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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 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.

Research Progress and Implications:

This report summarizes project activities after 20 months of a 36-month project. Descriptions of the various tasks, and results where available, are presented below.

Investigation of Kinetics of Urea Hydrolysis and Calcite Precipitation in Artificial Groundwater. Building on the understanding gained during the first year of the project with respect to the kinetics of calcite precipitation in artificial rich medium by model and Snake River Plain Aquifer (SRPA) isolates, this past year the University of Toronto researchers have focused on calcite precipitation kinetics in synthetic groundwater. Calcium and nutrient conditions in the synthetic groundwater (composition based on INEEL groundwater data) are more than an order of magnitude lower than in the medium used in the first year studies. As expected, the biologically induced precipitation reaction is much slower in the synthetic groundwater, but it appears to proceed according to thermodynamic predictions and previously established kinetic models for calcite precipitation.

Preparation for Field Experiments at the INEEL: Testing of Native Ureolytic Communities, Evaluation of Supplemental Nutrients, and Column Studies of Urea Transport. Laboratory testing at the INEEL of direct urea addition to SRPA groundwater samples suggests that native urea hydrolysis rates are likely to be relatively slow. Detection of urea removal for urea concentrations on the order of 10 to 50 mM required laboratory incubations of one to three weeks duration. This is likely in part a reflection of the low total microbial numbers in SRPA groundwater. Acridine orange direct counts of the tested groundwater samples generally estimated cell numbers on the order of 10^5 /ml, consistent with numbers previously

observed in INEEL groundwater. Most probable number (MPN) analyses for culturable urea hydrolyzing organisms in the groundwater also estimated low numbers, between 10 to 100 cells per ml. (These estimates, however, do not include attached cells, which are more numerous than unattached in most subsurface environments.) In order to stimulate the total microbial community, and in particular the urea hydrolyzing populations, several different nutrient supplements at various concentrations were tested for their ability to increase ureolysis rates. Lactate, glucose, brain heart infusion (BHI) medium, molasses and trace metal solutions were included in the evaluations. Molasses at a concentration of 0.1% was determined to be the most promising and practical supplement for field deployment, and the kinetics of urea hydrolysis by SRPA groundwater organisms in the presence of molasses are currently being investigated.

In order to gain a preliminary indication of the transport characteristics of urea in a basaltic aquifer such as the SRPA, transport studies were conducted with columns constructed of crushed basalt from the Test Area North (TAN) area of the INEEL. Crushed basalt may not accurately represent the consolidated rock with fractures and weathered surfaces typical of the INEEL subsurface, but we believe that it is an acceptable initial model for SRPA flow conditions. Results from pulse addition experiments with urea and the conservative tracer bromide in synthetic groundwater showed that urea breakthrough and elution was virtually synchronous with bromide; the data indicated that urea may in fact have been retarded even less than bromide. This may be explained by the larger size of urea, hindering its diffusion into pores in the basalt.

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. During the past year at the INEEL we have focused on the development of secondary ion mass spectrometry (SIMS) protocols to examine carbonates generated by bacterial activity in synthetic groundwater containing Ca and Sr. SIMS with sputter depth profiling allows the determination of changes in Sr to Ca ratios with depth in particulate carbonate samples. The sputter depth profiling results can be compared with analysis of the bulk composition by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Results of analyses on carbonates generated by *B. pasteurii* in synthetic groundwater with initial Ca and Sr concentrations of 80 ppm and 10 ppm, respectively, showed that SIMS could successfully measure ion ratios on the surface and within these particles. ICP-AES data indicated a bulk Sr:Ca ratio of 0.11, and sputtering SIMS data approached this value with increasing depth into the particle. The Sr:Ca ratio however, contrary to what would be expected from precipitation under batch conditions, was lower at the surface of the particles (~0.05) and increased with depth. One possible reason for this phenomenon is re-equilibration with solution conditions after initial fast precipitation; samples for analysis were collected after one week of incubation in the artificial medium. This possibility will be further explored in future work. Additional SIMS work will include analysis of carbonates generated in the presence of other ions such as cobalt and uranyl.

Molecular Methods to Characterize Microbial Community Response to Urea. Molecular methods to detect urease activity and associated changes in the subsurface microbiological community will be important in verifying the *in situ* success of the urea introduction and calcite precipitation scheme. In addition, these methods could be used to evaluate a candidate site's suitability for the proposed remediation approach. In the last year, significant progress has been made at the INEEL in the development of oligonucleotide primers for polymerase chain reaction (PCR) detection of bacterial urease genes. Known amino acid sequences for urease catalytic subunits were aligned and relationships inferred by phylogenetic analysis resulted in the delineation of three clades of microbial ureases. From these phylogenetic relationships, primers are being designed for each of the three clades. Testing of the primers for one of the clades is ongoing, and thus far results are very promising. Products of the anticipated size were amplified from known bacterial strains and also from 16 of 22 ureolytic bacterial isolates from the Snake River Plain Aquifer (SRPA). No products were observed in any of the 10 non-ureolytic SRPA isolates tested. A representative gel demonstrating the effectiveness of the primers is shown in Figure 1. Eight of the PCR products from SRPA ureolytic isolates have been sequenced thus far, and all show high homology with other urease gene sequences in GenBank. Testing of the aforementioned primers and primer pairs for the other clades will continue during the next year. 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).

Additional work on molecular methods to support this project is underway at Portland State University (PSU), where the native microbial communities in groundwater samples from the SRPA are currently being characterized. One hundred liters of groundwater from a well slated for urea introduction was passed through each of three capsule filters in the field at the INEEL, and the filters immediately frozen for shipment to PSU. There, DNA will be extracted and subjected to microbial community analysis by denaturing gradient gel electrophoresis (DGGE). Following the addition of urea to the well, triplicate

filters will again be processed, and the DGGE results compared to those for the pre-urea addition samples. Unique and common product bands in the gels will then be sequenced to provide information on the identity of individual community

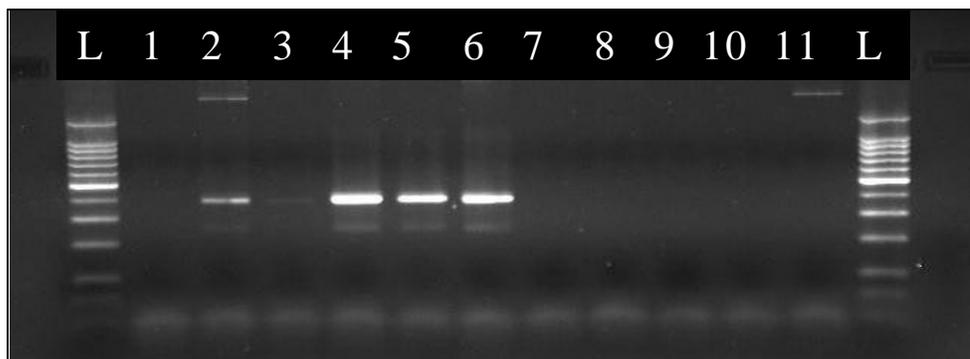


Figure 1. Agarose gel showing PCR results with urease primers L2F and L2R. Lanes L are DNA ladders; lane 1: negative control; lanes 2 and 3: urease positive known strains; lanes 4-6: SRPA ureolytic isolates; lanes 7-11: SRPA non-ureolytic isolates.

members. Such analyses could lead to the identification of “indicator” species or community structures that would signify the successful stimulation of urea hydrolysis and calcite precipitation.

Planned Activities:

During the remainder of the second year of this project and the following year the efforts described above in the development and testing of molecular methods to characterize microbial community responses will be continued. Spectroscopic method development and application as previously mentioned will also continue, in addition to the studies of ureolysis kinetics in INEEL groundwater. In addition, the relationships between precipitation rates and crystal size, shape, morphology and association with bacterial cells will be investigated. Such information can aid in the prediction of the stability of bacterially produced precipitates and also in the identification of such precipitates from field samples and thus verification of remediation success. Planning for field tests of the remediation approach is also underway, and we expect to initiate field work during the third year of the project. Finally, several manuscripts reporting on the progress described above are planned or are already in preparation for submission to peer-reviewed journals.

Information Access to Date:

T. L. Tyler, M. E. Watwood and F. S. Colwell. “Primers for Polymerase Chain Reaction to Detect Urease Genes in Subsurface Microorganisms.” Poster presentation at the American Society for Microbiology Annual Meeting, Orlando, FL, May 20-24, 2001.

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*. 17(4): 305-318.

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.” 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).