

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

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Abstract

Co-precipitation in carbonate minerals can slow the transport of divalent radionuclides and contaminant metals (e.g., ⁹⁰Sr, UO₂, Co) in the subsurface. It may be possible to accelerate this process in certain environments by stimulating the native microbial community to generate favorable chemical conditions. Arid western environments, where calcite precipitation is already an important process, are especially suitable candidates for such an approach. We are evaluating the potential for utilizing subsurface ureolytic organisms to produce conditions conducive to carbonate precipitation. Groundwater samples from the Snake River Plain Aquifer (SRPA) underlying the Idaho National Engineering and Environmental Laboratory (INEEL) in Idaho were screened for urea-hydrolyzing activity, and twelve ureolytic bacterial isolates were recovered. Three were selected for further characterization and evaluation in calcite precipitation experiments. 16S rRNA gene sequence data indicated that two were *Pseudomonas* and the other was a *Variovorax*. Urease activity appeared to be lower in the SRPA isolates than in the known urease-positive species *Bacillus pasteurii*, but in all cases the removal of dissolved calcium from the calcite precipitation medium matched equilibrium predictions based on measured ammonium concentrations. X-ray diffraction analyses indicated that calcite is the predominant carbonate polymorph precipitated. However, when strontium is included in the medium, aragonite and vaterite have also been observed.

Relevance to EM Needs

This project addresses the need for EM to develop new approaches to the remediation of subsurface environments contaminated by metals and radionuclides. Temporary immobilization of these contaminants in the vadose or saturated zones by incorporation into mineral phases can be a cost-effective means of reducing the risks posed by these contaminants. Our investigations of the geochemical and biological mechanisms involved in microbial calcite precipitation will provide the scientific underpinnings necessary for the development and practical implementation of this novel in situ remediation technology.

Isolation and Characterization

Several SRPA groundwaters collected from depths ranging from 75 to >210 m were screened for ureolytic organisms using differential medium (Figure 1). All samples tested positively for ureolytic activity, and twelve isolates were obtained. Fatty acid methyl ester (FAME) analysis indicated that they fell into three main groups (Figure 2). One member of each group was selected for further characterization and evaluation in the calcite precipitation experiments.

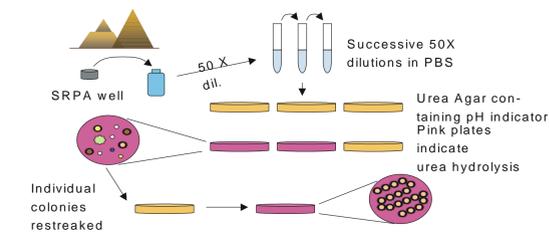


Figure 1. Protocol for isolation of ureolytic organisms.

All were gram negative, rod-shaped, facultative heterotrophs, but carbon source utilization profiles (BILOG GN) indicated differences between the isolates (Figure 3). Phylogenetic analysis of the isolates indicated that their 16S rRNA sequences were all similar to environmental eubacterial sequences reported previously in the literature. Two were identified as *Pseudomonas*, and one as *Variovorax*. Experiments with different growth conditions indicated that for all three isolates urease activity is inducible rather than constitutive.

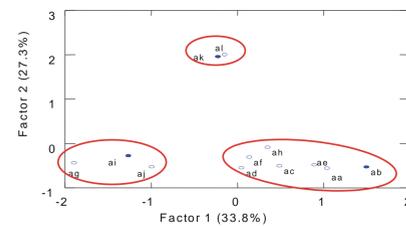


Figure 2. Plot of Factor 1 vs. Factor 2 from principal components analysis of fatty acid methyl ester (FAME) analysis of SRPA isolates.

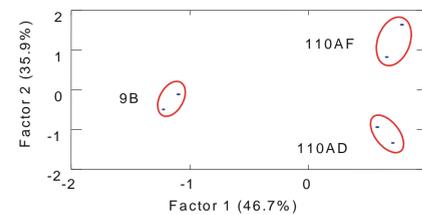


Figure 3. Plot of Factor 1 vs. Factor 2 from principal components analysis of substrate utilization results for incubation in BIOLOG GN microtiter plates (n = 2 for each isolate).

Urea Hydrolysis and Calcite Precipitation Experiments

Experimental Design

Cells grown overnight in Brain Heart Infusion (BHI) broth supplemented by 20 g/L urea were harvested by centrifugation and washed to remove residual medium. Cell suspensions at a fixed optical density (OD₆₀₀ = 0.149) were introduced into the calcite precipitation medium (CPM), consisting of 3.0 g/L Nutrient Broth (Difco), 25 mM CaCl₂, 25 mM NaHCO₃, and 333 mM urea (Ferris and Stehmeier, 1992). Positive controls containing *Bacillus pasteurii*, an organism known to express urease constitutively, were also prepared (OD₆₀₀ = 0.072 and 0.041). Triplicate experimental flasks were incubated at room temperature without shaking for periods of up to 8 hours. Calcium concentrations and pH were measured using ion selective electrodes. Urea hydrolysis was monitored routinely by measuring ammonium ion concentrations; comparison with direct measurements of urea confirmed the correlation of the two parameters.

Results

The SRPA isolates behaved similarly with respect to pH, apparently reaching a plateau (8.3) after approximately 4 hours (Figure 4). In contrast, the pH in the *B. pasteurii* cultures continued to increase over the course of the entire experimental period.

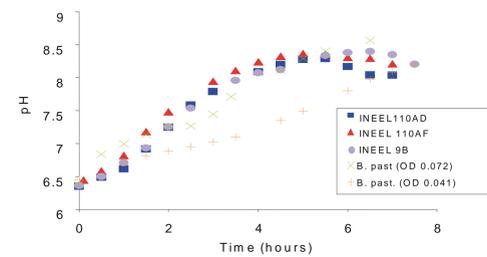


Figure 4. pH evolution during precipitation experiments.

Ammonium concentrations in all CPM cultures increased linearly with time (Figure 5), while dissolved calcium concentrations in the medium decreased linearly (Figure 6). Calcium concentrations plotted vs. ammonium concentrations match equilibrium predictions (MINEQL) for expected concentrations of calcium as a function of the measured ammonium (Figure 7). This result implies that dissolved calcium concentrations are effectively controlled by the equilibrium solubility of calcite. The average slope is 0.5, indicating that the generation of 2 moles of ammonium (or the hydrolysis of one mole of urea) results in the removal of 1 mole of calcium from solution (see equation 3).

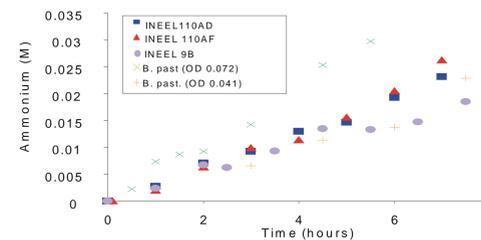
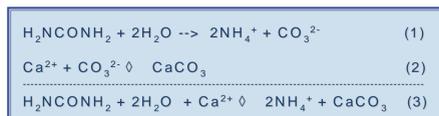


Figure 5. Production of ammonium during precipitation experiments.



Equations for urea hydrolysis (1), carbonate precipitation (2), and sum of the two reactions (3).

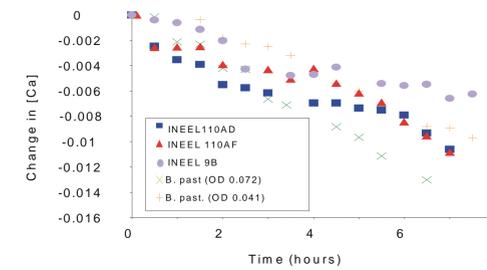


Figure 6. Decrease in [Ca²⁺] over time.

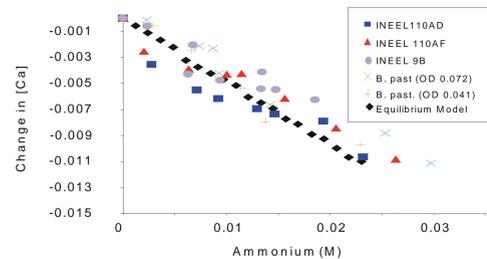


Figure 7. Change in [Ca²⁺] as a function of ammonium concentration.

Mineral precipitates formed during the experiments included spherical (Figure 8) and amorphous morphologies (Figure 9). X-ray diffraction analyses indicated that the predominant carbonate morphotype formed was calcite. However, when strontium was added to the medium, aragonite and vaterite were also observed.

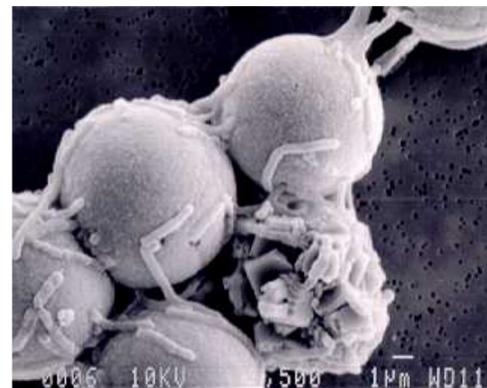


Figure 8. Calcite spheres produced during precipitation experiment by *B. pasteurii*.

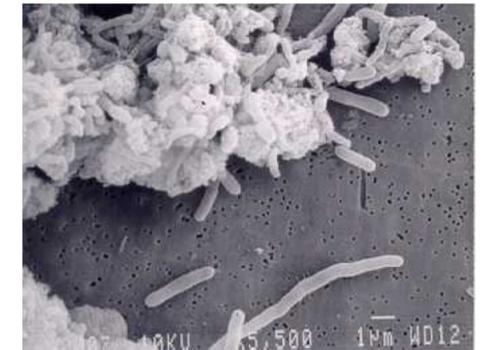


Figure 9. Calcite crystals produced during precipitation experiment by *B. pasteurii*.

Conclusions

Screening for urease activity in SRPA samples indicates that the ability to hydrolyze urea is widespread in the aquifer. Calcium carbonate precipitation is observed in microcosms inoculated with urease-positive isolates from the SRPA, and the predominant mineral produced has been identified as calcite. Equilibrium modeling results suggest that under the experimental conditions tested here, the rate of calcite precipitation can be linked directly to the rates of urea hydrolysis and ammonium production.

Current and Future Work

Carbonate precipitation experiments are currently being conducted in medium more closely simulating natural groundwater, to determine whether the observations derived from the artificial calcite precipitation medium are applicable to environmental conditions. Initial results are promising, with slower but still significant carbonate precipitation observed. Efforts are also currently underway to develop spectroscopic techniques (secondary ion mass spectrometry and Raman spectroscopy) that can be used to distinguish between uptake of trace metals by surface adsorption or by formation of solid solutions.

References

Ferris, F.G. and L.G. Stehmeier. 1992 Bacteriogenic mineral plugging. U.S. Patent No. 5,143,155

Acknowledgments

We thank Dan Lawson formerly at Idaho State University for assistance with the isolation and characterization of ureolytic microorganisms, Alex Trentview at the University of Toronto for assistance with the calcite precipitation experiments, and Amy Banta at Portland State University for assistance with the phylogenetic analysis. Funding for this work was provided by the INEEL, and by the U. S. Department of Energy's Environmental Management Science Program (DE-FG07-99ER15205).