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Microbially Mediated Immobilization of Contaminants Through *In Situ* Biostimulation: Scale up of EMSP project 55267

June 14, 2001

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Students / post grads: 1 Ph.D. candidate (pending)
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Purpose and Objectives

The overall goal of the proposed research is to provide an improved understanding and predictive capability of the mechanisms that allow metal-reducing bacteria to be effective in the bioremediation of redox sensitive toxic metals and radionuclides. The study is motivated by the likelihood that subsurface metal-reducing bacteria can be stimulated to effectively alter the redox state of contaminants so that they are immobilized *in situ* for long time periods. The work described in this proposal will advance the technological and scientific needs associated with the long-term management of the enormous in-ground inventories of Cr, U, Tc, and Co present at numerous DOE installations throughout the country. The objectives of our project are to (1) develop an improved understanding and predictive capability of the rates and mechanisms controlling microbially mediated reduction of toxic metals and radionuclides in heterogeneous field settings, (2) quantify the impacts of hydrological and geochemical processes on the effectiveness of indigenous microorganisms to transform and immobilize radionuclides and metals *in situ*, (3) provide an improved understanding of the importance of microbial consortia interactions in the bacterial immobilization of radionuclides and toxic metals, and (4) determine intrinsic bioreduction rate parameters to improve our generic predictive capability of *in situ* microbially mediated metal reduction.

This report summarizes 0.5 y of research for a 3 y project.

Progress and Relevance to EM Needs

Our approach involves a multiscale experimental and numerical endeavor that uses (1) a well-characterized, highly instrumented field facility for assessing biostimulation remedial strategies involving Cr(VI), U(VI), and Co(III)EDTA$^-$, (2) multiple tracer techniques designed to delineate the influence of coupled hydrological, geochemical, and microbial processes, (3) a novel microarray-based genomic technology for rapidly characterizing shifts in microbial community structure and activity, (4) sophisticated surface analysis techniques (x-ray absorption spectroscopy) for quantifying the distribution and chemical environment of the immobilized contaminants, and (5) next-generation, high-performance hydrobiogeochemistry modeling to assist the design and performance assessment of the proposed field scale experiment.

Previous results in our group demonstrated the sustained microbial reduction of ^{60}Co(III)EDTA to ^{60}Co(II)EDTA under dynamic flow conditions (Brooks et al., 1999). The net reduction of the ^{60}Co(III)EDTA dominated the fate and transport of this contaminant, even in the presence of strong mineral oxidants commonly found in the subsurface (e.g. Mn- and Fe-oxides). The environmental implications of these findings are pronounced since ^{60}Co(III)EDTA is extremely stable and soluble, and this enhances its persistence and transport in subsurface environments. By discovering a way to keep the bacteria healthy and growing, we were able to effectively stabilize ^{60}Co(II)EDTA in a flowing subsurface system. This is advantageous from a remedial perspective since ^{60}Co(II)EDTA is a weaker complex than ^{60}Co(III)EDTA, and therefore easier to decouple and remediate. The research findings, published by Brooks et al. (1999), provide new and important information on how to successfully implement a bioreduction strategy at the field scale. Their use of a dynamic flow system with sustained bacterial growth conditions in geochemically reactive media is consistent with contaminant migration scenarios *in situ.*

Progress to date includes:

- Microbial community dynamics of the groundwater from the field site has been characterized for direct counts (AODC), aerobic heterotrophs, anaerobic heterotrophs, fermenters (SO_4^{2-} reducers, methanogens, H_2 utilizers), nitrate reducers, Co(III)EDTA reducers, Fe(III) reducers, Mn(IV) reducers, SO_4^{2-} reducers, methanogens, and Fe(II) oxidizers.

- We have purified over 37 metal reducing isolates from the field-site groundwater. Each have been screened for Fe-citrate and Co(III)EDTA reduction.

- Several bacterial isolates were found to reduce redox sensitive metals such as Co(III)EDTA, Cr(VI), and U(VI) at a rate comparable to that of *Shewanella alga* (BrY). Reduction rates had time scales of hours which was sufficiently rapid to ensure that efficient toxic metal reduction could occur *in situ* even in fractured systems with fast moving groundwater (Fig. 1).

- Time coarse studies using WAG 5 groundwater are underway to follow cells (AODC), electron donor (e.g. lactate), and electron acceptor (Co(III)EDTA) with various amendments.

- Abiotic vs. biotic reduction rates and mechanisms regarding Co(III)EDTA, Cr, and U transformation to reduced products are also being investigated.

- Field site geochemical dynamics including temperature, pH, Eh, dissolved O_2, NO_3/NO_2, Fe(III)/Fe(II), SO_4^{2-}/S^{2-}, CO_2/CH_4, Cl^-, major cations and anions, TOC, and alkalinity are being quantified as a function of time in both the fracture and matrix regime.

- Field site hydrologic and geochemical processes have compiled and documented through publication and the design of the field scale contaminant injection and biostimulation experiment have been initiated.

Implications:

The experimental and numerical results from this research will provide knowledge and information in previously unexplored areas of *in situ* biostimulation for metal and radionuclide immobilization to support EM's mission of long-term isolation and *in situ* remediation of contaminated environments. By unraveling the fundamental mechanisms controlling the microbially mediated reduction and immobilization of contaminants *in situ*, we provide an enhanced opportunity for successfully implementing biostimulation strategies at numerous DOE installations that are plagued with legacy waste.

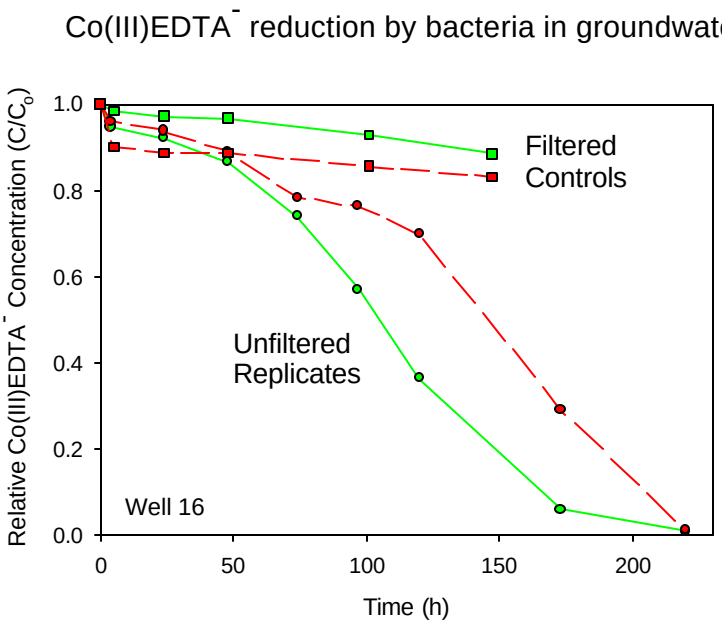
Relevant past publications:

Brooks, S.C., S.L. Carroll, and P.M. Jardine. 1999. Sustained bacterial reduction of $Co(III)EDTA^-$ in the presence of competing geochemical oxidation during dynamic flow. Environ. Sci. Technol. 33:3002-3011.

Jardine, P.M., W.E. Sanford, J.P. Gwo, O.C. Reedy, D.S. Hicks, R.J. Riggs, and W.B. Bailey. 1999. Quantifying diffusive mass transfer in fractured shale bedrock. Water Resour. Res. 35:2015-2030.

Publications:

Jardine, P.M., T.L. Mehlhorn, I.L. Larsen, W.B. Bailey, S.C. Brooks, Y. Roh, and J.P. Gwo. 2001. Influence of hydrological and geochemical processes on reactive contaminant transport in fractured shale bedrock. J. Contamin. Hydrol. (in press).



Reduction of Co(III)EDTA^{-} to Co(II)EDTA^{2-} by bacterial isolates obtained from the WAG5 field facility on the Oak Ridge Reservation