PROGRAM ELEMENT 6
Bacterial Transport

Top-Down Controls on Growth and Transport of Groundwater Bacteria From a Coastal Plain Aquifer

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The overall research goal is to examine the importance of protozoan predation and viral lysis, processes effecting so-called "top-down control," on the abundance and growth of groundwater bacteria at the DOE study site in South Oyster, Va. These findings will contribute to our increased understanding of bacterial transport within aquifers. The specific objectives are: (1) to quantify and characterize the indigenous microbial communities in both South Oyster groundwater and sediment samples; (2) in so doing, to provide a baseline for assessing the impact of transport experiments on the indigenous microbial community; (3) to quantify protozoan bacterivory of injected bacteria in South Oyster intact cores (with M. DeFlaun and M. Fuller, Envirogen, Inc.); (4) to assess the community response of protozoans to injections of aerobic bacteria into the South Oyster flowfield; (5) to quantify protozoan predation and viral lysis of groundwater bacteria in the aerobic portion of the South Oyster aquifer; (6) to compare bacterial community loss rates caused by viral lysis with those visited upon bacteria by protozoans; (7) to assess the ecological significance of protozoan predation and viral lysis through comparison with bacterial production. The methods used to study these processes will include a series of experimental manipulations on groundwater and sediment samples collected from the study site. Whole-core experiments will be performed as well. An initial emphasis will be placed on assessing top-down control of free-living bacteria, with the intent of switching to a focus on particle-attached bacteria in the latter half of the study (2001-2002).

Study results are as follows:

- The study area (South Oyster, Va.) is characterized by low microbial biomass. Protozoans (principally flagellates) ranged in abundance from 10 to 100 cells/g of sediment and from 1 to 8 x 10^3 cells/liter of groundwater. Bacterial densities varied between <1 to 10 x 10^6 cells/g of sediment and from <0.5 to 1.5 x 10^8 cells/liter of groundwater. Finally, virus-like particles (VLPs) were present in densities between 0.2 to 1.2 x 10^6 per g of sediment and about 4 x 10^8 per liter of groundwater. For each microbial taxon, there were differences in abundance, and for protozoans—in composition, between and within the two study sites.
- The aquifer exhibits high concentrations of organic carbon and nitrate; laboratory experiments indicate the bacterial community is phosphorus-limited.
- In laboratory experiments, protozoans' uptake and clearance of bacteria were 2.69 nl protist^{-1} h^{-1} (bacterial cell volume *ca*. 0.23 μm^{-3}) and 30.9 cells protist^{-1} h^{-1}, respectively. These rates would remove 0.5 to 2.2% of bacterial standing stock daily at the study site.
- In a field experiment ongoing at the time this abstract was submitted, we were following the time-course response of groundwater protozoans and VLPs following the injection of *Comamonas* into the flow-cell system described by Onstott et al. elsewhere in this compendium.

Ferrographic Tracking of Bacterial Transport at Oyster, Virginia

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Studies investigating enhancement of bioaugmentation require novel methods to track bacterial concentrations. Tracking techniques used to monitor the concentration bacteria added to groundwater must allow selective identification of the particular microbe added to the system. In addition, high-resolution counting (e.g., quantitation down to 100 cells/mL or less) is required due to the dramatic decrease (exponential) in suspended cell concentration with distance from the injection point. Furthermore, significant information regarding the kinetics of bacterial attachment and detachment can be determined from examination of cell concentrations many orders of magnitude below the injected concentration.

Ferrographic enumeration, an innovative technique recently developed for tracking bacterial concentration, is being applied to bioaugmentation studies at the Oyster, Va., field site. The technique employs immunomagnetic tagging and ferrographic separation. The technique provides selectivity due to reliance on antibody-antigen recognition to magnetically tag the bacteria of interest. It also provides high-resolution enumeration (enumeration down to ~10 cells/mL^{-1} for a 1 mL sample) due to deposition of the magnetically-tagged bacteria onto an exceedingly small area on a glass slide for visual identification under an epifluorescence microscope. The method is relatively inexpensive and rapid. Visual identification of the bacteria provides information on the relative shapes, sizes and possible aggregation of bacteria that may be important characteristics in their transport behavior.

Ferrographic enumeration of bacterial concentrations was performed during recent laboratory (intact and packed core) experiments conducted with Oyster site materials, and during field transport experiments at the Oyster site. The poster shows the high resolution of bacterial cell counts achieved using ferrographic enumeration.

The Influence of Heterogeneity and Growth on Microbial Transport in Saturated Porous Media

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The co-disposal of organic chelating agents with radionuclides at DOE sites has often resulted in enhanced mobility of these hazardous wastes. The success of biogeochemical alterations of these complexes is ultimately controlled by the transport and distribution of bacteria in physically and chemically heterogeneous subsurface systems. Much of the work to date on bacterial transport has focused on inert biocolloids or bacteria in non-growth states. Growth, however, increases the aqueous-phase concentration of bacteria, a first step in initiating transport in groundwater. To accurately represent bacterial transport during intrinsic bioremediation, the microbial growth and transport processes must be coupled.

The relationship between microbial growth and transport is being assessed in experimental systems at various scales. These include: (1) a microflow chamber (µm to mm); (2) small columns (mm to cm); and (3) intermediate-scale flow cells (cm to m). The microflow chambers are used to measure the attachment, detachment and residence time of bacteria to different mineral surfaces by direct observation with a confocal microscope. The residence time is used to measure the point at which irreversible adsorption to the mineral surface occurs. This process has been quantified by developing a mechanistic model that tracks the bacteria in space, time and the additional dimension of residence (or exposure) time. This model allows us to experimentally test the role of residence time on a mineral surface in adhesion processes.

Growth and transport are also affected by the dynamic nature of a contaminant plume, which creates redox extremes that can facilitate aerobic to anaerobic respiration within relatively short distances. We have created this type of small-scale heterogeneity in the redox properties in laboratory intermediate-scale flow cells. At any point in space and time the metabolic potential of an organism is dictated by its exposure time to the electron acceptor and donor. In our experiments the electron acceptor and donor are in the aqueous phase, thus local concentrations are controlled by the flow field. The mechanistic model used to track residence time can also be used to track the exposure time of the microorganisms to the electron acceptor and donor, and therefore, accurately assess the spatial distribution of metabolic potential.

Measurements of cell-level processes are of little use to field bioremediation efforts unless this information can be used to understand, simulate, and possibly improve the processes occurring at the field scale. The use of these small-scale measurements in large field-scale applications requires a formal mathematical process known as upscaling. An upscaling method has recently been developed to derive attachment/detachment kinetics from the surface interaction potential between a microorganism and a mineral. A method for measuring the microbe-mineral surface interaction potential by atomic force microscopy is currently being developed. This allows us a unique opportunity to test the upscaling theory by using the measurements at the cell-mineral scale to predict the attachment/detachment kinetics, and then compare these results with laboratory measurements of kinetic parameters at the bulk column scale. If successful, this same approach will be extended for applications to field-scale microbial transport at the Oyster site.

Enhancement of Bacterial Transport in Aerobic and Anaerobic Environments: Assessing the Effects of Metal-Oxide Chemical Heterogeneity

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Our research goals are to enhance our understanding of the fundamental processes required for successful, field-scale delivery of microorganisms to metal contaminated subsurface sites that exhibit both physical and chemical heterogeneity. The experiments being planned are designed to determine: (1) under what circumstances the preferential adsorption of bacteria to Fe, Mn and Al oxyhydroxides influences field-scale bacterial transport; (2) whether 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; (4) what level of site characterization, laboratory-scale experimentation and scaling-up approaches is required to accurately model field-scale bacterial transport; (5) which bacterial tracking methods yield the most reliable data for field-scale transport of living, viable bacteria; and (6) which other methods can be employed at the field-scale to enhance bacterial transport.

In the past year, two flow cells have been installed in a surficial aquifer located just south of Oyster, Va., on the DelMarVa peninsula. One flow cell was installed in an aerobic portion of the aquifer, and the other in a suboxic portion of the same aquifer. At the aerobic flow cell, 24 multilevel samplers (each with 12 sampling ports distributed over a depth of 3 m) were designed and installed by Oak Ridge National Laboratory and Golder Associates. The strata in both flow cells are comprised of quartz-rich fine-grained sands with significant grain size variations occurring over the cm to meter scale; significant variations in the concentrations of Fe, Mn, and Al oxyhydroxides also occur over the same scale. Higher levels of Fe(II) are present in the groundwater and sediment at the suboxic flow cell relative to the aerobic flow cell.

Five different strains of IRB have been recovered from groundwater and sediment samples by Florida State University and Pacific Northwest National Laboratory (funded independently by DOE/OBER) that satisfy the antibiotic resistance profiles and are currently being tested for field injection next spring by Envirogen, Inc. A 3-D hydrodynamic model of the aerobic flow cell was constructed based upon surface geophysical data gathered by Lawrence Berkeley National Laboratory and geological data gathered by Old Dominion University and pump tests and modeling performed by Golder Associates. Bacterial transport experiments confirm the existence of a dependency of the bacterial adhesion upon pH as expected for sediment containing Fe, Mn and Al oxyhydroxides. Bacterial transport models were applied to the intact core results to test hypotheses regarding bacterial transport and fate and to develop model parameterizations that were incorporated into the 3-D hydrodynamic model of the aerobic flow cell. An intact core experiment was also performed that tested the various bacterial tracking methods to be utilized during the bacterial field transport experiment. The *Comamonas* strain was labeled with both ^{13}C and a viable protein stain (CFDA) and injected into an intact core under conditions designed to simulate the field injection. The bacterial breakthrough was monitored using plate counts and CFDA direct counts and plate reader measurements (Envirogen), by ferrographic separation and counting (Univ. of Utah), by stable isotope analyses, DAPI counts and quantitative PCR (University of Montana) and by PLFA analyses (University of Tennessee). The success of the various tracking methods will be presented along with other results from a simultaneous injection of Br and ^{13}C/CFDA labeled *Comamonas* into the aerobic flow cell.

Vibration-Accelerated Transport of Microbes in Subsurface Media

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The low transport rates of microorganisms through porous subsurface media pose severe limitations on the impact and applicability of biological processes for in-situ remediation of subsurface contaminants. We are investigating the use of vibrational energies as a tool for accelerating the transport of microorganisms and nutrients in subsurface media. This basic research focuses on evaluating the applicability of vibration-induced transport through detailed hypothesis testing in laboratory experiments to be complemented by field-scale verification. We hypothesize that vibrational energies will increase microbial transport as a result of changes in subsurface porosity, increased dispersion and increased desorption.

Our results indicate that vibrational energies do indeed increase dispersion and transport in sediments. Comparisons between vibrated and non-vibrated control columns revealed stark differences in the flow, distribution and dispersion of tracers and microspheres. Current results suggest frequencies between 40-200 Hz with power levels of several Kilowatts per cubic meter may be best suited for microbial transport. Modeling by colleague C. Santamarina of Georgia Tech revealed that vibrations of these frequencies and powers should transmit more than 10 meters in radius within shallow sandy sediments. Our column experiments revealed that vibrations of 40-200 hertz typically increased aqueous flow in sediments by 60-100%. In addition to the increased flow, we observed faster breakthrough of iodide conservative tracers, although the fraction of the tracer recovered at the height of the peak (C/Co) was ~30% of that observed in non-vibrated controls.

These results, when combined with the dramatically increased tailing observed in vibrated columns, are indicative of increased dispersion with vibration. Although the flow dramatically increases with the onset of vibration, there is a drop in the velocity of water flow over time. In experiments using Abbott's Pit intact columns, the water velocity decreased ~5% per hour of vibration, and after long periods of vibration water flow through the columns dropped below levels in non-vibrated controls as a result of plugging within the column. These observations are consistent with the ~10% reduction column volume after vibration, resulting from the repacking of grains. As expected, upon cessation of excessive (>10 hr) vibration, water flow is always much less than in non-vibrated controls.

Transport of fluorescent microspheres as determined by flow cytometry is orders of magnitude greater than in non-vibrated columns (typically below detection limits after 0.5 m). Although the fraction of transported microspheres remains low, microsphere breakthrough appeared faster than the conservative iodide tracer. The faster breakthroughs are followed by long tails indicative of dispersion followed by secondary peaks corresponding to the iodide breakthrough. Analyzing slices of columns by confocal microscopy or UV light reveals greater transport and lateral dispersion of all sizes and colors of fluorescent microspheres in the vibrated columns. Current experiments are expanding to microbial transport, dissecting mechanisms of increased transport facilitated by vibration as well as field-testing procedures (e.g., multilevel samplers, sampling protocols and drive-point well installations) for a field-scale verification of vibration-facilitated microbial transport.

Enhanced Quantitative Methods as Integrating Elements of Multidisciplinary Bacterial Transport Research at the Oyster Site

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Experiments being conducted under NABIR at a field site near Oyster, Va., are identifying and quantifying microbial transport processes in sandy aquifers under varying biogeochemical conditions. At the field scale, multiple hydrologic and biogeochemical processes interact in a heterogeneous subsurface environment to complicate the interpretation of experimental results. In this complex environment, a well-designed suite of quantitative models can effectively serve as a focal point for the design and interpretation of microbial transport experiments, quantitative testing of research hypotheses, management and integration of data and transfer of information between different scales.

This project is developing and applying a series of advanced hydrogeological models of tracer and bacterial transport, drawing on and integrating data provided by collaborators (e.g., geophysical data from E. Majer/LBNL, hydrologic data from T. Griffin/Golder Assoc., and geological data from D. Swift/Old Dominion Univ.). Several levels of model complexity and various length scales are addressed through multiple linked models ranging from one-dimensional core-scale models of laboratory experiments to high-resolution heterogeneous models of field-scale transport. These models have been used for experimental design (e.g., location of multi-level samplers) and interpretation (e.g., testing of hypotheses regarding scaling of laboratory experiments for field-scale prediction).

Most recently, we have developed a novel approach to the simulation of microbial exclusion phenomena, based on a modified particle-tracking method. The application of these models in the areas of data management and integration, parameter and process scaling, collaborative interaction, and experimental design is the focus of several specific research elements. The scaling element will develop three-dimensional core-scale flow and transport models to quantify microbe/solid surface interactions and obtain field-scale process representations. Tracer test inversion techniques will be evaluated in terms of their ability to enhance model predictions relative to other types of characterization data. The experimental design element will employ a collaborative tool for identifying, guiding and documenting design decisions, and will integrate quantitative pre-modeling results with qualitative investigator input and practical considerations. The data management element will populate a web-based data and information repository with linkages to the numerical model framework, experimental design tools and collaborative databases.

This research will lead to specific results of relevance to the subsurface microbiological sciences, will increase the overall value of data and information collected at the Oyster site, and will develop a systematic approach and knowledge base applicable to future research at other sites (and ultimately to bioremediation applications).

Heterogeneity of Sedimentary Aquifers: Role in Microbial Dynamics Assessed by Radar Imaging and by Acoustic and Radar Tomography

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The Heterogeneity Program is an integrated, sedimentological, geochemical, geophysical and microbiological study designed to identify scales of physical heterogeneity that affect the biodynamics of natural subsurface environments. At the Oyster, Va., site, a regional grid of Ground-Penetrating Radar (GPR) lines have been constrained by Cone Penetrometer Test profiles (CPT profiles) in order to resolve the sub-regional stratigraphy. Special study areas have been cored and trenched. They have been subjected to more closely spaced radar lines in order to provide higher resolution stratigraphic information, and to compare with radar and seismic tomography. Cores have been split and subjected to infrared imagery and air minipermeameter analysis. More than 1,000 samples from cores and excavations have been analyzed for grain size. Of these, 300 have been analyzed for permeability, and a further subset has been analyzed for iron oxides.

The deposits of the Oyster site are highly permeable, with values on the order 0.1 to 50 Darcys. These deposits have been grouped into hydrofacies on the basis of grain size, permeability and stratal architecture, as observed in cores and GPR records. In order to estimate microbial activity, hydrogen uptake rates were measured under aerobic and anaerobic conditions across three of the five hydrofacies identified at the Oyster site. Activity was low and not highly variable, ranging from 6 to 60 nmol of hydrogen used per g per day. Microbial abundance has been measured using most probable number (MPN) analysis for aerobic and anaerobic heterotrophs and sulfate-reducing bacteria. MPN estimates for both heterotroph groups vary by 3 to 4 orders of magnitude**.** Phospholipid analyses and MPN determinations agree fairly well and indicate that microbial abundances ranged from 105 to 106 cells per gram. Phospholipid fatty acid analysis showed that all samples contained a relatively diverse microbial community structure. Principle component analysis showed that most samples clustered together in agreement with the low variability observed for microbial activity measurements.

Preliminary studies indicate a much closer relationship than heretofore envisaged between large-scale stratigraphy as resolved by Ground-Penetrating Radar and CPT profiles, and the meso- and small-scale physical heterogeneity seen in cores and excavations. In order to determine small-scale heterogeneity, a preliminary analysis of spatial correlation has been undertaken. It reveals a dominant log-permeability integral scale in the vertical direction of ~30-40 cm and a horizontal integral scale of ~1.5 m. It is apparent that the scale of spatial organization of microbial populations is significantly shorter (1-10 vertical cm; 10-40 horizontal cm). However grain size and permeability variations are apparent at this finer scale also, and appear to be significant controls of microbial population distribution. Present work focuses on relating these fine-scale physical parameters to physical parameters observed at larger spatial scales, and ultimately, to non-invasive geophysical images.