Final Report- Anna-Louise Reysenbach, Portland State University.

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Project ID Number: 70206

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

Lead Principal Investigator: Robert W. Smith, Idaho National Engineering and Environmental Laboratory, PO Box 1625 MS 2107, Idaho Falls, ID, rqs@inel.gov

Co-Investigators: F. "Rick" S. Colwell, INEEL, PO Box 1625 MS 2203, Idaho Falls, ID, fxc@inel.gov
Jani C. Ingram, INEEL, PO Box 1625 MS 2208, Idaho Falls, ID, uoa@inel.gov
F. Grant Ferris, University of Toronto, Dept. of Geology, Toronto, Ontario M5S 3B1, ferris@quartz.geology.utoronto.ca
Anna-Louise Reysenbach, Portland State University, Department of Environmental Biology, Portland, OR 97207, reysenbacha@pdx.edu.
Yoshiko Fujita, INEEL, PO Box 1625, MS 2203, Idaho Falls, ID, fujiy@inel.gov

Number of Graduate Students: INEEL – one; University of Toronto – one

Executive Summary.

As part of an interdisciplinary project to investigate whether we could demonstrate *in situ* remediation of metals and radionuclides that are present in the groundwater throughout the U.S. Department of Energy (DOE) weapons complex, we monitored the microbial communities associated with the contaminated zones. These results were correlated with the physical and chemical, and urease gene results, obtained by collaborators on the project. Shifts in the chemistry and successful immobilization of the metals was correlated with shifts in the community structure. These results set the framework for further monitoring of the wells as they respond to the increased pH and calcite precipitation, and ultimately *in situ* remediation.

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.

Summary of Project Accomplishments:

This report summarizes project activities after 36-month project. My laboratory was primarily involved in the molecular phylogenetic analysis of the communities as they responded to the geochemical changes.

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. Significant progress was been made at the INEEL in the development of oligonucleotide primers for polymerase chain reaction (PCR) detection of bacterial urease genes.

Additional work on molecular methods to support this project were the primary objective of our involvement here at Portland State University (PSU), where the native microbial communities in groundwater samples from the SRPA have been 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 was 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 were then be sequenced to provide information on the identity of individual community members. Such analyses provide a framework of the changes in community structures and could provide insights into identifying the communities that signify the successful stimulation of urea hydrolysis and calcite precipitation.

DGGE Analysis

Preliminary Bacterial community analysis:

DGGE analysis was very consistent between duplicate samples and DGGE patterns were reproducible between several PCR amplifications from the same template and also between duplicate gels. Several major changes were seen between timepoints (labeled A, B, and C in figure 1). Bands A and B are much stronger before treatment and band C, although visible in the pretreatment 06/14/01 sample, is at

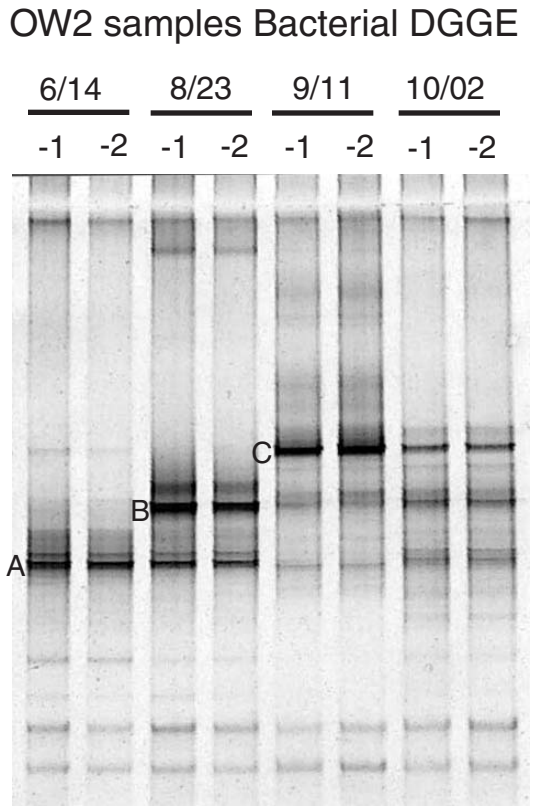
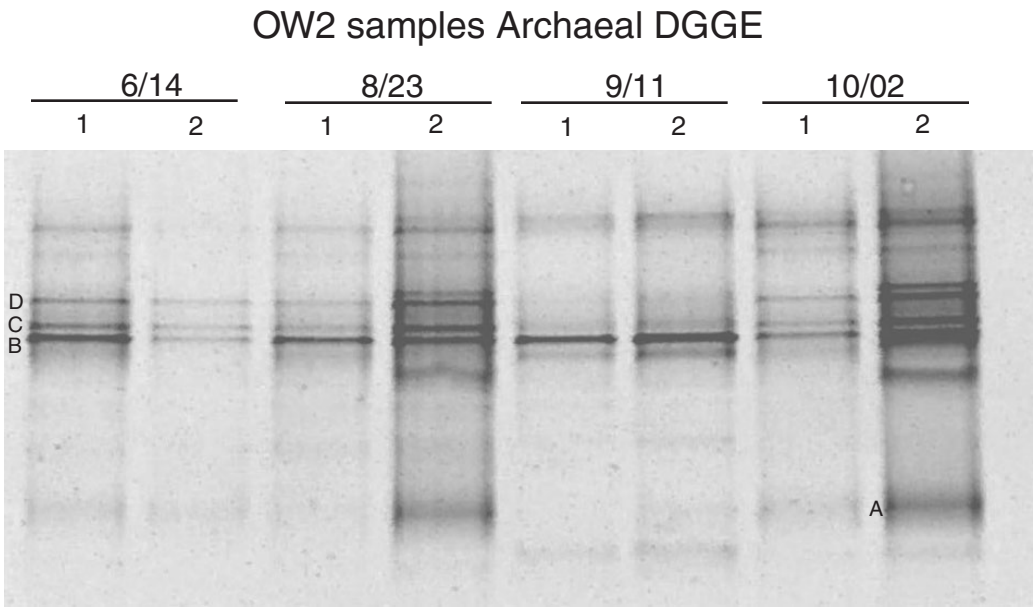


Fig. 1 Denaturant gradient gel of Owsley well 2 samples showing duplicate samples from the same test date. PCR products are ~350nt fragments of the 16S rRNA gene.



A Methanosarcina
B clone 33-P27A98
C Methanosarcina
D clone 33-P27A98

Fig. 2 Denaturant gradient gel of Owsley well 2 samples showing duplicate samples from the same test date. PCR products are ~400nt fragments of the 16S rRNA gene (344FGC/744RA).

its highest intensity 2.5 weeks after treatment on 9/11/01.

Band	Genbank match	Accession #	identities	% sim	comment
A	Uncultured bacterium clone GOUTA19	AY050588	288/306	94%	Closest match to any sequence
A	Magnetobacterium bavaricum	X71838	275/305	90%	Closest match to an isolate
B	Pseudomonas putida	U70977	329/330	99%	Closest match
C	Clostridium botulinum	X68171	302/305	99%	Closest match

Preliminary Archaeal community analysis:
DGGE analysis was run as above except with archaeal-specific primers 344FGC and 744RA. No obvious band intensity changes were seen which correlated with the treatment date under these conditions (Fig 2).

DGGE analysis was performed as above with the primer sets 338FGC/519R using DNA extractions normalized to cell count and also to filtrate amount. No significant differences were seen between these 2 sets of samples analysis (Fig 3).

Conclusions and Future directions. These results have laid the foundations for additional rapid assessment of the wells. Optimizing protocols are often the most time consuming aspect of a project, however, we can now rapidly and reliably analyze and compare samples taken from these wells at different time intervals. As samples become available, will continue to assist our collaborators in monitoring the microbial communities in these wells.

Journal Publications:

Lehman, R.M., S.P. O'Connell, A. Banta, J.K. Fredrickson, A.-L. Reysenbach, T.L. Kieft and F.S. Colwell. Microbiological analysis of a fractured basalt aquifer by sampling attached and free-living populations. Geomicrobiology J. In review.

Other papers of the project.
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.

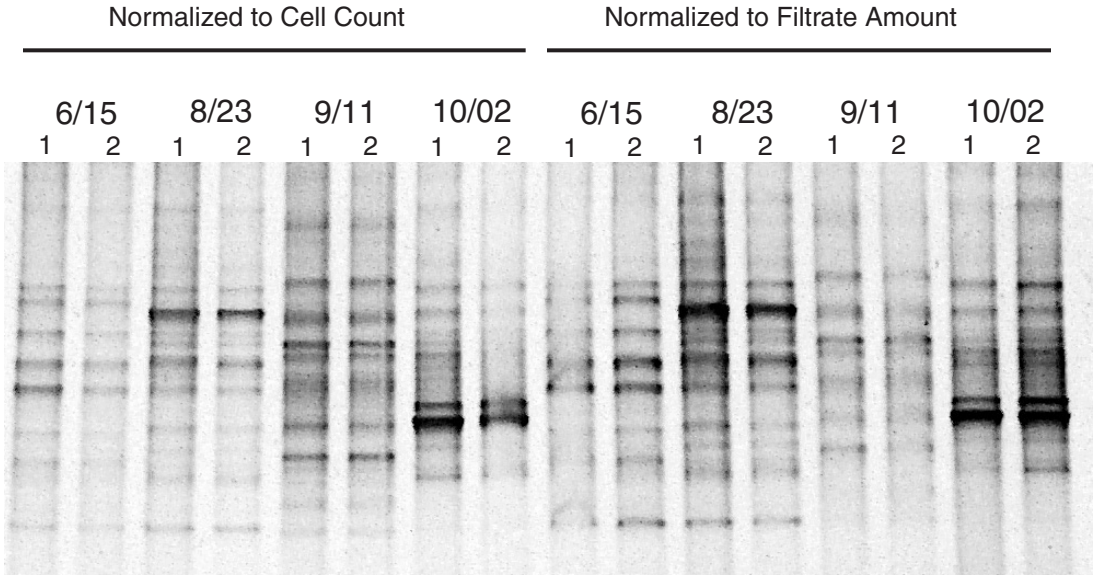


Fig. 3 Denaturant gradient gel of Owsley well 2 samples showing duplicate samples from the same test date. Template amounts used in the PCR are either normalized to cell count or filtrate amount. PCR products are ~350nt fragments of the 16S rRNA gene.