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Co-Precipitation of Trace Metals in Groundwater and Vadose Zone Calcite: In Situ
stabilization of ^{90}Sr and Other Divalent Metals and Radionuclides in Arid Western DOE
Sites

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Executive Summary

Radionuclide and metal contaminants are present in the vadose zone and groundwater throughout the U.S. Department of Energy (DOE) legacy sites. *In situ* containment and stabilization of these contaminants in vadose zones or groundwater is a cost-effective treatment strategy. Our facilitated approach relies upon the hydrolysis of introduced urea to cause the acceleration of calcium carbonate precipitation (and trace metal co-precipitation) by increasing groundwater pH and alkalinity (Fujita et al., 2000; Warren et al., 2001; Ferris et al., 2003; Mitchell and Ferris, 2005). Subsurface urea hydrolysis is catalyzed by the urease enzyme, which may be either introduced with the urea or produced *in situ* by ubiquitous subsurface urea hydrolyzing microorganisms. Because the precipitation processes are irreversible and many western aquifers are saturated with respect to calcite, the co-precipitated metals and radionuclides will be effectively removed from groundwater. The rate at which trace metals are incorporated into calcite is a function of calcite precipitation kinetics, adsorption interactions between the calcite surface and the trace metal in solution (Zachara et al., 1991), solid solution properties of the trace metal in calcite (Tesoriero and Pankow, 1996), and also the surfaces upon which the calcite is precipitating.

In this report we demonstrate that calcite precipitation induced by bacterial ureolysis is highly effective at co-precipitating Sr at low precipitation rates (Mitchell and Ferris, 2005), and while Sr inclusion reduces crystal size and increases crystal solubility, the majority of calcite precipitated and Sr co-precipitated occurs in larger crystals which are not effected by surface area controlled solubility (Mitchell and Ferris, 2006a). Similarly, biotic calcite precipitation produces larger, less soluble crystals, relative to abiotic calcite precipitation. This suggests bacterially induced calcite precipitation may enhance contaminant stabilisation relative to abiotically induced precipitation (Mitchell and Ferris, 2006b). These data confirm that calcite precipitation induced by the bacterial hydrolysis of urea is a highly effective and cost efficient strategy to combat metal and radionuclide contamination in groundwater environments.

1.0 Research Objectives

The Department of Energy (DOE) must evaluate or clean up extensive waste problems in the vadose zone and groundwater at federal sites due to years of radionuclide, transuranic and metal disposal. The costs associated with cleanup of DOE lands are estimated to be hundreds of billions of dollars over many decades. In addition, many of the contaminated sites cannot be remediated with existing technology. The development of new technologies will reduce some cleanup costs and make possible other remediation efforts that are currently not feasible due to the nature of the wastes and the complexity of the geologic environments in which the wastes reside. However, the cost-effective development of new technologies requires a sound understanding of the fundamental science upon which these technologies are based.

In situ remediation is an emerging technology that will play an important role in DOE's environmental restoration program and is an area where enhancement in fundamental understanding will lead to significantly improved cleanup tools. In situ remediation technologies have inherent advantages because they do not require the costly removal, transport, and disposal of contamination. In addition, these technologies minimize worker exposure because contaminated materials are not brought to the surface. Finally, these technologies will minimize the generation of secondary waste streams with their associated treatment and disposal. One of the roles of the Environmental Management Science Program (EMSP) has been to support fundamental research that provides the scientific understanding needed to develop in situ remediation technologies and strategies for the cleanup of contaminated DOE sites. The program is currently focused on the in situ remediation of radionuclides and their mixtures with hazardous material that are located in the vadose zone and in groundwater because these are remediation challenges unique to DOE facilities.

A particularly promising in situ remediation technology is bioremediation. In situ biological methods of destroying or immobilizing organic wastes in groundwater and the vadose zone are often cited as technologies that can provide significant advantages over conventional means of treating waste. Potential benefits of in situ bioremediation include:

- High biogeochemical specificity for targeted wastes
- Complete degradation of the organic constituents of contaminant mixtures yielding non-toxic metabolic byproducts
- Lower cost than conventional technologies
- Direct contact of the microorganisms with the waste in remote locations of the subsurface and therefore high reaction efficiencies for the desired biogeochemical processes

Many of the same advantages exist for the bioremediation of inorganic contamination such as radionuclides and metals except that biological processes are incapable of destroying the contaminants. However, bioremediation can be used to alter the mobility or reduce the toxicity of radionuclides and metals by

- Changing the valence state of the radionuclides and metals
- Degrading or producing complexing ligands
- Facilitating partitioning on to or off of solid phases (e.g., reductive dissolution of hydrous ferric iron oxides, co-precipitation of sulfides or carbonates)

The major objective of this research was to elucidate the mechanisms and rates for the sequestration of strontium by co-precipitation in calcite induced by microbial hydrolysis of urea. The work involved experimental and modeling investigations of (i) mechanisms of CaCO_3 nucleation and crystal growth, (ii) the effect of contaminants on CaCO_3 crystal size and solubility, (iii) the influence of bacteria surfaces on the nucleation and growth of CaCO_3 . These studies involved the novel and unique combination of biogenic crystal size data, crystal evolution modeling, crystal solubility and affinity based precipitation rate calculations to confirm that microbial induced calcite precipitation appears to be a highly effective bio-remediation strategy for aquifers contaminated with ^{90}Sr and other radionuclides and metals.

Catalysis of Calcium Carbonate Precipitation Rates: Microbial Contributions

Microorganisms contribute to the precipitation of a wide variety of authigenic minerals including oxides, phosphates, carbonates, sulfides, and silicates. The mechanisms of microbial mineral precipitation are diverse, but conform generally with chemical equilibrium constraints that regulate nucleation and crystal growth reactions (Stumm and Morgan 1996).

Nucleation is the most critical stage for mineral precipitation, and occurs either homogeneously or heterogeneously. In homogeneous reactions, mineral nuclei are formed by the random collision of ions in solution. Heterogeneous nucleation involves formation of crystal nuclei on the surfaces of foreign solids such as microbial cells on mineral surfaces. Once a stable nucleus has formed, crystal growth can proceed spontaneously providing that the solution remains oversaturated. The free energy of crystal nucleation is controlled thermodynamically by the bulk free energy of the solution, and the interfacial free energy of the corresponding solid phase (Stumm and Morgan 1996).

Microbial metabolic activity will often trigger a change in solution chemistry that leads to oversaturation (e.g., production of sulfide by sulfate-reducing bacteria). This can induce mineral precipitation by lowering the bulk free energy term for both homogeneous and heterogeneous nucleation reactions; however, chemically reactive sites on microbial cell surfaces that facilitate metal sorption at the nucleation sites will tend to reduce the interfacial surface tension of the solid phase and decrease the surface area of the nucleus that is in contact with the bulk solution. The result is a reduction in the overall interfacial free energy of the nucleus which is favorable towards heterogeneous nucleation and precipitation.

In laboratory experiments, carbonate precipitates commonly develop on the external surfaces of individual microbial cells (Ferris et al. 2003). Epicellular carbonate mineral deposition is also common with microorganisms in natural samples. This pattern of mineralization is generally consistent with the inherent ability of microbial cells to serve as templates for heterogeneous nucleation and crystal growth. The effect is apt to be quite important under disequilibrium conditions (e.g., in groundwater springs where degassing of CO_2 may occur), and in metastable solutions that are oversaturated (Stumm and Morgan 1996). Cell surface precipitation contributes further to the development of extremely fine-grain precipitates (i.e., often less than 1.0 mm in diameter), as expected from a lower free energy of nucleation and rapid onset of precipitation.

Carbonate minerals are precipitated by several rather divergent groups of microorganisms. These include photoautotrophic cyanobacteria, various anaerobic respiratory bacteria (i.e., sulfate- and iron-reducing bacteria), and bacteria that produce ammonia from the degradation of nitrogen-rich organic matter. In each case, the metabolic activities of these different microorganisms contribute directly to the development of highly oversaturated solutions

from which carbonate mineral deposition subsequently occurs. Here we limit our discussion to a single process, the production of ammonia.

The production of ammonia during microbial decomposition of nitrogen-rich organic compounds ($R-NH_2$) tends to drive pH upwards and results in formation of relatively alkaline conditions. When solution concentrations of Ca^{2+} and HCO_3^- are high enough, calcium carbonate precipitation may take place. Of interest in this proposal is the in situ manipulation of calcite precipitation rates by the microbially catalyzed hydrolysis of urea via the overall reaction:

Urea is an important organic nitrogen carrier in natural environments, and is widely used as an agricultural fertilizer. Hydrolytic decomposition of urea to ammonia and carbon dioxide is catalyzed by the urease enzyme with a rate many times that of the uncatalyzed reaction (Ferris et al. 2003). This enzyme is widely distributed among bacteria, as well as higher organisms, and contributes directly to marked pH increases in soils treated with urea fertilizers. The high pH associated with urea hydrolysis will promote calcite precipitation. Bacterial hydrolysis of urea has been used to actively precipitate carbonates as mineral plugging and cementing agents in porous geological media. Because urea is relatively inexpensive, widely available, and degrades to innocuous compounds (carbon dioxide and ammonia) it is an ideal agent for in situ manipulation of subsurface biogeochemical environments. Furthermore, the use of urea to cause an increase in pH is preferable to direct addition of base because the gradual hydrolysis of urea is likely to promote a wider spatial distribution of calcite precipitation in the subsurface formations than the direct addition of base, which would in contrast cause immediate precipitation only in the borehole.

Mineralogy and Trace Metal Uptake of Microbially Precipitated Carbonates

In natural sediments, the most common carbonate minerals are the $CaCO_3$ polymorphs calcite and aragonite. Both will form under the influence of bacterial activity, although calcite deposition is more commonly reported. Ultimately, the type of carbonate mineral phase that is precipitated by bacteria will be controlled by the chemical composition of the aqueous environment in which they are growing. For example, microbial calcite precipitation proceeds in waters containing mostly Ca^{2+} , whereas huntite or hydromagnesite precipitates from Mg^{2+} -rich waters.

Although Sr concentrations of up to 1.0 weight percent are common in aragonite, normal values for Sr substitution in calcites tend to be much lower. This is generally attributed to the availability of more space in the aragonite structure in comparison to that of calcite; however, high precipitation rates sustained by microbial growth have been implicated in the partitioning of up to 1.0 wt. % Sr into calcite. Because the larger Sr ion is expected to 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 in the stability of the Sr-calcite solid solutions. In a broader context this implies that solid solution formation involving trace metal species like Sr may actually be significantly enhanced in natural waters, through rapid microbial precipitation of fine-grained carbonate minerals.

2.0 Methods

2.1. Nucleation, crystal growth and solubility of CaCO_3 precipitates induced by bacterial ureolysis from a Sr-free and Sr-inclusive artificial groundwater

2.1.1. Materials and Methods

Differences in the nucleation and growth of calcite precipitates induced by the bacterial hydrolysis of urea (ureolysis) from a Sr-contaminant inclusive, and a Sr-free artificial groundwater (AGW) mimicking the composition of the ^{90}Sr contaminated Snake River Plain aquifer, were investigated in a 8-day time-series experiment, using microcosms containing AGW and urea, and inoculated with the ureolytic bacteria, *Bacillus pasteurii*. Control experiments were performed with the urease deficient bacteria, *Bacillus Subtilis*. This resulted in two open system test and two open control systems: (i) *B. subtilis* suspension and AGW (control experiment) (ii) *B. subtilis* suspension and AGW + Sr contaminant (control experiment), (iii) *B. pasteurii* suspension and AGW (treatment experiment), and (iv) *B. pasteurii* suspension and AGW + Sr contaminant (treatment experiment). Experiments were performed over 8 days in an incubator at 20°C.

Solution chemistry and reaction kinetics. Throughout the experiment the experimental solutions were monitored for ammonium, dissolved calcium and strontium content and pH. From the solution data, the calcite saturation state was calculated from the product of the activities of dissolved Ca^{2+} and CO_3^{2-} divided by the equilibrium calcite solubility product. The critical saturation state at which precipitation began and the absolute reaction rate of calcite precipitation was determined as previously described (Ferris et al., 2003; Mitchell and Ferris, 2006) using affinity-based rate laws applied to the experimental data using unconstrained non-linear estimation and a quasi-Newton optimisation routine for parameter estimation in STATISTICA v 6.0.

Crystal collection and analysis. On each day of the experiment, each of the four experimental systems were sampled for any precipitates that may have formed. The bottle wall below the solution level was scraped with a sterile pipette tip, and these, and any other precipitates collected at the base of the bottle were collected by pipette suction. These were fixed using glutaraldehyde to a final concentration of 2 %, and were vacuum filtered through 0.2 μm pore-size filters. Mineralogical analysis of the precipitates was performed by powder X-ray diffraction (XRD). Filtered precipitates were gold coated and imaged by scanning electron microscopy (SEM). For each day, a random area of the coated precipitates were imaged at $\times 50$, and from this image < 20 randomly chosen areas were imaged at $\sim \times 1.5\text{k}$. Any crystals in the field of view were measured at their maximum diameter using ImageJ v.1.33.

Modelling nucleation and crystal growth. Raw crystal size data was entered into the USGS CRYSTAL COUNTER software (Eberl et al., 2000) and a CSD with a 100 nm bin size was generated for each day of the experiment up to day 6. The observed CSDs from the Sr-inclusive or Sr-free AGW were then matched with simulated CSDs, modelled using distinct crystal nucleation and growth mechanisms using the USGS GALOPER (Growth According to the Law of Proportionate Effect) software (Eberl et al., 2000). GALOPER assumes crystal growth rates are proportional to crystal size, rather than the same linear growth rate once crystals have

been nucleated (Kirkpatrick, 1981; Marsh, 1988). This ‘Law of Proportionate Effect’ (LPE) is defined as:

$$X_{J+1} = X_J + \varepsilon_J X_J, \quad [1]$$

where X_J is the linear crystal dimension after J calculation cycles, and ε_J is a random number that generally varies between 0 and 1. Equation 3 is iterated many times for each crystal with each iteration termed a growth cycle (Kile et al., 2000). CSDs that can be simulated using GALOPER include constant-rate nucleation and growth, nucleation with a decaying nucleation rate and growth, surface-controlled growth, supply-controlled growth, constant-rate and random growth, Ostwald ripening, random ripening, and crystal coalescence (Eberl et al., 2000).

Mass, surface area and solubility. The mass and surface area of individual crystals was estimated assuming spherical precipitates. Masses were determined from the product of the sphere volume ($4/3\pi(d/2)^3$ (where d is the measured maximum crystal diameter) * the density of calcite (2.71 g cm^{-3}) (Deer et al., 1992) and surface areas were estimated as the surface area of the sphere ($4\pi(d/2)^2$). The molar surface area (A), moles m^{-2}) for individual crystals was calculated from the estimated mass and surface area, according to $A = \text{Moles} / \text{Surface Area} (\text{m}^2)$. The solubility of individual crystals was estimated as the solubility constant of a given molar surface area ($Ks0_{(A)}$) (Stumm and Morgan, 1996), according to;

$$\ln Ks0_{(A)} = \ln Ks0_{(A=0)} + \frac{2\gamma A}{3RT} \quad [2]$$

where $\ln Ks0_{(A=0)}$ is the published solubility constant at equilibrium (for an infinite molar surface area, $A = 0$), γ is the solid-liquid interfacial free energy for calcite ($0.094 \text{ joules m}^{-2}$) (Stumm and Morgan, 1996), R is the gas constant and T is the temperature in Kelvin.

2.2. The influence of bacteria surfaces on the nucleation and growth of CaCO_3

2.2.1. Materials and Methods

Microcosm experiments were performed using a novel dialysis technique to identify the influence of bacterial surfaces and exudates on the morphology, mineralogy, size and solubility of CaCO_3 precipitated in response to the enzymatic hydrolysis of urea in AGW by the ureolytic bacteria, *Bacillus pasteurii*. Control experiments were performed with the urease deficient bacteria, *Bacillus Subtilis*. Individual microcosms were prepared as follows: 100 ml of bacteria-free AGW was decanted into pre-autoclaved acid washed 250 ml polypropylene cylinders containing acid washed stir bars. 75 ml of either *B. pasteurii*-inclusive (test) or *B. subtilis*-inclusive (control) AGW was then decanted into a tube of inert Spectrum Spectra/Por® 6 Regenerated Cellulose Dialysis Membrane with a 29 mm diameter and a 10K Dalton molecular weight cut off (MWCO), which had been thoroughly soaked for 1 day and rinsed 10 times in SUPW, and sealed with autoclaved nylon clamps. This pore size will contain the bacteria, and organic macromolecules including low molecular weight proteins, polysaccharides from bacteriogenic exudates, but will allow inorganic ions to pass through. While amino acids $< 10\text{K}$ MWCO from the degradation of proteins may possibly pass through the dialysis membrane, most organic molecules will be contained within the *B. Pasteurii*-inclusive AGW. Each dialysis tube containing bacteria-inclusive AGW was immediately immersed into one of the polypropylene

cylinders containing bacteria-free AGW, covered with Parafilm®, and placed on a magnetic stirring plate at a controlled room temperature of ~ 20°C. This resulted in control and test microcosms with equivalent starting AGW chemistry, with bacteria-inclusive AGW inside the dialysis membrane, and bacteria-free AGW outside the membrane (Figure 1). *Reverse experiments* were also undertaken in duplicate as above, but with the bacteria-free AGW inside the dialysis tubing, and the bacteria-inclusive AGW outside the dialysis tubing, in order to assess any experimental artefacts. Solution chemistry determination, ureolysis and calcite precipitation kinetics, crystal collection, CSD generation and nucleation and growth modelling was undertaken as performed and presented in section 2.1.1.

Figure 1. Schematic diagram of experimental setup.

3.0 Results and Discussion

The critical saturation state ($S_{critical}$) at which precipitation was induced was greater in the Sr-inclusive ($S_{critical} = 91$) than the Sr-free ($S_{critical} = 73$) experiments, indicating Sr inhibits the saturation state at which calcite nucleation can occur. This is likely to reflect the blocking of

potential nucleation sites on the bottle walls and bacteria, since the poisoning of potential nucleation sites by inhibitory ions is commonly observed in experimental systems (Gutjahr et al., 1996; Lebron and Suarez, 1996; Zhang and Dawe, 2000). Sr-free experiments exhibited a gradual increase in mean calcite crystal size (< 1000 nm) from day (D) 1 to 6, while in the Sr-inclusive experiments, daily mean sizes were approximately constant from D1 to D6, and crystals were smaller (mean < 840 nm) (Figure 1). While crystals were most frequent in the ~ 250 to 2000 nm size range, the occurrence of larger but far less frequent crystals (maximum sizes = $11000 < 83000$ nm) accounted for a significant proportion of the calcite precipitated and Sr co-precipitated (Figure 2). These data demonstrate a steady state had been attained early in the Sr-inclusive experiments from growth inhibition by Sr. Speciation modelling suggests growth inhibition in the presence of Sr does not reflect the formation of complexes between the inhibitory Sr^{2+} ion and the reactive CO_3^{2-} ions, which would reduce the activity of the CO_3^{2-} ions, the saturation of the calcite, and thus the rate at which ionic collision by molecular diffusion would occur (Morse, 1986). Rather it must reflect the effect of Sr upon the surface of the precipitating solid, by adsorption of metals on to high energy calcite growth sites (kinks), thus making them unavailable for growth, or on to the advancing terrace or step sites of a growing crystal, thus impeding the velocity of growth (Morse and Mackenzie, 1990; Parsiegla and Katz, 1999; Freij et al., 2004). Such incorporation is thus as a trace species, not as stoichiometric metal-carbonate precipitate, and is therefore in support of our XRD data, which indicates that strontanite is not precipitated.

Figure 2. (a) Crystal size distributions (CSDs) of calcite crystals generated from the bacterial hydrolysis of urea from Sr-free and Sr-inclusive artificial groundwater, on days 1, 2, 4 and 6 of the experiment generated using the USGS CRYSTAL COUNTER software (Eberl et al., 2000). Crystals larger than 4000 nm were recorded, but their frequency was always < 0.0063 . (b) Lognormal fits of the raw CSDs generated in the CRYSTAL COUNTER software. The strength of the lognormal CSD fit, expressed as the residual squared of the crystal size frequency between the raw and fitted CSD, was always > 0.91 .

Figure 3. (a) Mass of individual calcite crystals as a percentage of the total precipitated mass on each day of the Sr-free and Sr-inclusive experiments. (b) Surface area of individual calcite crystals as a percentage of the total precipitated surface area on each day of the Sr-free and Sr-inclusive experiments. Crystals to the right of dashed line are $> 12,000$ nm.

Modelling of the crystal growth mechanisms on the USGS GALOPER software suggested D1 CSDs in the Sr-inclusive and Sr-free experiments were generated by a decreasing nucleation rate with surface-controlled growth, followed by supply-controlled and random growth despite the availability of Ca^{2+} and HCO_3^- (Figure 4). The requirement of surface-controlled growth during the nucleation processes suggests the initial rate of growth was limited by the available surface area and not the supply of Ca^{2+} and CO_3^{2-} . Conversely, subsequent supply-controlled growth, even though Ca^{2+} and CO_3^{2-} were at significant concentrations in the bulk solution suggests the transport of reactants to the crystal surface was limited by advection or diffusion. This is likely given the exponential increase in the demand for reactants required to maintain size proportional growth over time (Kile et al., 2000), and is most likely to reflect advection limited growth even in unstirred experiments, since solution velocity is considerable relative to the mean crystal size for small crystals ($< 100 \mu\text{m}$) due to convection and Brownian motion (Kile and Eberl, 2003). Calculation of the K_{S0} suggests the co-precipitation of Sr increases crystal solubility, reflecting the higher molar surface area of the smaller crystals generated (Figure 5). However, this is unlikely to dramatically reduce the long term effectiveness of Sr immobilisation since the majority of calcite precipitated and Sr co-precipitated occurs in low frequency large crystals which are far less soluble than the high frequency smaller crystals.

Figure 4. Results of crystal nucleation and growth evolution modelling using USGS GALOPER software (Eberl et al., 2000). (a) Initial nucleation and surface-controlled growth. (b) Growth from nucleation to observed day 1 crystal size distribution (CSD) by supply-controlled growth in Sr-free AGW. Subsequent growth in Sr-free AGW to observed day (c) 2 and (d) 4 CSD by random growth. (e) Growth from nucleation to observed day 1 CSD by supply-controlled growth in Sr-inclusive AGW. (f) Subsequent growth in Sr-inclusive AGW to the observed day 2 CSD by random growth.

(a) Initial nucleation

(d) Sr-free: Day 4 and 6

(b) Sr-free: Day 1

(e) Sr-inclusive: Day 1

(c) Sr-free: Day 2

(f) Sr-inclusive: Day 2

Figure 5. (a) Molar surface area as a function of crystal size, (b) K_{s0} as a function of crystal size on each day of the Sr-free and Sr-inclusive experiments.

Ureolysis by *B. pasteurii* resulted in the production of ammonium and an increase in pH in the whole AGW solution. This initiated predominantly rhombohedral calcite precipitation (Figure 6) at the same critical saturation state ($S_{critical} = 11$ to 12) in the *B. Pasteurii*-inclusive and bacteria-free zone of the AGW, indicating the mineralogy and morphology of $CaCO_3$ precipitation is not controlled by *B. Pasteurii* surfaces and organic macromolecule exudates, which will be predominately contained within the dialysis membrane. Calcite formation is more likely to reflect the influence of organic and inorganic species slowing the rate of $CaCO_3$ precipitation (Kitano and Hood, 1965; Meldrum and Hyde, 2001; Braissant and Verrecchia, 2002; Braissant et al., 2003; Bosak et al., 2004; Bosak and Newman, 2005). Organic and inorganic species can complex with cations and reduce cationic activity and decrease mineral supersaturation, or poison growth sites on the mineral surface (Meldrum and Hyde, 2001). Indeed, precipitation rates in our experiments are lower by one to two orders of magnitude than those measured in seeded precipitation studies at similar values of saturation, if rates are un-normalised for the surface area of the seed material (Zhang and Dawe, 1998; Zuddas and Mucci, 1998; Nilsson and Sternbeck, 1999). The predominantly rhombohedral morphology is consistent with the standard polyhedral prismatic form of calcite, (Deer et al., 1992) often precipitated without the presence of bacteria or organic macromolecules (Meldrum and Hyde, 2001; Braissant et al., 2003; Roque et al., 2004), which suggests the influence of *B. Pasteurii* surfaces and exudates on crystal morphology is somewhat limited. The stepped and rounded edges of crystals from both the *B. Pasteurii* and bacteria-free AGW (Figure 6) probably reflect the effect of inorganic ions within the precipitating solid, which have been shown to distort $CaCO_3$ mineral structures (Tracy et al., 1998; Raz et al., 2000; Kontrec et al., 2004; Kralj et al., 2004). Interestingly, the rhombohedral form with rounded stepped edges is very similar to mature calcite crystals precipitated from solutions containing urea and $CaCl_2$ in the presence of purified urease enzyme (Sondi and Matijevic, 2001). This suggests NH_4^+ or urea may be controlling the dominant morphology of the crystals, since urease should be contained within the dialysis membrane and only organic macromolecules $< 10K$ Dalton MWCO will be present in both the *B. Pasteurii* and bacteria-free AGW. However the more ordered nature

Figure 6. Example SEM images of calcite precipitates generated in response to bacterial ureolysis. Calcite crystals generated in the *B. Pasteurii*-inclusive AGW by (a) day 1, and (b) day 7, and in the bacteria-free AGW by (c) day 1, and (d) day 7. (e) and (f) show examples of calcite crystals generated by bacterial ureolysis from *reverse experiments* are also shown from the *B. Pasteurii*-inclusive and bacteria-free AGW. (g) and (h) show the stepped surface topography of crystals in both the *B. Pasteurii*-inclusive and bacteria-free AGW which causes somewhat rounded crystal edges.

(a) Day 1: Bacteria-inclusive

(b) Day 1: Bacteria-free

(c) Day 7: Bacteria-inclusive

(d) Day 7: Bacteria-free

(e) Reverse experiments: Bacteria-inclusive

(f) Reverse experiments: Bacteria-free

(g) Bacteria-inclusive surface topography

(h) Bacteria-free surface topography

and smoothed surfaces of crystals from the *B. Pasteurii*-inclusive AGW suggests the presence of bacteria and organic macromolecules is somewhat stabilising the rhombohedral form of the crystals. Warren et al. (2001) demonstrated a wider range of crystal morphologies than in our current study, including pseudopolyhedral ‘dumbell’, globular, as well as rhombohedral morphologies. Some of this variation is likely to reflect the formation of vaterite, which often exhibits a more spherical or globular morphology (Braissant et al., 2003; Roque et al., 2004). However, the high concentration of urea and ammonium generated (< 660 mM) is probably responsible for much of the deviation of morphology away from standard rhombohedral calcite (Sondi and Matijevic, 2001).

However, the evolution of distinctly different lognormal crystal-size-distributions in the *B. Pasteurii*-inclusive and bacteria-free zone of the AGW resulted from identical changes in bulk solution chemistry (Figure 7). Specifically, *B. Pasteurii* increased the size and size variance of crystals, and led to a greater crystal growth rate throughout the experiments, relative to bacteria-free AGW. Estimates of the mass of *B. Pasteurii* encased within the mature calcite crystals suggest *B. Pasteurii* can only account for < 4.4 % of the crystal diameter, and was not sufficient to account for the ~ 46 % larger median crystal size exhibited by day 7 in the *B. Pasteurii*-inclusive, than the bacteria-free AGW. This indicates a direct influence of bacterial surfaces and bacteriogenic organic macromolecules.

Figure 7. Crystal size distributions of calcite crystals generated from bacterial ureolysis from *B. Pasteurii*-inclusive and bacteria-free artificial groundwater, on (a) day 1 and (b) day 7 of the experiment, generated using the USGS CRYSTAL COUNTER software (Eberl et al., 2000). Lognormal fits of the raw CSDs from the *B. Pasteurii*-inclusive and bacteria-free artificial groundwater, generated in the CRYSTAL COUNTER software, are shown in (c) and (d) respectively.

Figure 8. Results of crystal nucleation and growth evolution modelling using USGS GALOPER software (Eberl et al., 2000). (A) *B. Pasteurii*-inclusive AGW: (i) Initial nucleation and surface-controlled growth with a critical nucleus size of 3nm and a 0.63 probability of nucleation. (ii) Growth from nucleation to observed day 1 CSD by supply-controlled growth. (iii) Growth from day 1 to day 7 by supply-controlled growth then Ostwald ripening, with a 0.2 probability of crystal coalescence. (B) Bacteria-free AGW: (i) Initial nucleation and surface-controlled growth with a critical nucleus size of 3nm and a 0.8 probability of nucleation. (ii) Growth from nucleation to observed day 1 CSD by supply-controlled growth. (iii) Growth from day 1 to day 7 by continued supply-controlled growth. Quality of simulation expressed as the residual squared of the crystal size frequency between the raw and simulated CSD.

(A) Bacteria-inclusive AGW

(i) Initial nucleation

(ii) Day 1. r^2 of simulated v measured fit: 0.96

(iii) Day 7. r^2 of simulated v measured fit: 0.81

(B) Bacteria-free AGW

(i) Initial nucleation

(ii) Day 1. r^2 of simulated v measured fit: 0.99

(iii) Day 7. r^2 of simulated v measured fit: 0.92

Figure 9. (a) Estimated spherical surface area of crystals as a function of crystal size, (b) Estimated spherical surface area as a function of calcite precipitation rate, (c) Molar surface area as a function of crystal size and (d) K_{s0} as a function of crystal size, from the bacteria-free and *B. Pasteurii*-inclusive AGW on days 1 and 7.

Quantifying the effect of bacteria and bacteriogenic macromolecules on the CSD of precipitates has received little attention. Roqué et al., (2004) found mixtures of acidic organic proteins or polyaspartic acid with $CaCl_2$ inhibited $CaCO_3$ crystal growth relative to blank $CaCl_2$ solutions. This contradicts our findings (Figure 7) and suggests an alternative explanation for the larger crystals formed in the presence of bacteria. If crystals had formed earlier in the presence of bacteria, the size difference could be propagated throughout the growth processes such that crystals are always larger in the presence of *B. Pasteurii*. However, there was no evidence of crystals forming earlier in the bacteria-inclusive AGW, and crystals were visually apparent slightly earlier in the bacteria-free AGW. Therefore, it seems more plausible that *B. Pasteurii* or their exudates may subtly distort the mineral structure, leading to larger crystals without the formation of alternative morphologies or metastable $CaCO_3$ polymorphs. Indeed, the more ordered smoother crystals apparent by day 7 in the presence of *B. Pasteurii* than the bacteria free-AGW (Figure 6c and d) suggest a stabilisation of the calcite mineral structure and rhombohedral morphology. However, the nucleation and growth mechanisms modelled on GALOPER suggest the greater size and variance of crystals in the presence of *B. Pasteurii* is propagated by a reduction in the probability of nucleation relative to the bacteria-free AGW once a critical saturation has been attained (Figure 8). This generates a lower frequency of smaller crystals but a higher frequency of larger crystals than in the bacteria-free AGW, with subsequent size-

dependent supply-controlled growth propagating this size difference throughout the growth process (Figure 7c and d, and Figure 8).

Calculated crystal solubility ($\ln K_{s0}$) was lower for crystals < 4000 nm in diameter, reflecting smaller molar surface areas (Figure 9). This suggests that the larger crystals generated in the presence of *B. Pasteurii* have a lower affinity for re-dissolution than those generated in the bacteria-free AGW, which may act as a positive feedback to maintain larger crystal sizes in the presence of *B. Pasteurii*. Indeed, the proportion of crystals that are apparently affected by surface area controlled solubility decreases from only 81 to 35 % from day 1 to day 7 in the presence of *B. Pasteurii*, but from 92 to only 48 % over the same period in the absence of *B. Pasteurii*.

Calcite precipitation induced by bacterial ureolysis is highly effective at co-precipitating Sr at low precipitation rates (Mitchell and Ferris, in press), and while Sr inclusion reduces crystal size and increases crystal solubility, the majority of calcite precipitated and Sr co-precipitated occurs in larger crystals which are not effected by surface area controlled solubility (Mitchell and Ferris, 2006a). Similarly, biotic calcite precipitation produces larger, less soluble crystals, relative to abiotic calcite precipitation. This suggests bacterially induced calcite precipitation may enhance contaminant stabilisation relative to abiotically induced precipitation (Mitchell and Ferris, 2006b). These data further demonstrate that calcite precipitation induced by the bacterial hydrolysis of urea is a highly effective and cost efficient strategy to combat metal and radionuclide contamination in groundwater environments. On going field investigation will elucidate the field scale effectiveness of the approach.

We have also undertaken experiments to investigate the rate dependent competitive co-precipitation of Sr – Ba, Sr – Cs and Ba – Cs. binary mixtures, as well as U, Zn and Co. These metals were chosen on the basis of common radionuclide and metal mixtures found in contaminated DOE sites which exhibit similar chemical behaviour (Riley et al., 1992). Crystal size distributions are also being generated from these experiments and crystal size evolution modelling undertaken on GALOPER to investigate the effect of such contaminants on the nucleation, growth and solubility of the resulting precipitates. These data are currently being processes in preparation for publication in *Chemical Geology* and *Applied Geochemistry*.

4.0 Relevance, Impact and Technology Transfer

Demonstration of Sr^{2+} solid phase capture in response to microbial induced carbonate mineral precipitation, initiated 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 mineral precipitation reactions in subsurface environments.

5.0 Project Productivity

This project has accomplished the goal of developing a better quantitative understanding of microbiological and geochemical controls on carbonate mineral precipitation reactions that are caused by ureolytic bacteria, and has determined key kinetic parameters to assess potential contributions and constraints of these processes for

the solid phase capture and immobilization of strontium in the subsurface. In addition, we have made a number of presentations at major scientific meetings and produced three peer reviewed journal publications with several manuscripts in preparation.

6.0 Personnel Supported

6.1 Graduate students

R. James
Y. Litwin

6.2. Postdoctoral research associates

Dr. V. Phoenix
Dr. A. Mitchell
Dr. S. Smith

7.0 Publications

7.1 Published in peer-reviewed journals

Mitchell, A.C. and Ferris, F.G. 2005. The Co-Precipitation of Sr into Calcite Precipitates Induced by Bacterial Ureolysis in Artificial Groundwater – Temperature and Kinetic Dependence. *Geochimica et Cosmochimica Acta* 68:4199-4210.

Mitchell, A.C. and Ferris, F.G. 2006a Mechanisms of strontium contaminant immobilisation in calcite precipitates induced by bacterial ureolysis – evidence from crystal size distributions. *Environmental Science and Technology* 40: 1008-1014.

Mitchell, A.C. and Ferris, F.G. 2006b The Influence of *Bacillus Pasteurii* on the Nucleation and Growth of Calcium Carbonate. *Geomicrobiology Journal* 23: 1-24.

7.2 Published in proceedings

NA

7.3 Accepted/submitted/in preparation for publication

Mitchell, A.C. and Ferris, F.G. Competitive co-precipitation of Ba, Sr and Cs in calcite precipitates induced by bacterial ureolysis in artificial groundwater. In preparation for *Chemical Geology*.

Mitchell, A.C. and Ferris, F.G. The influence of Ba, Sr and Cs co-precipitation on the nucleation growth and solubility of calcite precipitates induced by bacterial ureolysis. In preparation for *Applied Geochemistry*.

7.4 Graduate student theses

NA

8.0 Interactions

8.1 Participation at meetings

Colwell, F.S., R. W. Smith, F. G. Ferris, J. C. Ingram, and A. L. Reysenbach, Y. Fujita, J. L. Taylor, T.L. Tyler, M. E. Delwiche, and M. M. Cortez. 2002. 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. U.S. Department of Energy EMSP meeting, Washington, D.C., U.S.A.

Mitchell, A.C. and Ferris, F.G. The Co-Precipitation of Sr into Calcite Induced by Bacterial Ureolysis in Artificial Groundwater – Temperature and Kinetic Dependence. *AGU 2003. San Francisco, December 2003.*

Mitchell, A.C., Magalhaes, L., Mancini, A. and Ferris, F.G. Mechanisms of calcite precipitation by bacterial ureolysis – evidence from crystal size distributions. *Goldschmidt 2004. Processes in Geochemistry, Copenhagen, June 6th – 12th 2004.*

Mitchell, A.C. and Ferris, F.G. Mechanisms of strontium contaminant immobilisation in calcite precipitates induced by bacterial ureolysis – evidence from crystal size distributions. *The Joint International Symposia for Subsurface Microbiology (ISSM 2005) and Environmental Biogeochemistry (ISEB XVII) Jackson Hole, Wyoming, August 14th-19th 2005.*

9.0 Transitions and Future Work

These investigations are being continued at INEEL under the direction of F.S. Colwell and Y. Fujita, with a greater focus on field experimentation.

10.0 Patents

NA

11.0 Literature Cited

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