Final Report:
Enhancement of Bacterial Transport in Aerobic and Anaerobic Environments:
Assessing the Effect of Metal Oxide Chemical Heterogeneities
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ABSTRACT:

The scope of our research continues to be to understand the fundamental processes that control microbial transport in physically and chemically heterogeneous aquifers and from this enhanced understanding determine the requirements for successful, field-scale delivery of microorganisms to metal contaminated subsurface sites. Our specific research goals were to determine; 1) the circumstances under which the preferential adsorption of bacteria to Fe, Mn, and Al oxyhydroxides influences field-scale bacterial transport, 2) the extent to which the adhesion properties of bacterial cells affect field-scale bacterial transport, 3) whether microbial Fe(III) reduction can enhance field-scale transport of Fe reducing bacteria (IRB) and other microorganisms and 4) the effect of field-scale physical and chemical heterogeneity on all three processes. Some of the spin-offs from this basic research that can improve biostimulation and bioaugmentation remediation efforts at contaminated DOE sites have included; 1) new bacterial tracking tools for viable bacteria; 2) an integrated protocol which combines subsurface characterization, laboratory-scale experimentation, and scale-up techniques to accurately predict field-scale bacterial transport; and 3) innovative and inexpensive field equipment and methods that can be employed to enhance Fe(III) reduction and microbial transport and to target microbial deposition under both aerobic and anaerobic conditions.

SUMMARY OF RESULTS

At the time of our BERAC technical review in October 2000, we had completed the first two research goals and successfully field-tested a suite of microbial tracking tools. Since then we have successfully completed the third field research goal. With the requested funds we will be able to complete sample and laboratory analyses, finish publication of the extensive set of laboratory and field data, to help assemble the detailed data sets into a relational data base that will be much more useful to DOE, to finish development of spin-off field technologies so that they will be readily available for use at contaminated DOE sites and to complete a monitoring program that will test for long term effects as well as provide essential data on seasonal fluctuations in the geochemical and microbiological properties of the aquifer at Oyster.

1) Data analyses leading to the completion of graduate student Ph.D. theses and publications resulting from the excavation (Aug. '99) and all four field experiments (Nov. '99, Aug. '00, Aug. '01 and Sept. '01) at the Oyster site.

Microbiological, physical and geochemical analyses of the vadose zone at the Narrow Channel Flow Cell have been published (Musselwhite et al., 2002). A similar characterization of several cores collected in the saturated zone of the Narrow Channel

Flow Cell is underway as part of Musselwhite's (University of Oklahoma) and Green's (Old Dominion University) Ph.D. dissertations. This includes analyses of the core samples for their adsorbed, exchangeable and oxyhydroxide Fe, Mn and Al contents (Princeton Univ.) and adsorbed organic carbon (Envirogen, Inc.).

The Nov. '99 injection of the adhesion-deficient, *Comamonas* sp., DA001, at Narrow Channel Focus Area (NCFA) was the largest and most detailed field-scale bacterial transport study that has ever been performed. It demonstrated that physical heterogeneity and heterogeneity in microbial adhesion properties control microbial transport at the 10-meter scale and that chemical heterogeneity was not an important parameter. The results have been covered in several publications (Johnson et al., 2000; Mailloux et al., 2002a; DeFlaun et al., 2001) and the innovative microbial tracking tools developed specifically for this injection (Holben and Ostrom, 2000; DeFlaun et al., 2001; Fuller et al., 2000b; Lytle et al., 2000, 2001) were successfully field-tested (Fuller et al., 2001; Kovacic and Holben, 2002). The results were also significant in that they provided a basis for scaling-up bacterial transport from the laboratory to the field (Fuller et al., 2000a; Dong et al., 1999, 2002a,b,c; Mailloux et al., 2002a; Scheibe et al., 2002a; Ginn, 2002) and protocols for adequately characterizing subsurface heterogeneity prior to remediation and strategies for bioaugmentation (Scheibe et al., 2001; Hubbard et al., 1999, 2000, 2001; Mailloux et al., 2002c; Scheibe et al., 2002b; Zhang et al., 2001).

Validation of some of these scale-up models (in fact all microbial transport models), however, required determination of the attachment efficiencies of a bacterium during the course of a field transport experiment, since the natural groundwater and its constituent colloids and even other bacteria may affect the bacterial attachment efficiency of the injected microorganisms. This had never been done before so two new approaches were developed, one implemented prior to the second field experiment and the other during the second field experiment (June and Aug. '00). In the first method, repacked, sized, Fe-coated quartz and clean quartz columns were run in the field using the groundwater pumped from the MLS's of the flow cell (Hall et al., 2002). These experiments were conducted with DA001 and a Fe(III) reducing bacteria, FER 1, to investigate the effects of microbial properties, i.e. size and attachment efficiency on transport. The results indicated that the dissolved organic carbon, DOC, in the NCFA groundwater ameliorates any tendency on the part of either DA001 or FER 1, to preferentially adsorb to Fe oxyhydroxides.

In the second approach, an anaerobic, dual, bacterial injection was performed at the sub-oxic focus area (SOFA) and cores from the flow cell were subsequently collected to determine the concentrations of adsorbed, injected bacteria. This injection also examined the effects of microbial properties by comparing the transport and adsorption of a Fe(III)-reducing Beta-Proteobacterium, OY107, to the adhesion-deficient DA001. The transport distance of each bacteria species was monitored in the field using viable stains (Fuller et al., 2002). From the core samples the concentrations of OY107 and DA001 are being directly compared to the grain size and to the Fe, Mn, Al oxyhydroxides concentrations (Streger et al., 2002). The subsurface of the SOFA flow cell exhibits a much greater physical and chemical heterogeneity and a more complex structure than that of Narrow

Channel and comparisons of the geophysical tomography data (Chen et al., 2001), the geochemical and grain size data (Chen et al., 2002) and the hydraulic properties is still underway. Prof. H. Dong and his students at Miami University are currently measuring the surface charge of the sediments and the adsorbed organic carbon concentrations are being determined by Envirogen, Inc., to determine to what degree that latter masks the former. In principle, the more Fe, Mn and Al oxyhydroxides present in the sediment, the more positive the surface charge. A more positive surface charge should attract more bacteria.

The adsorbed bacterial results varied depending upon the analytical method (i.e. qPCR and CFU's) and the planktonic bacterial results did not vary down the flow axis in a straightforward manner. Consequently, a third injection was performed at SOFA (Aug. '01) following the same procedure in the second injection with a slightly different injection strategy. The analyses of the bacterial breakthrough curves and geochemical data on the cores have been completed, but the results have not yet been integrated with the results of the Aug. '00 injection.

The third hypothesis, that microbial Fe-reduction enhances microbial transport, was tested by a push-pull, biostimulation experiment performed after the Aug. '01 injection at SOFA. Several thousand groundwater samples were collected for geochemical and microbial analyses. The preliminary results do indicate a successful stimulation of Fe(III) reduction concomitant with an increase in suspended biomass in the stimulated zone. Several simultaneous current investigations related to this experiment include:

- Microcosm experiments to ascertain the causes of the biomass increase, i.e. growth versus desorption (Mailloux and Devlin, Princeton University; Fuller, Envirogen, Inc.).
- Geochemical measurements record a progressive reduction of electron acceptors in the SOFA flow cell during the experiment and have revealed evidence of an adsorbed nitrate reservoir associated with the sediments. The origin and distribution of this reservoir is currently being delineated using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate (Princeton University), because it has significant implications for biostimulation strategies for nitrate-contaminated groundwater.
- Geophysical measurements were coordinated with the stimulation experiment (Hubbard et al., 2002) and detected the formation of a gas phase tentatively believed to be N_2 from nitrate reduction. Comparisons of the two data sets are currently underway. These investigations are being conducted by a Ph.D. student at UC Berkeley under the direction of Dr. Susan Hubbard of LBL.
- Comparison of the biostimulation results with Fe-reduction potential measurements of cores from the SOFA and performed by Prof. Roden's students at the Univ. of Alabama (Chen et al., 2002) are underway.
- Comparison of the stimulation results with microbial sulfate reduction activity measurements performed on the same cores by Prof. McInerney and his students at the Univ. of Oklahoma are in progress.
- To determine whether biomineralization that occurs during Fe reduction in the laboratory (Dong et al., 2000; Fredrickson et al., 1998) bears any resemblance to

that which occurs in the field, sediment implants were used to collect potential Fe(II) mineral precipitates. Prof. H. Dong and his students at Miami University are currently analyzing these.

2) Data analyses and publication of on-going laboratory experiments and their integration with the field data into a relational database.

With thousands of data points from the three injections into two very well characterized flow cells with dramatically different physical and chemical heterogeneity, the Oyster experiments represent the most detailed, field scale bacterial transport experiments performed to date. To model the results of these experiments, a 3-D, continuum, transport model and a stream tube, ensemble model (Ginn, 2000d) of the injection has been developed by Tim Scheibe and research associates of PNNL and Tim Ginn and students of UC Davis for both NCFA and SOFA. These models will be used to compare the results of the Nov. '99 injection into the aerobic NCFA with the anaerobic injections at SOFA. Preliminary models approximate the migration of the plumes through the flow cells. The preferential adsorption of strains DA001 and OY107 onto the mineral phases of both flow cells and their velocity enhancement relative to water represent microbial properties that are critical to an accurate model replication of the field observations.

Microbial community interactions also appear to play a critical role in field-scale bacterial transport and potentially anaerobic biostimulation. The field experiments have detected predation of injected bacteria (Zhang et al., 2001; Choi et al., 2001), a phenomenon that continues to be investigated by Prof. Dobbs and students at Old Dominion University, as well as the apparent enhanced release of indigenous, injected strains (Johnson et al., 2001). Additional bench-scale experiments using SOFA sediment and artificially coated, repacked control sediments, using OY107, DA001 and Oyster protists and utilizing natural SOFA and NCFA groundwater are currently underway examining the fundamental processes controlling these two phenomena. These experiments include:

- the effects of predation on microbial Fe(III)-reduction (Mailloux and student, Princeton University). The protists activity appears to be related to the dissolved O₂ concentrations and the presence of solid phase Fe(III).
- the effects of “cryptic” growth under aerobic and anaerobic conditions (Mailloux and student, Princeton University and Fuller at Envirogen, Inc.) and the consequences for microbial tracking tools. One potential application that has resulted from this research is a demonstration that growth can be detected in the field using flow cytometry coupled with viable stains.

Samples were also collected during the biostimulation experiment for the characterization of microbial community structure using 16S rDNA (Mailloux and students, Princeton University). The sequences from the clone libraries will be compared with those extracted from the frozen archive samples to determine whether the microbial communities that resided on the grain surfaces were desorbed into the aqueous phase during the biostimulation experiment.

All of these data will be utilized by Scheibe, Ginn and their students and postdoctoral research associates to examine the effects of the microbial properties of injected strains and of the microbial community interactions on field-scale bacterial transport.

A large information data base for Oyster is almost complete and includes; 1) a 3-D spatial characterization of the geology, geochemistry, geophysics, hydrology and microbiology of the flow cells, 2) a 3-D, short timeframe, temporal data set related to the field-scale bacterial transport experiments, including the characteristics of the injected bacterial strains, the movement of these bacteria in the subsurface and the response of the geochemistry and the indigenous microbial community during the transport experiment, and 3) the beginnings of a long time-frame data set that includes limited 3-D characterization of the geochemistry and microbial communities and how they vary with season. Several hundred isolates of indigenous microorganisms were examined during the selection of the injected bacterial strains and their physiological, antibiotic-resistance and molecular phylogenetic traits are being determined prior to their being archived at SMCC as part of a Ph.D. dissertation by a Florida State University student under the direction of Prof. Balkwill. Characterization of the physical and chemical features in the cores collected during the second injection is still underway at Princeton and Old Dominion Universities. LBL geophysical characterization of SOFA relies upon these analyses and has yet to be published and it provides the basis for construction of the 3-D continuum model by Scheibe at PNNL.

Old Dominion University is currently integrating all Oyster data sets with their grain size and permeability data within a GIS formatted, searchable, web-accessible, database. Princeton University will be contributing both solid phase chemistry and microbial 16S rDNA data on the sediments, as well as the geochemistry and 16S rDNA of the groundwater and its temporal variability in 3-D. When published, the fully integrated, searchable database will provide a valuable resource to NABIR program for other investigators interested in correlating geochemical, microbiological and geophysical data for granular porous, freshwater aquifers. The database can also be utilized to test theoretical reaction-transport models for remediation scenarios in similar environments. Finally, the database will also represent an educational resource from the NABIR program to the environmental science community.

3) Verification that the geochemical and microbiological properties of the aquifer have returned to the state they were in, prior to field injections.

The Site Closure Plan was submitted to The Nature Conservancy (TNC) and the Virginia Department of Environmental Quality (VaDEQ) in Dec.'01 and approved by them. The required monitoring program was initiated in January 2002. To date, we believe that the South Oyster groundwater system begins to return to its natural state within about 6 months following an injection, but currently the dissolved inorganic carbon and microbial abundance of the SOFA are elevated compared to pre-stimulation measurements. This may be simply the result of seasonal variations in these parameters that we have not yet documented or a longer-term disturbance of the microbial community following stimulation and bioaugmentation. DOE is obligated to monitor both the NCFA and the

SOFA at the site for two years following the last field experiment (ending Dec. '01) and we cannot begin to plan final closure until additional data is gathered by a program of systematic and frequent groundwater sampling for geochemical and microbial analyses. In collaboration with Tim Griffin of Golder Associates, monthly groundwater sampling from the monitoring wells at NCFA and SOFA has been initiated for the following:

- Aqueous inorganic and organic chemistry and microbiology
- pH, temperature, conductivity, rain gauge and water level measurements downloaded from the in situ probes installed at NCFA and SOFA
- field measurements of the pH, Eh, dissolved O₂, and nitrate.

The hydrogeological model developed by Drs. Scheibe, Ginn and their graduate students will be continuously updated with water level and other information to guide the selection of monitoring wells for sampling as well as to aid in the interpretation of results.

All of the amendments, including chemicals and microbes, introduced to the aquifer during the field experiments will be monitored. The analyses of these various parameters are being performed by students and personnel in the laboratories of Drs. Onstott, Balkwill, McCarthy, Fredrickson and DeFlaun. The data, however, will be utilized by the majority of the investigators and their students involved in the project. This information is vital to the completion of several studies and publications. The evolution of the aquifer chemistry and microbiology to its pre-perturbed state is an important study by itself, with implications for site remediation and restoration. The extensive culture collection and characterization of the microbial community developed prior to the field experiments will be compared to the post-experimental community to assess the return to its 'native' state. This information will be of use to the scientific and remediation community in general and to the NABIR program in particular.

Of particular interest to TNC and the local community planners (Accomack-Northampton Planning District Commission) is the control of nitrate concentrations emanating from the farm fields upgradient of both the NCFA and the SOFA. As a service to TNC, warm season grasses, known to scavenge nitrate from groundwater, were planted around and downgradient of the two flow fields. The efficacy of these grasses in protecting the coastal and estuarine surface water from high nitrate concentrations is being evaluated as part of this monitoring program. Envirogen will produce a report on this nitrate study and present the results to TNC and other local community groups at the discretion of TNC.

A record of the seasonal variations in the microbial communities and aqueous geochemistry of a Coastal Plain aquifer will also be of immense value to TNC and the local communities in planning their future development. The detection of an adsorbed nitrate reservoir in the sediment has significant implications in understanding the impact of the use of artificial fertilizers in these delicate, coastal plain environments.

As of September, 2005, the Oyster site is closed and the monitoring program terminated. Results were reported to the TNC and VaDEQ and other community groups at TNC's discretion.

PEER-REVIEWED PUBLICATIONS

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