S. alga* (BrY) and HFO

OBJECTIVE

to quantify the relative abilities of *Shewenella alga* (strain BrY), hydrous ferric oxide (HFO; $Fe(OH)_3$) and *S. alga* coated with HFO to sorb Sr through construction of adsorption isotherms and subsequent calculation of surface complex formation constants (K^S_{Sr}) and maximum sorptive capacities.

METHODS

Sr partitioning to the solid phase was evaluated in two hour laboratory assays over a range of pH (2.5 to 8 at 0.5 pH units) and total Sr concentration (0.005 to 10 mM). *S. alga* (BrY) cell densities equivalent to an $OD_{600} \cong 0.4$ and an HFO concentration of 50 mM were used in the experiments. Cells coated with HFO were prepared according to the method of Warren and Ferris (1998, *E.S.&T.* in press). All assays were pH adjusted and shaken gently over the 2 hour incubation time. At the end of this period, samples for total and dissolved Sr (0.2 µm filtered) were taken and analyzed by ICP or FAAS. The association of Sr directly at bacterial cell and HFO surfaces was confirmed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS).

RESULTS

Isotherms can be constructed by relating solid phase Sr concentrations to solution Sr concentrations and the equilibrium proton condition (eqn.1; **Fig. 1**)

$$BH + Sr^{2+} + H_2O \longleftrightarrow BSrOH + H^+ \qquad (1)$$

where

$$K^S_{Sr} = [BSrOH][H^+]/[H^+][Sr^{2+}]$$

where BH represents a surface site; BSrOH can be estimated by solid Sr (Sr_S) and Sr^{2+} can be estimated by dissolved Sr (Sr_D) (double reciprocal plots of these data yield estimates of K^S_{Sr} and maximum sorption capacity; **Table 1**).

Solid phase Sr concentrations are highest in the *S. alga* treatment as is reflected by the higher surface complex formation constants (K^S_{Sr}) and maximum sorptive capacities values calculated for the bacteria (**Table 1**).

In addition, significant solid phase Sr partitioning was initiated at lower pH values in the presence of the bacteria compared to the HFO with the bacteria coated with HFO between these two, as evidenced by pH adsorption edge data (**Fig. 2**; $Sr_T = 10^{-5}$ M).

CONCLUSIONS

***S. alga* (BrY) sorb significantly greater quantities of Sr than either *S. alga* coated with HFO or HFO alone. The bacteria shifts the effective pH range over which significant Sr sorption occurs to lower values compared to HFO (6 vs 7.5).**

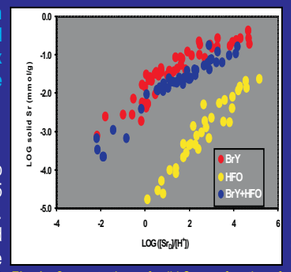


Fig. 1. Concentrations of solid Sr as a function of solution Sr concentrations and pH (see equation 1) for *S. alga* (BrY), $FeOH_3$ (HFO) and *S. alga* coated with HFO.

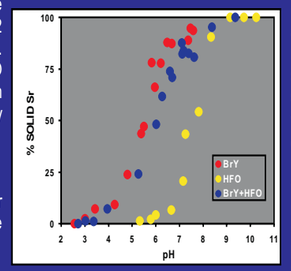
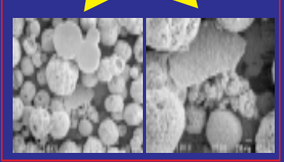


Fig. 2. Sr adsorption edge diagrams for *S. alga* (BrY), $FeOH_3$ (HFO) and *S. alga* coated with HFO.

Table 1. Apparent Surface Complex Formation Constants (K^S_{Sr}) and Maximum Binding Capacities ($\Gamma_{Sr_{max}}$) for *S. alga* (BrY) and HFO

	LOG K^S_{Sr}	$\Gamma_{Sr_{max}}$ (mmol.g^{-1})	r^2
BrY	0.5	0.04	0.94
BrY+HFO	0.1	0.03	0.98
HFO	-1.4	0.0004	0.81

$FeCO_3$(Fe, Sr and C identified by EDS analyses) formed by *S. alga* (strain BrY) in conjunction with HFO reduction



2. Coprecipitation of $SrCO_3$ and $FeCO_3$ by *S. putrefaciens* (CN-32)

OBJECTIVE

To determine if strontianite and siderite will co-precipitate under iron-reducing conditions using *Shewanella putrefaciens* (strain CN-32) in batch cultures.

METHODS

Strain CN-32 cells were prepared under anaerobic conditions in a Coy hood (2.0% H_2 atmosphere). 50ml of our standard *Shewanella* media (Roden & Zachara., E.S.&T. 1996) containing 30 mmol/L hydrous ferric oxide (HFO) and 20 mM Na-Lactate (in 100ml Wheaton bottles) were inoculated with the cells. $SrCl_2$ was added from a sterile anaerobic stock (100mM) to a final concentration of 1mM. $CaCl_2$ was added prior to sterilization at 10mM (final). Cultures were incubated at 30C with minimal agitation and were sampled over 1-2 months.

Samples were obtained using 3cc syringes fitted with 21G needles. Sampling was performed in a Coy anaerobic chamber. A wet-chemical approach was used to quantify solid-phase carbonate mineral formation (Roden and Wetzel, L.& O. 1996). A first 1 ml aliquot was directly added to a sealed 6ml Wheaton bottle containing 1ml of 4% nitric acid. A second 1 ml aliquot was centrifuged for 5 minutes in the Coy chamber. The supernatant was collected and passed through a 0.45mm filter into a second acid-containing Wheaton bottle. The samples (whole and filtered) were allowed to stand overnight before the headspace CO_2 levels were determined by GC. Reduced iron content of the samples were determined using the ferrozine assay (1) or by ICP analysis. Sr and Ca concentrations were determined by ICP as well. pH was determined using a microelectrode.

RESULTS

The addition of Sr had no effect on the rate of HFO reduction by BrY or CN-32 (data not shown). CN-32 reduced almost 100% of the HFO (**Fig. 3**). After an initial increase, inorganic carbon levels decreased over time (**Fig. 4**). Sr initially sorbed to the bacteria and HFO before being released during iron reduction (**Fig. 5**). During Fe(III) reduction, a precipitate formed in the cultures. As the precipitate developed, total concentrations of Fe (II) and Sr decreased implying Sr incorporation into $FeCO_3$. Analysis of the precipitate identified Fe (II), inorganic carbon and Sr at levels to balance the total concentrations added (data not shown). Sr, Fe and C were detected in these precipitates by EDS.

Ca was added to cultures to induce $CaCO_3$ precipitation and solid phase capture of Sr by solid solution. The addition of Ca had little effect on HFO reduction (**Fig. 6**). However, Ca reduced the initial sorption of Sr (**Fig. 8**), and underwent rapid precipitation (**Fig. 9**). The culture pH and Sr concentrations decreased as $CaCO_3$ and $FeCO_3$ began to precipitate (**Figs. 8** and **10**). These results confirm that microbial mineral transformations at the Fe(II)/Fe(III) redox boundary induce carbonate mineral precipitation and contribute to solid phase capture of Sr.

CONCLUSIONS

The benign impact of Sr on bacterial activity implies that toxicity is not a major concern for bioremediation applications. At the same time, bacterial Fe(III) reduction contributes to substantial mineralogical transformation of substrate HFO including the formation of the Fe(II) carbonate, siderite with associated decreases in dissolved Sr levels of up to 30% in 40 days.

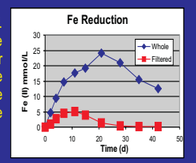


Fig. 3. Iron reduction by CN-32. Note how the unfiltered sample concentration decreases over time, this is due to the precipitation of carbonate minerals to the culture bottle surface.

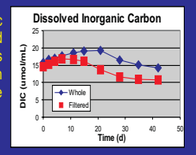


Fig. 4. Dissolved Inorganic Carbon. Note how the unfiltered sample concentration decreases over time, due to the precipitation of carbonate minerals to the culture bottle surface.

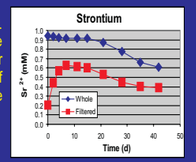


Fig.5. Strontium Concentrations. Note how the unfiltered sample concentration decreases over time, due to the precipitation of carbonate minerals to the culture bottle surface.

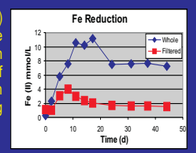


Fig. 6. Effects of Ca on Fe(III) Reduction. Note how the unfiltered sample concentration decreases over time. The rate of Fe(II) decrease occurs much sooner in the Ca containing cultures (Fig. 3).

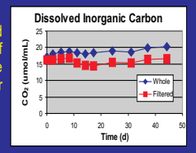


Fig. 7. Ca Effects on Dissolved Inorganic Carbon. The rate of the unfiltered sample concentration decrease over time is comparable to Fig. 4.

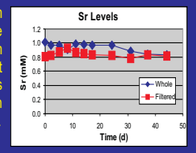


Fig. 8. Ca Effects on Strontium Concentrations. Note how little Sr sorbed to HFO in comparison to the Ca-minus experiment (Fig.5.). In addition, the aqueous Sr levels do not decrease as in the Ca-minus experiment (Fig.5).

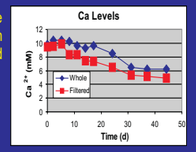


Fig. 9. Ca Concentrations. Note the decrease in Ca concentration follows the decrease in filtered DIC (Fig. 7).

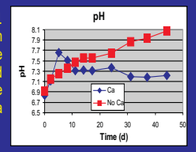


Fig. 10. Ca Effect on Culture pH. Note how the pH rises above 8 in the absence of Ca. The decrease in the Ca-ammended cultures appears to follow the decrease in aqueous Ca concentrations (Fig. 9).

3. Sr and UO_2 Incorporation in Calcite Associated with Urea Degradation

OBJECTIVE

to elicit $CaCO_3$ formation and Sr/UO_2 incorporation associated with urea degradation by *Bacillus pasteurei*

METHODS

B. pasteurei was exposed to a Ca, CO_3 and CH_4N_2O amended growth media with a total radionuclide concentration of 10^{-3}M (as either Sr and UO_2 individually or combined) in the laboratory as 24 hour time series experiments (cell concentrations equivalent to an $O.D._{600} \cong 0.4$). $CaCO_3$ formation and Sr and UO_2 inclusion for the *B. pasteurei* treatment were compared to results for bacterial controls (bacteria no radionuclides) and abiotic controls (no bacteria, radionuclides). Total and dissolved (0.2 µm filtered) element concentrations and pH were monitored over the 24 hour period. SEM and EDS were used to evaluate $CaCO_3$ formation and the presence of Sr and UO_2 in the the $CaCO_3$ crystals.

RESULTS

$CaCO_3$ formation was observed in the presence of *B. pasteurei* within 3 hours, associated with pH values at or above 9 in both the bacterial control and bacteria plus Sr, UO_2 or Sr+UO_2 treatments.

pH values did not increase to the same extent in the abiotic control (see **Fig. 11** for an example of pH time course for the 3 treatments) and no $CaCO_3$ formation occurred unless the pH was experimentally adjusted to 9.

Sr incorporation followed an increasing linear trend with time while UO_2 indicated a rapid initial incorporation that remained static over the first 6 hours of the experiment but decreased considerably by 24 hours (**Fig. 12**). EDS analyses confirmed the presence of both radionuclides in the $CaCO_3$ crystals (identified as calcite and some vaterite by X-ray diffraction analyses; not shown). Results for the Sr and UO_2 combined treatment mimic the results for each radionuclide independently indicating no additive effects (**Fig. 12**).

Total Sr inclusion was approximately 100% of that added (**Fig. 13**) resulting in a 3% by weight inclusion (**Table 2**); while UO_2 reached a maximum of 30% of the total added (**Fig. 13**) but resulting in the same weight % (**Table 2**) as that seen for Sr.

CONCLUSIONS

Extremely rapid calcite formation (3 hours) with significant incorporation of both Sr and UO_2 (~3%) can be achieved using bacterial degradation of urea. This discovery demonstrates 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.

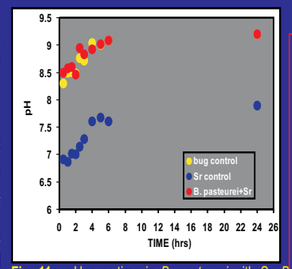


Fig. 11. pH over time in *B. pasteurei* with Sr, *B. pasteurei* alone (control) and in the abiotic control (Sr, media, no bacteria). Results are identical to those observed for UO_2 alone and Sr and UO_2 together.

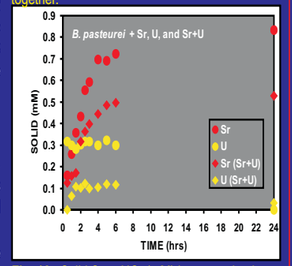


Fig. 12. Solid Sr or UO_2 (mM) incorporation in developing $CaCO_3$ versus time in the presence of *B. pasteurei* and urea.

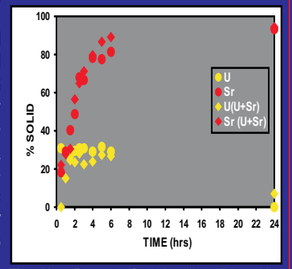
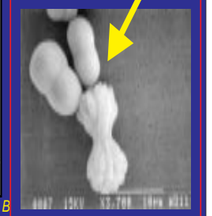
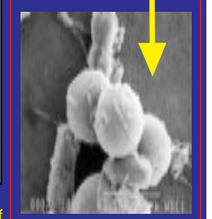
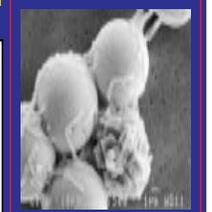


Fig. 13. Percent of total radionuclide added incorporated into developing $CaCO_3$ versus time in the presence of *B. pasteurei* and urea. Total concentration of radionuclide added was 1 mM (e.g. 1 mM Sr in Sr alone; 0.5 mM Sr in Sr+UO_2).

Table 2. Sr and UO_2 Incorporation in Calcite Formed By Urea Degradation with *B. pasteurei*

RADIONUCLIDE	%WEIGHT (g/g)	$LOGK_D$ (L/kg)
Sr	2.8	3.7
U	2.7	2.2
U+Sr - Sr	0.9	3.8
U+Sr - U	0.5	2.1

$CaCO_3$ precipitated abiotically



$CaCO_3$ formed in association with urea degradation by B. pasteurei (subsequent 4 images - note morphological variation in $CaCO_3$ precipitates)