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Project Title: 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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Research Objectives:

Radionuclide and metal contaminants are present in the vadose zone and groundwater throughout the U.S. Department of Energy (DOE) weapons complex. Demonstrating *in situ* immobilization of these contaminants in vadose zones or groundwater plumes is a cost-effective remediation strategy. However, the implementation of *in situ* remediation requires definition of the mechanism that controls sequestration of the contaminants. One such mechanism for metals and radionuclides is co-precipitation of these elements in authigenic calcite and calcite overgrowths. Calcite, a common mineral in many aquifers and vadose zones in the arid western U.S., can incorporate divalent metals such as strontium, cadmium, lead, and cobalt into its crystal structure by the formation of solid solutions. 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, solid solution properties of the trace metal in calcite, and also the surfaces upon which the calcite is precipitating. A fundamental understanding of the coupling of calcite precipitation and trace metal partitioning and how this may occur in aquifers and vadose environments is lacking.

The focus of the research proposed here is to investigate the facilitated partitioning of metal and radionuclides by their co-precipitation with calcium carbonate. Our specific research objectives include

- Elucidating the mechanisms and rates of microbially facilitated calcite precipitation and divalent cation adsorption/co-precipitation occurring in a natural aquifer and vadose zone perched water body as a result of the introduction of urea.
- Assessing the effects of spatial variability in aquifer host rock and the associated hydro/biogeochemical processes on calcite precipitation rates and mineral phases within an aquifer and a vadose zone perched water body.

Research Progress and Implications:

This report summarizes project activities at the INEEL after 8 months of a 36-month project. Descriptions of the various tasks, and results where available, are presented below.

Initial screening, isolation and characterization of ureolytic subsurface bacteria. Water samples were collected from wells in the Snake River Plain Aquifer (SRPA); three were located on the INEEL property and one just south of the site boundary. All groundwater samples tested positively for urease activity, in both liquid and solid media. Other groundwater samples collected from elsewhere in eastern Idaho also universally tested positively, indicating that urease activity is indeed common in the SRPA. Twelve ureolytic SRPA isolates were obtained from the urea agar plates. Fatty acid methyl ester (FAME) profiles were obtained for all twelve isolates. Principal component analysis of FAME results indicated that the isolates could be divided into three main groups. Based on these results, one member of each group was selected for further characterization. All three of the isolates were gram negative rod-shaped heterotrophs, but the carbon source utilization potential results indicated differences between the isolates. However for all three organisms amino acids appeared to be oxidized most rapidly. Limited phylogenetic analysis performed on the isolates indicated that all three likely had 16S rRNA gene sequences very similar to previously described isolates or clones. One was of the genus *Variovorax*, and two were of the genus *Pseudomonas*. Because the SRPA isolates were so similar to previously reported sequences, the acquisition of sequence data for the entire 16S rRNA gene was not pursued further.

Calcite precipitation experiments. The three selected SRPA isolates were sent to F. G. Ferris at the University of Toronto, for testing of their ability to precipitate calcite when introduced into an artificial medium supplied with calcium and urea (See report for EMSP grant DE-FG07-99ER15025). Briefly, the SRPA isolates were demonstrated to induce calcite precipitation similarly to the known constitutive urease-positive organism *Bacillus pasteurii*, although the experimental results suggested that the urease activity of all of the SRPA isolates was significantly lower than that of the *B. pasteurii*. In all cases however calcite precipitation appeared to proceed according to predictions based on the equilibrium solubility of calcite, given the measured pH and ammonium concentrations. The results indicate that the generation of two moles of ammonium (or the hydrolysis of one mole of urea) results in the removal of one mole of calcium from solution.

Subsequent to the demonstration of calcite precipitation induced by SRPA isolates in an artificial medium with high calcium concentrations, a synthetic groundwater "recipe" was developed based on average data for the inorganic composition of SRPA water. The pH of the synthetic groundwater, in an open system, was 8.3, which is within the range of typical pH values reported for SRPA waters. The calculated log saturation index of the synthetic groundwater was approximately 0.45, similar to values calculated for real groundwater samples from the aquifer. This recipe was sent to Ferris, and he has been using it for calcite precipitation experiments, after adjusting the pH to 6.5 and adding 33 mM urea; the calcium and urea concentrations are an order of magnitude less than what was used in the previously described experiments. Initial results are promising; calcite precipitation, although slower than in the artificial precipitation medium, is indeed linked to urea hydrolysis. In addition, INEEL recently sent Ferris two additional ureolytic SRPA isolates.

Spectroscopic Methods to Characterize Carbonates. In order to predict the extent and stability of trace element incorporation into calcite precipitated under our remediation approach, it is necessary to understand how much, and in what form, the trace elements are being incorporated into the carbonates. To help us with this task we are investigating two spectroscopic techniques: Secondary Ion Mass Spectrometry (SIMS) and Raman spectroscopy. SIMS can distinguish between the presence of a trace metal on the surface (providing information concerning adsorption) and in the near surface region (indicating absorption into the solid solution). In addition, it is capable of spatial chemical imaging on a sub-micron scale. By comparing the SIMS data with bulk chemical data (collected by inductively coupled plasma atomic emission spectroscopy), trace metal uptake mechanisms can be inferred. The initial SIMS results indicate that the Sr to Ca ratio for calcite precipitated both biotically and abiotically under similar solution conditions (Ca concentration 1050 ppm, Sr concentration 23 ppm) is ≈ 0.008 (atomic ratio). Raman spectroscopy is of interest because it can distinguish between molecular interactions of carbonate with Ca, Sr and other metals. Initial Raman spectra have been collected for pure carbonate minerals and on biogenic calcite produced at INEEL under conditions similar to Ferris'. These spectra will be utilized as references for the carbonate specimens investigated throughout this project.

Molecular Methods to Characterize Microbial Community Response to Urea. Because it is likely to be difficult to verify that urea hydrolysis (and thus carbonate precipitation) is in fact occurring in the aquifer as a result of urea introduction, efforts are underway to identify microbial community responses to urea that can be used as indicators of *in situ* urea hydrolysis. Work thus far on this task has focused on the genes which encode for the protein responsible for urea hydrolysis, urease. In order to develop nucleic acid-based methods for detecting the presence of urease, or urease activity as expressed by mRNA, in environmental samples, conserved regions of the genetic information for urease must be identified. Known urease peptide sequences from approximately twenty clinical and environmental isolates were aligned, and five reasonable priming sites showing a high degree of homology were initially identified. These priming sites are within the *ureC* gene, which codes for the large catalytic subunit of urease in most ureolytic eubacteria. Oligonucleotide primers corresponding to these sites were designed, and are currently being tested in polymerase chain reaction (PCR) protocols. The goal is to develop amplification protocols that can be used reliably on environmental ureolytic organisms, present as mixed communities in groundwater. Such protocols would be the foundation for the development of methods such as MPN-PCR (to estimate numbers of urease-positive organisms in a sample) or RT-PCR (to estimate urease activity, as opposed to the presence of the gene, in a sample).

Preparation for Field Experiments. In planning field experiments in the SRPA, an estimate of the numbers of urea-hydrolyzing organisms in the aquifer is desirable. Five water samples from across the INEEL site were collected for Most Probable Number (MPN) estimates of culturable ureolytic organisms in SRPA groundwater. The samples were inoculated into Urea R Broth, a urea medium that contains a pH sensitive dye to indicate when urea has been hydrolyzed. In addition, samples for total direct counts of cells have been preserved, to allow estimates of the proportion of the cells in the samples that are ureolytic. Results from the MPN analyses are not yet available. Other activities related to preparation for field experiments include the identification of wells where field experiments can be conducted. Three wells at the INEEL north of Test Area North (TAN) have been set aside for multi-level sampler experiments. During the fall of 2000 these wells will be evaluated for the ability to stimulate calcite precipitation by microbially-induced urea hydrolysis.

The results thus far of our evaluation of the potential for utilizing urea hydrolysis as the basis for an *in situ* remediation technique for ^{90}Sr and other divalent contaminants in SRPA groundwater are promising. Urea hydrolyzing bacteria were isolated successfully from SRPA samples, and can catalyze precipitation of calcite similarly to the known urease-positive organism *B. pasteurii*. Early indications suggest that this is also true under conditions more closely simulating natural groundwater, and the development of analytical and molecular biology methods to assess the success of the bioremediation approach is proceeding steadily.

Planned Activities:

During the remainder of the first year of this project and into the second year experiments under conditions more closely simulating natural SRPA groundwater will be continued, to determine whether the observations derived previously in this study are applicable to environmental conditions. The spectroscopic methods will be applied to additional solid samples, including those formed during experiments in the synthetic groundwater and synthetic groundwater amended with trace elements. Development work on the molecular verification of urease activity will also continue. During the second year an *in situ* experiment is planned in a single groundwater well at the INEEL. Initial review of potential candidate wells at the site has commenced. We will also continue discussions started in April 2000 with R.M. Thompson (DOE-RL) related to the feasibility of this technology to remediate a strontium plume at the Hanford 100 Area.

Information Access:

Y. Fujita, F. G. Ferris, R. D. Lawson, F. S. Colwell and R. W. Smith. 2000. Calcium Carbonate Precipitation by Ureolytic Subsurface Bacteria. *Geomicrobiology Journal*. Accepted.

Y. Fujita, F. G. Ferris, F. S. Colwell, J. Ingram and R. W. Smith. "Accelerated Calcium Carbonate Precipitation by Aquifer Microorganisms: A Possible *In Situ* Remediation Technique for Radionuclides and Metals." Accepted for oral presentation, Chemical-Biological Interactions in Contaminant Fate Session, American Chemical Society National Meeting, Washington, D. C., August 20-24, 2000.

Y. Fujita, F. G. Ferris, F. S. Colwell and R. W. Smith. "Microbially Accelerated Calcium Carbonate Precipitation for Radionuclide Sequestration: Preliminary Laboratory Studies." Oral presentation at the Northwest/Rocky Mountain Regional Meeting of the American Chemical Society, Idaho Falls, ID, June 15-17, 2000.

Y. Fujita, R. D. Lawson, F. G. Ferris, F. S. Colwell, and R. W. Smith. "Microbially Accelerated Calcium Carbonate Precipitation for Radionuclide Sequestration: Preliminary Laboratory Evaluations of Urea-Hydrolyzing Organisms." Poster presentation at the American Geophysical Union Fall Meeting 1999, San Francisco, CA. *EOS* **80**:H31B-06 (1999).