Natural and Accelerated Bioremediation Research

South Oyster Science Plan

Field Experimentation in Bacterial Transport

Bacterial Transport Element

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Glossary

CPT	Cone penetrometer test
DAPI	A chemical stain used in counting bacteria. Its full name is 4',6-diamidino-2-phenylindole.
DIRB	Dissimilatory iron-reducing bacteria
DOC	Dissolved organic carbon
DOE	U.S. Department of Energy
EMSL	Environmental and Molecular Sciences Laboratory
GC-CRIMS	Gas chromatography-chemical reaction interface mass spectrometry
HPLC-ESI	High Performance Liquid Chromatography-Electrospray Ionization.
INEEL	Idaho National Engineering and Environmental Laboratory
IR	Infrared
IRB	Iron-reducing bacteria
ISE	Intermediate-scale experiment
LBL	Lawrence Berkeley Laboratory
MIRP	Microbial FE reduction potential
MLS	Multi-level sampler
NABIR	Natural and Accelerated Bioremediation Research
NC	Narrow Channel Focus Area
ORNL	Oak Ridge National Laboratory
PCR	Polymerase chain reaction
PCR-DGGE	Denaturing gradient gel electrophoresis
PNNL	Pacific Northwest National Laboratory
SMCC	Subsurface microbial culture collection
SOFA	South Oyster Focus Area
TNC	The Nature Conservancy
TOC	Total organic carbon
VaDEQ	Virginia Department of Environmental Quality

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Introduction

Bacterial transport is of increasing interest to those involved in remediating subsurface environments because adding and dispersing bacteria that can degrade or transform contaminants (bioaugmentation) is an attractive and viable remedial option. Treatment regimes for contaminated soils and aquifer sediments have most often involved separating the contaminant from the solid, followed by treatment or disposal.

Soils and near-surface sandy aquifers commonly contain iron (Fe), manganese (Mn), and aluminum (Al) oxyhydroxides. Toxic metals and radionuclides can strongly adsorb onto the surfaces of these oxyhydroxides, and their desorption kinetics into the groundwater can be extremely slow. Simply pumping the groundwater to the surface for treatment, therefore, is not always an effective remediation strategy for aquifers contaminated with metals or radionuclides.

Bacterial interactions with metals are varied and complex. These biogeochemical reactions can result in the physical removal of metals or radionuclides by mobilization, or the precipitation of metals or radionuclides to immobilize them. Bioaugmentation—the addition of microorganisms—is being considered (Caccavo et al. 1996; Gorby et al. 1994; Phillips et al. 1999) as a means to modify the behavior of metals and radionuclides concentrated in the oxyhydroxides of sandy aquifers.

Bioaugmentation promises to be very useful where natural attenuation or biostimulation are inappropriate or do not work. For example, biostimulation can be ineffective where: 1) the necessary nutrients/inducers to promote the desired microbial activity are absent and cannot be added to the environment, or 2) organisms with the appropriate activity are absent from the indigenous population. In these cases, bioaugmentation may be an effective biological treatment strategy and perhaps the only feasible option. There are also cases where the natural microbial population may be insufficient to achieve remediation with biostimulation; in this case, bioaugmentation is essential to increase the rate and thereby shorten the time frame and costs for full-scale remediation.

There are two distinct approaches for using bioaugmentation to remediate contaminated areas (Unterman et al. 1999):

- **Growth Strategy**

  In the first bioaugmentation approach, microorganisms that are selected for long-term survival and the ability to occupy a selective niche within the contaminated environment are introduced; electron donors, nutrients, electron acceptors, or selective co-substrates may then be added to aid survival. Thus, the goal of this approach is to achieve prolonged survival and growth of the introduced organisms and the concomitant treatment of the target contaminants.

  This approach is most effective in subsurface environments that contain a suitable growth substrate or where a selective growth substrate can be added to promote the survival of the microbial amendments. This approach has been most effective when the microorganisms are immobilized in the subsurface and form a permeable barrier or "biocurtain" that intercepts the migrating contaminant

plume. To predict the effectiveness of this approach, the size of the treatment zone and the *in situ* transport behavior and physiological characteristics of the introduced strain must be known.

Although microbial barriers represent very effective containment strategies, they are still a variation of subsurface pump and treat technology, and therefore, are constrained by the same problems associated with pump and treat remediation programs. Specifically, pump and treat methods may require a decade or more to complete clean up, and are controlled by the rate of the flow of the contaminated groundwater passing through the permeable barrier. Because contaminants sorb strongly to aquifer sediments, remediation by pump and treat is limited by desorption kinetics, which are typically very slow.

- **Biocatalyst Strategy**

  In the second bioaugmentation approach, large numbers of bacteria are introduced into a contaminated environment as biocatalysts that will degrade, mobilize, or immobilize a significant amount of the target contaminant before becoming inactive or perishing. The long-term survival, growth, and establishment of the biocatalyst is not a primary goal of this treatment approach. Additional injections of bacteria (biocatalyst) can be added as needed to further the remediation process.

  Applications of this approach are controlled by the cost of culturing sufficient masses of organisms and the extent to which organisms are distributed *in situ* throughout the area of contamination. A pivotal requirement for successful subsurface remediation using this second approach is reliable delivery and dispersion of the injected microorganisms throughout the contaminated area. Development of this bioaugmentation technology, however, is currently limited by a paucity of data on field-scale migration of bacteria through porous sedimentary aquifers (DeFlaun et al. 1997; Dybas et al. 1998; Steffan et al. 1999).

The transport of bacteria in groundwater is inversely related to the extent of their attachment to sediment grain surfaces. The extent of bacterial attachment to grain surfaces is related to the surface chemistries of the bacteria and sediment (Van Loosdrecht et al. 1989; Van Loosdrecht et al. 1990; Coston et al. 1995; Neu 1996; Johnson and Logan 1996; Jucker et al. 1998), and to the physical properties of the sediment, as described by filtration theory (Rajagopalan and Tien 1976; Logan et al. 1995).

Hence, the extent of bacterial transport varies with changes in both the physical and chemical characteristics of the aquifer, i.e., physical and chemical heterogeneity in groundwater aquifers, as well as with variations in the phenotype of the bacterial strain (DeFlaun et al. 1999).

Whereas the effects of physical heterogeneities on bacterial (colloidal) transport has been a subject of significant investigation over the past decade (Bales et al. 1989; Buddemier and Hunt 1988; Champ and Schroeter 1988; Toran and Palumbo 1992; Harvey et al. 1993; McKay et al. 1993; Vilks and Bachinski 1996), the significance of chemical heterogeneity in bacterial transport has received less attention.

The best known, and arguably the most common transport-relevant, chemical heterogeneities in sedimentary materials are Fe and Al oxyhydroxides. At pH values less than 8, these oxides may display positively charged surfaces that may serve as points of bacterial attachment within a sedimentary matrix dominated by negatively-charged silicate minerals.

The extent of bacterial transport in a number of sedimentary materials has been shown to

be inversely related to the amount of Fe and Al oxyhydroxides present in the sediment (Johnson and Logan 1996; Knapp et al. 1998). Obfuscating the effects of Fe and Al oxyhydroxide are variations in groundwater pH and ionic strength, both of which serve to alter the extent of bacterial attachment to sediment with a given oxyhydroxide content (Fontes et al. 1991; Li and Logan 1999; Scholl and Harvey 1992; Ryan et al. 1999).

Additionally, the presence of dissolved natural organic matter (such as humics) in groundwater may further complicate the effect of oxyhydroxide on bacterial transport due to their ability to mask the positive surface charge of the oxyhydroxide (Tipping and Cooke 1982; Davis 1982; Thurman 1985; Johnson and Logan 1996). Another potential effect of the natural organic matter in groundwater is that its transport may enhance the transport of organic contaminants, metals, and radionuclides and perhaps even injected bacteria (McCarthy et al. 1996).

Suboxic conditions may prevail in mixed metal-organic contaminated sediments. Within this setting, Fe oxyhydroxide frequently becomes a major electron acceptor in suboxic microsites, promoting the growth of iron-reducing bacteria (IRB). The ability of IRB to transform, mobilize, or immobilize toxic metals and radionuclides makes them good candidates for bioaugmentation (Caccavo et al. 1996). Unfortunately, models for suboxic bioaugmentation are inadequate given the absence of either column-scale or field-scale bacterial transport experiments under suboxic conditions (Ginn 1995).

In many cases, the best source of microorganisms for bioaugmentation is the contaminated zone itself. The environment already may have selected for bacteria with the desired metabolic and enzymatic capabilities, but their activity may be limited by nutrient availability. A potentially successful bioaugmentation strategy is to isolate and culture bacteria from the contaminated zone and select strains that 1) possess low adhesion for the aquifer minerals, 2) tolerate starvation, 3) can be readily grown to high densities, and 4) possess the appropriate enzymatic capabilities to remediate the contaminant.

The most important advantage to isolating and the reinjecting members of the *in situ* bacterial populations is that indigenous strains are "pre-adapted" to *in situ* biogeochemical conditions. Another advantage of this approach is that use of natural microbiota may be more attractive to stakeholders and local government agencies, because such bacteria already exist in the environment and are not genetically altered.

To obtain high-quality data on the transport of injected, indigenous bacterial strains, however, reliable and sensitive detection methods specific to the injected bacteria are required (Errampalli et al. 1999). Selective plate counts, fluorescent cell stains, and whole-cell labeling with the stable isotope of carbon (^{13}C) (DeFlaun et al. 1997) have all been tested to monitor microbial transport in groundwater; however, each of these techniques has potential limitations.

The detection limit for plate counts may be higher than is needed to document the presence of the injected target organism in a downgradient sampling point, and it may not be able to enumerate those target organisms that are still able to carry out the function for which they were injected but are no longer able to form colonies on solid media (Oliver 1993). Fluorescent cell stains allow all the injected cells to be detected regardless of their culturability, but most stains are known to affect the activity, viability, or adhesive properties of the cells (Parolin 1990). Recent work with viable stains shows promise for tracking bacteria without affecting their physiology (Fuller et al. 1999; Fuller et al. in prep). Injection of cells with high amounts of ^{13}C incorporation, with subsequent detection of the stable isotope enrichment of the particulate carbon downgradient, is not

dependent on cell culturability and is not expected to alter cell activity, viability, or adhesive properties. However, these bulk determinations of ^{13}C enrichment are not able to unequivocally document that the ^{13}C represents live target cells, since the ^{13}C in the injected organisms may have been transferred or incorporated into other microbes as target organisms died and lysed or became prey for protozoa. Compound-specific ^{13}C analyses when applied to microbial lipids should be able to ascertain the relative importance of these processes

These new bacterial tracking methodologies, as well as refinement of traditional ones, merit further investigation, particularly in light of recent scientific and technological advances.

The feasibility of remediating metal and radionuclide contamination in either aerobic or suboxic porous aquifers using bioaugmentation strategies is theoretically conceivable, but it cannot be rigorously evaluated because so few field experiments have been performed. The purpose of this field research project is to 1) develop new insights into the basic processes that control bacterial transport in aquifers, and 2) extend this experience to metal-contaminated DOE sites. A pristine site was sought for initial field experiments to elucidate the basic mechanisms controlling field-scale transport before transitioning to contaminated sites where this basic understanding could be applied. To meet these needs, the Oyster Scientific Team has selected an appropriate field site, are developing procedures for selecting bacteria strains from *in situ* communities, developing new procedures for tracking bacteria, testing scale-up approaches, and generally designing strategies to facilitate the transport of bacteria within heterogeneous Fe and Al oxyhydroxide-bearing subsurface systems where so much toxic metal and radionuclide contamination resides.

Integrated Approach

Numerous published studies of bench-scale, aerobic bacterial transport experiments in columns packed with glass beads and quartz sand are the basis for filtration theory (Martin et al. 1996; Johnson et al. 1995) and the realization of the importance of various bacterial desorption kinetic parameters. Applying these theories to field-scale bacterial transport experiments shows promise (Harvey and Garabedian 1991; Bales et al. 1997), but the veracity of these theories is compromised by the physical and geochemical heterogeneity of the field environment (Harvey et al. 1993; DeFlaun et al. 1997).

Field experiments are essential to confirm that process-level understanding can be used to reliably predict bacterial transport.

Field transport experiments are essential to confirm whether an understanding of the processes believed to be responsible for adhesion and detachment of viable bacteria from the aquifer solids can be used to reliably predict the rate and extent of bacterial migration at a well-characterized field site with an accurate monitoring technique. Field transport experiments also provide a test-bed for future studies at NABIR Field Research Centers and ultimate transfer to a variety of industrial and federal sites.

To perform bacterial transport experiments under field conditions, the following major questions must be addressed:

- What level of field characterization is required to develop an accurate bacterial transport model?

  Heterogeneity in grain size, porosity, hydraulic conductivity, and mineral composition all affect the migration of bacteria. To address this question, the subsurface environment must be characterized using: 1) surface geophysical techniques; 2) coring and logging; 3) subsurface geophysical tomography; 4) examination of nearby stratigraphically equivalent strata; and 5) conservative, reactive, and particle tracers. The suite of observations and the spatial resolution required for adequate characterization have yet to be determined, but an overall approach recently has been developed (DeFlaun et al. 1997).

- How can results from laboratory transport experiments be used and scaled-up to the field?

  Laboratory transport experiments are currently being performed under conditions simulating the natural groundwater environment using intact cores. These studies document the effect of bacterial adhesion on the transport distance, bacterial concentrations, and flow rates that maximize transport, the stability of the labels used to track the microorganisms, and the effects of sedimentary facies on transport. These data are crucial to flow-cell design and to the bacterial growth, labeling, and injection protocols.

  Intact core studies also provide a way to test methods for facilitating bacterial transport in advance of field trials. Examination of the cores following a bacterial transport experiment enables documentation of the bacterial attachment/detachment process at the millimeter scale.

Although the effects of physical and chemical heterogeneity on bacterial transport can be tested by intact core experiments, heterogeneities are difficult to quantify because of the challenge of characterizing the core in three dimensions. A useful approach to quantifying attachment/detachment phenomena in heterogeneous media is the use of intermediate (meter-scale) laboratory flow cells in which defined heterogeneities can be built into the porous media, and their role in controlling bacterial transport can be assessed (Murphy et al. 1997).

Finally, parametric models of laboratory-scale bacterial transport studies using intact cores and meter-scale flow cells have been developed that will yield bacterial attachment/detachment parameters for sub-facies-scale physical and chemical heterogeneity (Ginn 1995). These parameterizations can be combined with facies-scale characterization of physical heterogeneity to develop quantitative models for field-scale bacterial transport. A comparison of the success of this scaling-up approach in capturing field-scale heterogeneity and reproducing the observed bacterial breakthrough profiles, as opposed to alternative scaling-up strategies, is one of this program's goals.

Hypotheses to be Tested

This document outlines the major research tasks that, in total, comprise the South Oyster Science Plan. The plan is a tool that seeks to 1) promote collaborations among various investigators and institutions, and 2) assist scientists outside of the program to better understand the research in progress.

The principal goal of the experiments outlined herein is to increase our scientific understanding of the role of microbial adhesion and biogenic Fe(III) reduction on field-scale bacterial transport. The field experiments will result in protocols that will generally lead to a new understanding of bacterial transport in sediments containing Fe, Mn, and Al oxyhydroxides, the sites of metal and radionuclide contamination, in circum- to subneutral pH groundwater.

A specific focus of the planned research is to develop field strategies for bioaugmentation whereby the preferential adsorption of facultative IRB to Fe oxyhydroxides and the total microbial Fe(III) reduction rate are increased. The plan relies on a series of integrated field and laboratory experiments, each of which is designed to test one of the following core concepts:

- Physical heterogeneity controls bacterial transport—Wild-type and adhesion-selected strains of bacteria typically yield a range of collision efficiencies and charge densities (Glynn et al. 1998; Baygents et al. 1998). Theoretically, hydrophilic bacterial strains with low or neutral surface charge should be insensitive to the variable mineral surface charges encountered in a chemically heterogeneous aquifer. Bacterial adhesion to aquifer sediment is controlled primarily by physical heterogeneity, and specifically, by grain and pore throat size distributions. In sediments with variable grain size, e.g., silt to sand, the bacterial strains will move faster through the coarse-grained layers than a conservative solute tracer. The scale at which this is observed is on the order of meters and controlled by the spatial scale of the sedimentary layering and on the inter-layer variability of grain size. This phenomenon, often accredited to either "pore exclusion" or "size exclusion," has been documented in the laboratory, but not observed in the field. The mechanisms governing this behavior are poorly understood.

- Chemical heterogeneity controls bacterial transport—For quartz-bearing sediments containing Fe, Mn, and Al oxyhydroxide cement and circum- to sub-neutral pH groundwater, the quartz has a negative surface charge, whereas the oxyhydroxide cement has a positive surface charge. Bacterial strains that are hydrophobic or that bear high negative surface charge will be repelled by quartz grains lacking oxyhydroxide coatings and attracted to surfaces dominated by oxyhydroxide phases.

  If the oxyhydroxides are uniformly distributed as grain coatings throughout the aquifer sediment, then these negatively charged bacterial strains will not migrate as far as neutrally charged bacteria. If the concentration, mineralogy, and grain size of the oxyhydroxides are heterogeneously distributed in layers with uniform groundwater chemistry, however, then the nega-

tively charged bacteria are likely to move faster than the conservative solute tracer through the layers containing little oxyhydroxide.

The concentrations of negatively charged bacteria entrained in the groundwater will diminish rapidly, however, as more of the population adheres to the layers rich in oxyhydroxide phases. Bacterial adhesion in aquifer sediment, therefore, is controlled by a combination of *in situ* chemical and physical heterogeneity.

- Microbial Fe(III) reduction will indirectly enhance IRB transport by several mechanisms—Caccavo et al. (1997) has proposed that IRBs are hydrophobic and adhere reversibly to hydrophobic Fe, Mn, and Al oxyhydroxide minerals. Membrane proteins then cause strong bacterial adhesion to Fe oxyhydroxide mineral surfaces.

  The high affinity of IRB for oxyhydroxides improves their chances of adhering to near-field contaminated microsites but impairs their ability to move through the sediment to far-field contaminated oxyhydroxide loci. In those cases where high collision efficiency limits bacterial penetration of a formation, bacterial transport can be significantly improved if the desorption rate of the bacteria is increased.

  Enhancement of the Fe(III) reduction rate of the IRB may increase the desorption rate through several mechanisms. Fe(III) reduction will reduce the bioaccessible Fe(III) surface area and should encourage the IRB to desorb. Increases in the adsorbed, microbially produced Fe(II) on the Fe(III) surfaces may also reduce IRB adhesion.

  One approach to enhancing Fe(III) reduction is to provide a humic acid analogue, e.g., anthroquinone disulfonate, which acts as an electron shuttle from the Fe(III) mineral surface to the membrane-bound respiratory system (Fredrickson et al. 1999). Naturally occurring humic acids may also increase Fe(III) reduction activity and thereby increase IRB desorption rates and transport. Elevated Fe(III) reduction rates could locally increase the pH, which in turn, may promote desorption of IRB by reducing the positive surface charge of the oxyhydroxide minerals. Finally, if the Fe(III) reduction activity is high enough to induce significant IRB growth, the desorption of daughter IRB cells will increase net transport rates. Under growth conditions, however, the effects of bacterial predation by protists must be considered (Kinner et al. 1997).

The results of prior lab and field bacterial transport studies provide a conceptual framework for the planned series of aerobic and anaerobic injection experiments.

The pH of the groundwater where published field-scale bacterial transport experiments have been conducted to date, e.g., Cape Cod and Borden sites, ranges from 5 to 6.5. In this range, the surface charges of Fe, Al oxides, and oxyhydroxides are positive as opposed to the negative surface charges of bacteria, quartz, and feldspar (Sverjensky and Sahai 1996). These minerals may act as strongly adsorbing and perhaps irreversible sites for bacterial adhesion, and ample laboratory evidence indicates that bacterial retention in columns packed with by Fe(III)-coated quartz is higher than columns packed with just quartz (Mills et al. 1994; Johnson et al. 1996; Johnson and Logan 1996; Knapp et al. 1998).

Variations in the amount of bacterial breakthrough detected in the multi-level sampler (MLS) arrays at either the Borden or Cape Cod site could be mitigated by variations of Fe, Mn, Al oxyhydroxide coatings of the sediments, but extensive characterization of these chemical parameters has not been performed for these sites. In preliminary intact core experiments, the spatial distribution of bacteria remaining in the sediment appears

to correlate with the distribution of Fe, Mn, Al oxyhydroxide minerals (Dong et al. 1999). But grain size also controls bacterial adhesion, and if grain size and oxyhydroxide concentrations are correlated in the aquifer sediment, then deconvolution of the transport controlling mechanisms will require sophisticated experimental approaches.

Absorption/desorption experiments performed on Cape Cod sand (Scholl and Harvey 1992) that contain Fe(III) oxyhydroxide minerals indicated that significant desorption of bacteria from these sand-sized materials occurred when the pH was increased from 5 (typical for uncontaminated Cape Cod groundwater) to 8. This is attributed to the reversal of the Fe(III) oxyhydroxides surface charge from positive to negative as the pH was increased from 5 to 8.

Scholl and Harvey (1992) also performed a field bacterial injection experiment with DAPI-stained cells at Cape Cod and then followed the bacterial injection experiment with an injection of groundwater adjusted to pH 8 using a phosphate buffer. In this case, little desorption was reported, which they attributed to the neutralization of the pH 8 water as it moved away from the tracer injection well.

Bales et al. (1997) appear to have been more successful at introducing a high pH solution subsequent to an injection of viruses and microspheres at the Borden site. As the pH increased from 7.2 to 8.4, a pulse of bacteriophage, which had been deposited on the sediment by the first injection, traveled through the MLS array. Differences between field and laboratory results were attributed to the uncharacterized chemical heterogeneity in the field.

Ryan et al. (1999) followed a bacteriophage and Si-colloid injection into the Cape Cod aquifer with a high-pH (8-10) solution. Release and breakthrough of adsorbed bacteriophage and Si-colloids were correlated with pH breakthrough.

Quantifying the influence of chemical heterogeneity on field-scale bacterial transport, therefore, requires multiple experiments where either the pH of the groundwater is adjusted to weaken mineral surface charge differences, or the surface charge of the bacterial strain (or fluorescent microspheres) is varied, or some competitive anion is added to saturate the positively charged sites. With respect to remediation of toxic metals and radionuclides adsorbed to Fe, Mn, and Al oxyhydroxides, a bacterial strain that preferentially adsorbs to the oxyhydroxides, i.e., a negatively charged bacterium, is most desirous.

Capillary electrophoresis can be used to identify strains with a specific charge (Glynn et al. 1998). Adsorption/desorption or adhesion experiments performed as a function of pH and synthetic Fe, Al oxyhydroxide concentrations can be used to evaluate the dominance of electrostatic interactions versus hydrophobicity effects.

Laboratory experiments performed by Johnson et al. (1996) suggest that dissolved organic carbon (DOC) will reduce the bacterial adsorption on quartz surfaces and increase bacterial adsorption on Fe(III) oxide-coated quartz. Scholl and Harvey (1992) reported reduced bacterial adsorption on Cape Cod sediments with groundwater containing 4 ppm dissolved organic carbon versus artificial groundwater containing <0.4 ppm dissolved organic carbon. This relationship switched, however, at pH >6.5.

Dissolved organic carbon is frequently characterized by negatively charged moieties, which, if present in groundwater over a long period of time, will preferentially coat the Fe, Mn, and Al oxyhydroxides, thereby masking the differential surface charges. In repacked core experiments, Johnson and Logan (1996) showed that humic coatings on Fe(III) oxides with sedimentary humics reduces bacterial adhesion to Fe(III) oxides by 60%.

If laboratory experiments do suggest a correlation between bacterial absorption/desorption rates, and either high pH or the concentration of Fe, Mn, and Al oxyhydroxide phases, then the surface charge of the site sediments should be measured as a function of pH by using the "streaming potential" (Ryan et al. 1999). Such measurements can be used to determine if charges are masked compared to artificially coated sand grains. If they are, then the addition of high pH water to intact sediment cores should result in the release of adsorbed dissolved organic carbon or colloids from the sediment, thereby enhancing the charge differential between quartz and Fe, Mn, and Al oxyhydroxide phases.

Provided that intact core experiments with Oyster sediments look promising, then a field-scale bacterial injection might then be followed by a high pH injection. Measuring pH breakthrough at the MLS's is straightforward. The challenge will be to see if the number of injected bacteria that adsorbed to the sediment and released during the high pH injection can be correlated with the chemical heterogeneity of the sediment as determined from core analyses. The total organic carbon (TOC) content of the water also should be monitored during the high pH injection, in the event that organic matter adsorbed to the sediment is partially desorbed.

Another approach for enhancing the surface charge differences between the quartz and the Fe, Al oxyhydroxide phases is to decrease the ionic strength. Camesano and Logan (1998), Johnson et al. (1996), and Hornberger et al. (1992), have all reported enhanced transport of bacteria through quartz sands by lowering the ionic strength of the water. Low ionic strength groundwater would produce a thick, repulsive double layer on the surface of quartz grains, leading to lower collision efficiencies (Spielman and Friedlander 1974). The same may be true for Fe and Al oxyhydroxides at pH 8, but, at a pH of 5, the electrostatically repulsive barriers of quartz will be increased relative to the electrostatic attraction of the Fe, Mn, and Al oxyhydroxides. Preferential attraction of negatively charged bacteria to the Fe and Al oxyhydroxides should be increased. Such laboratory or field experiments, however, have not been reported.

Combined with pH changes, ionic strength variations could prove to be a powerful tool for manipulating electrostatic processes and facilitating the attachment of bacteria to metal-contaminated sediments. Such experiments could be designed to investigate the bacterial adsorption process by injecting the bacteria with low ionic strength artificial groundwater. The bacterial desorption process could be investigated by injecting bacteria with natural groundwater, followed by an injection of low ionic strength water. It may also be possible to lower the surface charge differential by injecting the bacteria with artificial groundwater with the same salinity as the natural groundwater, but with greater proportion of divalent ions.

Such field experiments should be preceded by measurements of bacterial surface charge as a function of ionic strength and by bacterial transport experiments using Oyster intact cores and low ionic strength artificial groundwater. If these experiments produce promising results, then a field injection of bacteria in the presence of varying ionic strength natural groundwater might be useful. Measuring conductivity breakthrough at the MLS's is straightforward. The challenge would be to determine if the number of bacteria adsorbed or desorbed during the low ionic strength injection can be correlated with the chemical heterogeneity of the sediment. TOC and colloids in the groundwater should

be monitored during the low ionic strength injection, in the event that they are partially desorbed during the injection.

Groundwater temperature is another possible parameter that affects bacterial transport, but published reports are inconclusive. Laboratory experiments indicate that the higher the temperature, the greater the adhesion of bacteria to sediment (Hendricks et al. 1979; Fletcher 1977). Bellamy et al. (1985) observed 100 times greater adsorption of bacteria to sand at 17°C versus 2°C.

For bacteria that are motile at high temperature, but not at low temperature, the opposite behavior was observed (McCaulou et al. 1995). Strain A0500, a motile subsurface strain, exhibited a lower collision efficiency when passed through a repacked sand column at 18°C, where it was motile, versus 4°C, where it was nonmotile. Strain A0500 also exhibited faster detachment rates compared to nonmotile bacteria. Enhanced column transport of motile bacteria over nonmotile bacteria under low flow velocities (0.6 m/day) has also been reported by Camesano and Logan (1998).

Low temperature injections could enhance bacterial transport for nonmotile species relative to ambient temperature injections and the electrostatic potential difference between positively and negatively charged minerals surfaces could also increase. The metabolic activity and growth of the adsorbed bacteria will also be affected by changes in groundwater temperature. Although no papers report varying the groundwater temperature during field-scale bacterial transport studies, Davis et al. (1985) did report injecting hot water and monitoring the temperature breakthrough in a sandy aquifer using thermistors.

The effect of Fe(III) reduction upon bacterial transport requires performing experiments under anaerobic conditions. To date, almost all laboratory experiments on bacterial transport and all field bacterial transport experiments have been conducted under aerobic conditions. An anaerobic field injection of xenobiotic compounds has been recently reported by Rügge et al. (1999), but bacteria were neither injected nor was the transport of indigenous bacteria investigated. Bioaugmentation has been performed in the field at contaminated sites that are suboxic (Steffan et al. 1999), but the injected microorganisms were aerobic, and the injection medium was oxygenated. Although parts of the Cape Cod aquifer are anaerobic due to organic contamination, the mechanisms mitigating bacterial transport in that portion of the aquifer have not yet been delineated.

To determine whether long-term Fe(III) reduction leads to enhanced bacterial transport, field experiments must be conducted in an undisturbed, uncontaminated aquifer where natural Fe(III) reduction has been in progress since the deposition of the sediments.

By altering the laboratory flow system to prevent leakage to air and by co-injecting deoxygenated artificial groundwater with the bacteria, the same experiments can be repeated on intact cores obtained from the suboxic portion of the field site. These cores must be collected and stored so as to best maintain original physical/chemical conditions before incorporation into the laboratory flow system. Numerical models of these intact core experiments will be used to define bacterial adhesion and desorption parameters for scaling up for the suboxic field bacterial transport study.[1]

(1) An Intact Core Workshop was held in April 1999 to review and improve procedures for conducting intact core experiments. Representatives from ORNL, PNNL, Princeton University, LBNL, INEEL, and Envirogen, Inc. attended the workshop.

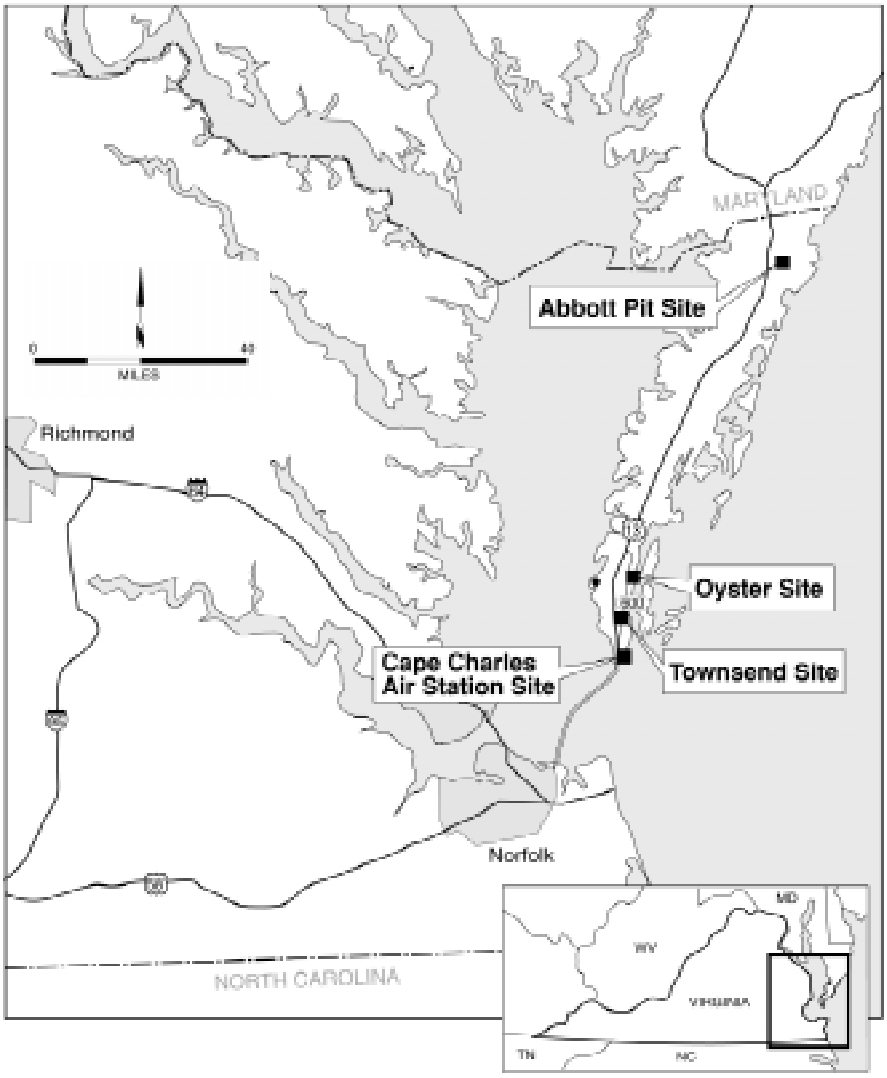

Figure 1. *Location of Oyster Field Site on the Delmarva Peninsula. Alternate candidate sites included the Cape Charles Air Station and Townsend. The Abbott Pit site is a satellite site that provides ready access to sediments for laboratory microbial adhesion and transport studies.*

strong bacterial adhesion to the mineral grains by suppression of the electrical double layer. Finally, the presence of relatively high, aqueous Fe(II) in the suboxic portion of the aquifer is important as it indicates that microbial Fe reduction is probably taking place.

A field site south of Oyster, Virginia (Figure 1), has been identified that meets all these requirements. At this location, the dissolved oxygen in the groundwater of the surficial, unconfined aquifer decreases from 6 mg/L to <1 mg/L as it flows from a local topographic high toward the tidal marsh. This trend toward hypoxia is observed in numerous locals in the Delmarva Peninsula and is related to the upwelling of suboxic water from deeper confined aquifers (Figures 2a,b; Speiran 1996).

To test the stated hypotheses in the field, however, flow cells[2] to support this research must be located at a site where aerobic and suboxic groundwater exist in proximity within approximately the same hydrogeological unit. The pH of the site groundwater needs to be neutral to sub-neutral to test the effects of oxyhydroxide surface charge on bacterial transport. The salinity of the site groundwater needs to be <0.1 wt% to avoid IRB have been enriched from indigenous populations at this site. Once adhesion-deficient, indigenous facultative IRB strains have been selected, they will be labeled and injected with conservative tracers into both the aerobic and suboxic flow fields.

Satellite field sites, established on the Delmarva Peninsula are also being used throughout this project as sources of both

(2) The "flow cells" being constructed are forced gradient cells with a series of injection and extraction wells designed to create desired groundwater flow.

bulk sediments and for comparative purposes. Sites such as Abbott's Pit (Figure 1) have sediments that are sandy, but differ from South Oyster in the degree of heterogeneity, distribution of grain sizes, and metal oxyhydroxide content. Sediments from these sources will be used to both verify results obtained with South Oyster sediments as well as to determine differences in results obtained in sediments with differing physical/chemical heterogeneity.

A number of researchers from all the DOE NABIR scientific program elements will use materials from the Abbott's Pit, South Oyster, and other established satellite sites on the Delmarva Peninsula (Figure 1). Use of these common reference materials provides unique opportunities for collaboration among researchers from many scientific disciplines and elements in the NABIR program. This sediment collection and distribution is administered by Dr. Gary Jacobs (Oak Ridge National Laboratory).

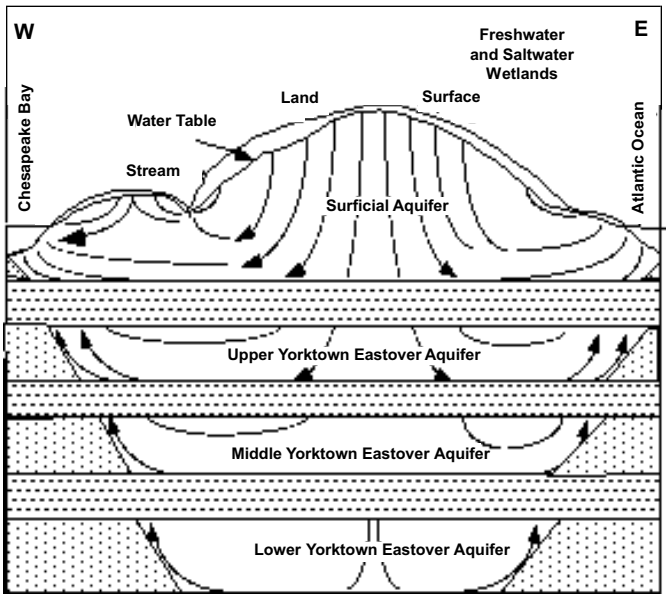

Figure 2a. *Cross-Section of Delmarva Peninsula Illustrating Hydrostratigraphy and Regional Groundwater Streamlines.*

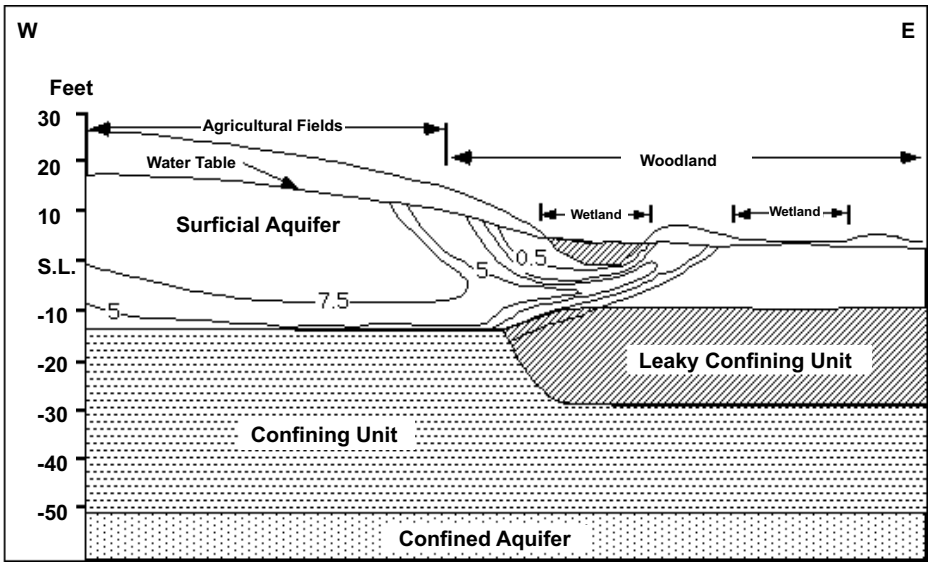

Figure 2b. *Cross-Section of Townsend Site Illustrating the Decrease in Dissolved O_2 in Proximity to the Margin of the Delmarva Peninsula.*

Plan Reviews

Visiting scientists will conduct periodic reviews to evaluate the status of the research and the science plan. Annual "stocktaking meetings" are also held annually to review progress and to promote collaborations.

Collaborating Team Members

Successful field experiments in bacterial transport require seamless integration of the results from bench-scale core studies with the characterization and sample acquisition activities in the field. Timely feedback from the investigators responsible for modeling bacterial transport is pivotal to the design and execution of the laboratory and field experiments. Drs. T.C. Onstott, M.F. DeFlaun, and Mr. Tim Griffin share overall responsibility for coordinating laboratory research, theoretical modeling, and field research activities among the Principal Investigators (PI's) on this project.

Successful field experiments require seamless integration of results.

The following summarizes research activities associated with the South Oyster bacterial transport project and lists co-PIs and collaborators responsible for or participating in each activity.

- Enrichment and selection of IRB from the site—Drs. D. Balkwill (Florida State University[3]) and J. Fredrickson (Pacific Northwest National Laboratory).
- Intact core bacterial transport studies—Dr. M. DeFlaun (Envirogen, Inc.) and Dr. W. Holben (University of Montana). Dr. W. Johnson (University of Utah).
- Understanding the effects of predation by protozoa on bacterial transport—Dr. F. Dobbs (Old Dominion University).
- Adhesion studies of obligate anaerobic and facultative bacteria—Dr. M. Fletcher (University of South Carolina).
- Theoretical analysis of intact core experiments—Dr. T. Ginn (University of California at Davis).
- Coring, logging, excavation, and logistical support at the field site—Mr. T. Griffin and Dr. B. Hallett (Golder Associates).
- Installation of flow cells—Drs. DeFlaun, Onstott (Princeton University), Holben, T. Phelps (Oak Ridge National Laboratory), and Mr. Griffin.
- Methods for tracking bacteria in the field—Drs. Holben, Johnson, D. White (University of Tennessee), and M. Fuller (Envirogen, Inc.).
- Photographic infrared imaging of the South Oyster excavations and cores—Dr. P. Long (Pacific Northwest National Laboratory) and J. Wilson (New Mexico Institute of Mining and Technology).[4]
- Intermediate flow cell experiments examining heterogeneity and growth of IRB on scaling bacterial transport—Dr. E. Murphy (Pacific Northwest National Laboratory/EMSL).[4,5]
- Geophysical characterization of the site—Drs. E. Majer and S. Hubbard (Lawrence Berkeley Laboratory).

(3) SMCC (Subsurface Microbial Culture Collection) supports this research by archiving and characterizaing the Oyster culture collection.
(4) Environmental Molecular Science Laboratory at PNNL.
(5) Related projects in other NABIR program elements at the South Oyster Site.

- Alternative methods to enhance transport; genetic engineering to reduce bacterial cell size—Dr. A. Matin (Stanford University).(6)

- Analyses of the effects of colloids on bacterial transport—Drs. Phelps, Onstott, and DeFlaun.

- Geostatistical analysis of geological, geophysical, and microbiological data—Drs. C. Murray (Pacific Northwest National Laboratory) and D. Swift (Old Dominion University), and M. McInerney (University of Oklahoma).(6)

- Geochemical analyses of groundwater and sediments—Dr. Onstott.

- Development of advanced bacterial deployment strategies in the field—Dr. Phelps.(6)

- Nitrate/Fe geochemistry—Dr. E. Roden (University of Alabama).(6)

- Three-dimensional finite difference modeling of conservative tracer and bacterial transport field data—Dr. T. Scheibe (Pacific Northwest National Laboratory) and Mr. T. Griffin.

- *In situ* assessment of effective reactive surface area of chemically heterogeneous porous media using reactive tracers—Dr. R. Smith (Idaho National Engineering and Environmental Laboratory).(6)

- Physical characterization of sediments, stratigraphy and structure—Dr. Swift.

- Microbiological site characterization—Drs. White, Balkwill, and T. Marsh (Michigan State University).(6)

- Repacked core studies to assess effects of microbial reduction of Fe oxyhydroxides on bacterial transport—Drs. J. Fredrickson and J. Zachara (Pacific Northwest National Laboratory/EMSL).

- Spatial heterogeneity of microbial Fe reduction potential—Drs. C. Murray, E. Roden, S. Hubbard, E. Majer, Y. Gorby, and F. Brockman (Pacific Northwest National Laboratory).(6)

- Ferrographic method for tracking bacteria—Dr. W. Johnson (University of Utah).

- Role of humics in enhancing bacterial transport—Dr. J. McCarthy (Oak Ridge National Laboratory) and M. Fuller.

(6) Related projects in other NABIR program elements at the South Oyster Site.

Research Plan

1.0 Site Design and Development

Expected Results

- Selection and characterization of a field research site.
- Design and installation of flow cells for detection of heterogeneity effects on bacterial transport.

1.1 Site Selection

Task Leader and Collaborators

Griffin, DeFlaun, Onstott, and Swift

Goal

- To select a site that meets the criteria set forth in the proposed research.

Approach

The challenges of site selection include accessibility and the presence of geochemical, geological, microbiological, and hydrological properties that are adequate to test the stated research hypotheses. In this case, site-selection efforts focused on the Delmarva Peninsula.

In the past, the DOE Subsurface Science Program, under direction of Dr. Frank J. Wobber, conducted bacterial transport research at a site owned by The Nature Conservancy (TNC) in Oyster, Virginia. In the interest of building on the experience developed at that site, sites located in similar geological formations on the Delmarva Peninsula were screened. Selected sites had to meet the following criteria:

- Areas of oxic and suboxic groundwater of sufficient extent (100 m x 100 m) to construct two bacterial transport flow cells.
- Circumneutral pH, low salinity, and elevated solid Fe(III) or dissolved Fe(II).
- Close proximity to a site of exposed sediments for collecting intact cores for aerobic and suboxic experiments and to develop stratigraphic/structural information for incorporation into 3D models of the field flow cells.
- Groundwater not contaminated by organic pollutants.
- Groundwater or sediments containing indigenous facultative or obligate IRB.
- Satellite sites (e.g., Abbott's Pit) available to support comparative research.

Site selection began with a thorough analysis of existing information on the local and regional hydrology and geology. A model of deep upwelling groundwater as the source of suboxic groundwater along the flanks of the Delmarva Peninsula (Figures 2a,b; Speiran 1996) was used as a guide. The literature survey was followed by visual inspection for the presence of outcrops or pit exposures and discussion with local agencies regarding institutional ownership and access. More than 20 potential sites were investigated in Northampton and Accomack counties. Based on visual inspection, the number of candidate sites was narrowed to three located at the Cape Charles Air Base, Townsend, and South Oyster (Figure 1). The Townsend and South Oyster Sites are owned by TNC, and the Cape Charles Air Base is occupied and operated by the U.S. Fish and Wildlife Service.

In January 1998, a field investigation was completed at these three sites to verify

whether they met the stated criteria. Holes were advanced by direct-push cone penetrometer test (CPT) technology, using to the degree possible "real-time" probe data for site characterization. CPT holes are small diameter (~2 in.), require no circulation media, and are plugged immediately after the test is completed.

A cluster of CPT holes was made at each location to acquire all the data desired. Field confirmation of suboxic groundwater oxygen levels of <2 mg/L determined whether additional CPTs were conducted at that location. If suboxic conditions were encountered, a second CPT cluster was installed 50 to 100 m away, either on or perpendicular to depositional strike (depending on physical site constraints), to help determine the lateral continuity of the suboxic groundwater. The following parameters were evaluated in samples from each location:

Groundwater

- Dissolved oxygen, pH, salinity, Fe(II), and total Fe and were measured in the field (Griffin).
- Volatile organic carbon samples were collected and measured in the lab to check for potential anthropogenic contamination (DeFlaun).
- Suboxic groundwater samples (sealed in serum bottles) were collected and shipped overnight to a microbiology laboratory to enrich for IRB (Fredrickson and Balkwill).

Sediment

- Sediment samples sealed in tubes were collected for stratigraphic and grain size analyses (Swift).
- Sediment samples sealed in tubes (suboxic locations only) were collected and shipped overnight to a microbiology laboratory to enrich for IRB (Fredrickson and Balkwill).
- Sediment samples sealed in tubes (suboxic locations only) were shipped overnight to a geochemical lab for analysis of total Al, Fe, and Fe(III) in sediment (Onstott).

A brief sampling plan was prepared to outline the sample procedures and protocols to be used in the field. This plan was made available to personnel at the National Wildlife Refuge, located at the old Cape Charles Air Station Site, and TNC on request, and was circulated to all PI's for input before the field work.

Finally, all field-related activities were recorded in detail in field logbooks, including CPT cluster configurations, samples collected (with ID numbers), and results of field analyses.

A brief report was prepared that included results of all field measurements and laboratory analyses of groundwater and sediment samples. It was circulated to PI's at a meeting held in Salt Lake City, January 24-25, 1998. The report identified accessible suboxic groundwater at South Oyster and Cape Charles Air Base, but not at the Townsend Site (Figures 2a,b). Based on the data obtained, the consensus among the PI's was that the South Oyster Site would be the primary site and Cape Charles Air Base the back-up site. The South Oyster Site is located in a large open field just south of the small village of Oyster, Virginia (Figure 3).

1.2 Detailed Site Characterization

Task Leader and Collaborators

Griffin, Onstott, Swift, Majer, Balkwill, and Scheibe

Goals

- To determine *in situ* geological heterogeneity across the site; to assess hydrogeological units.

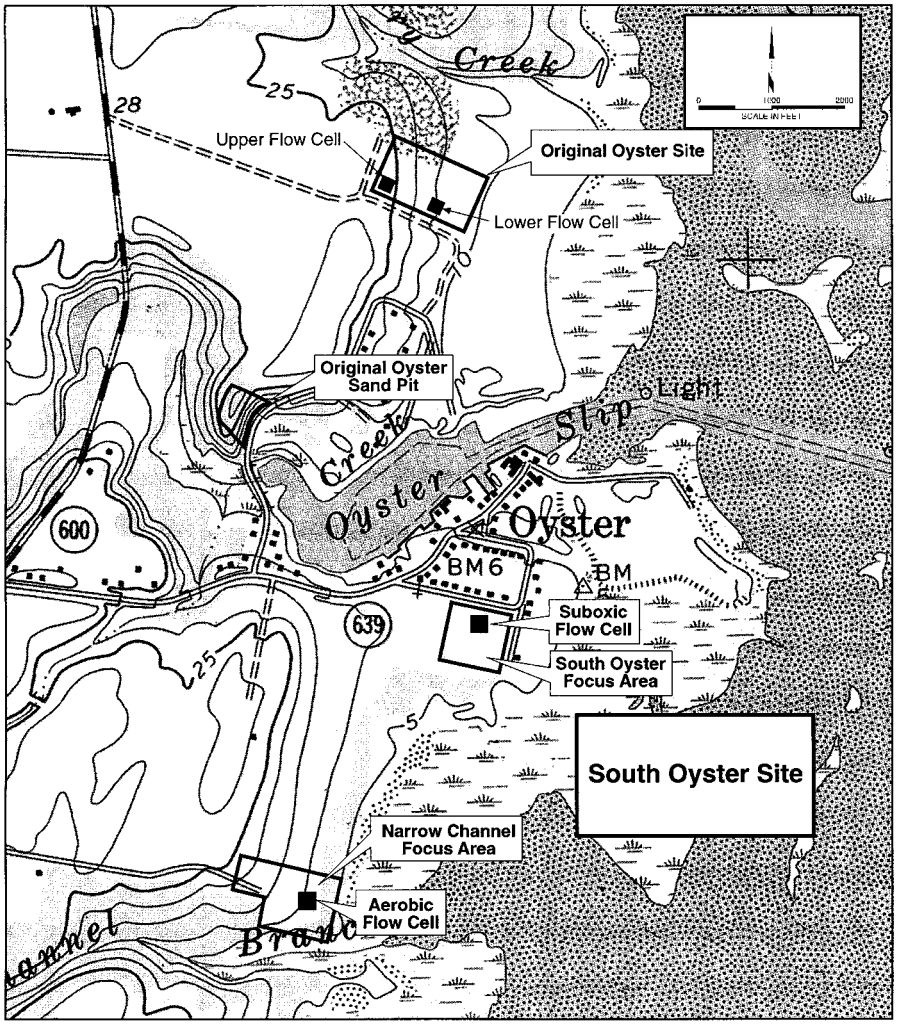

Figure 3. *South Oyster Field Site and Locations of Suboxic and Aerobic Flow Cells*

- To identify any hidden, man-made structures in the subsurface.
- To locate any saline groundwater intrusions.
- To establish direction of groundwater flow.
- To locate the boundary between oxic and suboxic groundwater.

Approach

For purposes of the detailed site characterization, the site was partitioned into three focus areas: 1) South Oyster Focus Area in the northeastern portion of the field near the town limits, 2) Narrow Channel Focus Area on the southern flank of the field adjacent to Narrow Channel Branch, and 3) the north-south trending Wheatfield Focus Area located between the first two focus areas.

Geologically, all three focus areas are located in the Wachapreague Formation, which overlaps from the east onto the flank of the Mappsburg Scarp. The South Oyster Focus Area is situated farther down the flank of the scarp, while the Narrow Channel Focus Area (SOFA) to the south is well up the scarp flank. The Wheatfield Focus Area is oriented north-northeast along the scarp flank, parallel to deposition strike. Based on results from the initial field reconnaissance, the detailed site characterization efforts were focused on the South Oyster Focus Area, where suboxic groundwater conditions had been identified, and on the Narrow Channel Focus Area, where aerobic groundwater had been identified.

To address the first three goals, a non-intrusive surface geophysical survey was performed with ground-penetrating radar at the site in March 1998. The purpose of the survey was to identify any hidden subsurface structures that would interfere with the natural groundwater flow gradient or chemistry and correlate the stratigraphy between the respective flow cells and any potential excavation point(s) (Majer).

To address the other goals, a limited series of 23 CPT investigations was performed between April 6 and 18, 1998, along the geophysical survey lines. The purpose of the investigations was to establish the boundary between the suboxic and aerobic water, determine the hydrological gradient, and establish a stratigraphic and geophysical correlation. Measurements of depth to water were taken, and a geographic survey was performed to accurately establish surface topography and water table elevations. Sediment and groundwater samples were collected and processed anaerobically in the field. These holes were plugged immediately after coring and tests were completed. Results of the field work were compiled as a report to the PI's and posted on the Princeton University web site in June 1998 (Griffin).

Based on the results of this more detailed site characterization, the suboxic flow cell is located in proximity to sample station SO-3 in the South Oyster Focus Area (Figure 4) while the aerobic flow cell is located near NC-4 in the Narrow Channel Focus Area (Figure 5). Major cation and anion concentrations from representative groundwater samples from these two locations are similar, but the redox-sensitive species are distinct (Table 1). The size of the flow cells is approximately 20 m x 30 m with the long dimension oriented parallel to the groundwater flow direction (east-southeast for both flow cells). The CPT logs and ground-penetrating radar indicate that the sandier portion of the aquifer located between 6 m and 9 m will be the target zone for the injections and monitoring wells.

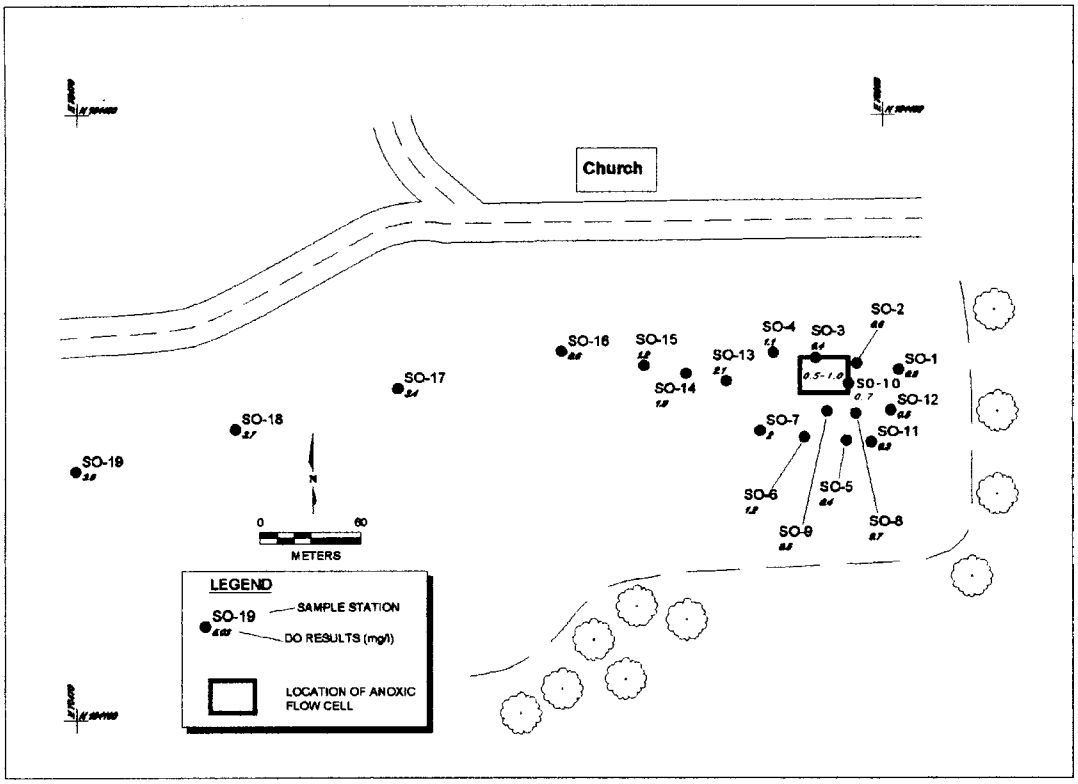

Figure 4. *South Oyster Focus Area Showing Approximate Location of Suboxic Flow Cell (dissolved O_2 in mg/L)*

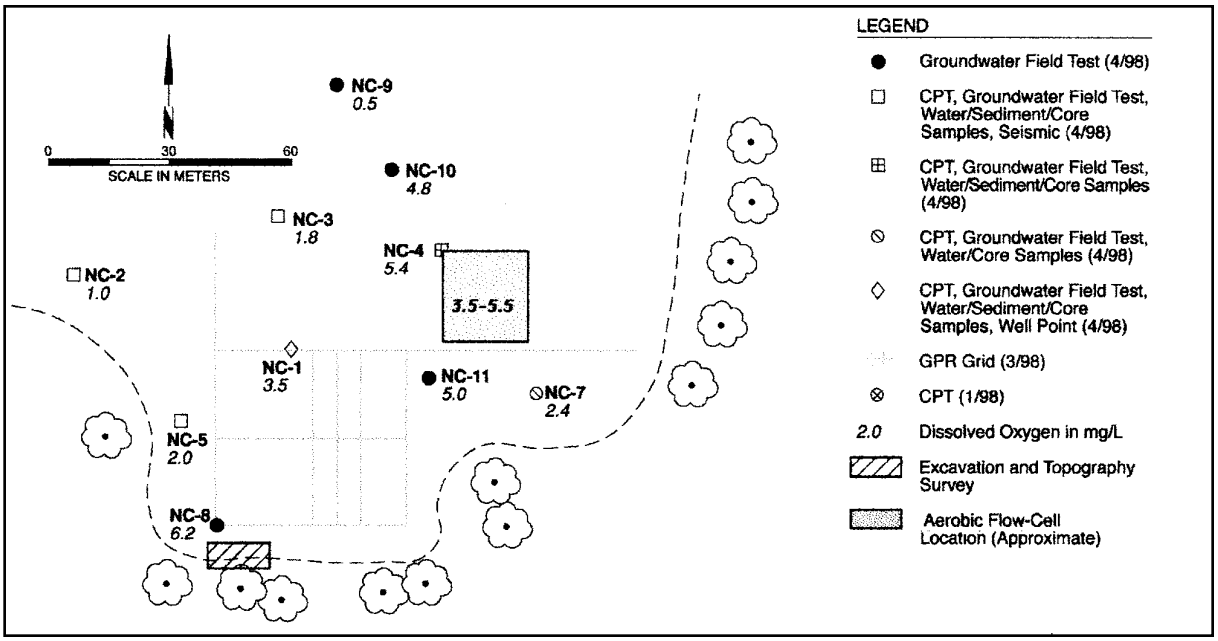

Figure 5. *Narrow Channel Focus Area Showing Approximate Location of Aerobic Flow Cell (dissolved O_2 in mg/L)*

The Nature Conservancy, which owns the South Oyster site, has a number of requirements that must be fulfilled to obtain and maintain a research permit at this site. These requirements include: 1) working with county and state officials and the local Oyster community to educate, inform, and gain public acceptance of the field research to be conducted there; 2) fostering and fulfilling collaborations with other researchers involved at TNC sites; 3) working with TNC to design a field site that will be both physically attractive and educational; 4) keeping the county Office of Planning and Zoning informed of our progress; 5) providing results of our research to TNC; and 6) prior permission from the Virginia Department of Environmental Quality to conduct injection experiments.

The Nature Conservancy also requires a single contact person for each project, which is Dr. DeFlaun's responsibility. Dr. DeFlaun, with the assistance of Drs. Swift and Onstott, have met with a number of county officials as well as with the Water Quality Consortium of Northampton County to inform them of our plans for the site and to elicit their comments. The research application to TNC for a research permit was approved by TNC in September 1988.

A variance to the groundwater quality standards from the Virginia Department of Environmental Quality and a variance for injection into a drinking water supply from the U.S. Environmental Protection Agency (EPA) Region III were approved in August 1998. The NEPA Categorical (CX) Exclusion was approved by the Chicago Operations Office in September 1998. Although these experiments have no impact on groundwater quality in the area, a variance must be granted nonetheless to conduct injection experiments.

1.3 Flow Cell Parameter Integration

Task Leader and Collaborators

Onstott, Swift, Griffin, Long, and Majer

Goals

- To provide a detailed 3D-depiction of the permeability structure of the flow cells.

- To document the physical, chemical, and mineralogical variations in the flow-cell target horizons.
- To calibrate geophysical signals that will be used to map physical and chemical variations in 3D.
- To obtain more refined geological and geophysical surveys of the site to constrain the exact location and orientation for the forced gradient flow cells and MLS's.
- To provide a detailed 3D picture of the permeability structure of the flow cells to constrain the vertical distribution of samplers in the MLS's.

Table 1. Groundwater and Sediment Properties for Narrow Channel Aerobic (NC) and South Oyster Suboxic (SOFA) Flow Cells. Concentration are in parts per billion unless otherwise specified.

Groundwater Parameters	**NC** (6-7 m-bgs) Aerobic Flow Cell	**SO** (6-7 m-bgs) Suboxic Flow Cell
Dissolved O_2 (mg/L)	3.5-5.5	0.4-1.0
Eh (mv)	431 to 437	-25 to 69
pH	5.6-6.1	5.6-5.9
Total organic carbon	1000	10,000
Total inorganic carbon	40,000-55,000	65,000-80,000
Cl	19,400-33,210	45,975-85,300
NO_3	25,180-43,110	5330-15,870
SO_4	22,560-64,100	52,905-69,450
Na	13,300-17,300	13,300-46,500
Mg	3090-6240	3600-10,800
Ca	20,300-29,200	23,400-25,000
Filtered Fe total	100	430-1980
Filtered Fe(II)	0	330-1960
Unfiltered Fe total	20	1440-3720
Sediment Parameters		
Total Fe (ppm)	1892-8847	3731-22,413
Fe(II)/Fe Total	0.02-0.13	0.09-0.73
HCl-Amine extractable Fe	24-442	14-1626
HCl-Amine extractable Mn	3-12	1-78
HCl extractable Al	48-1072	18-653
Porosity (%)	20-45	15-36
Hydraulic conduct. (10^{-5} m/s)	5-28	0.7-4.1
Mean grain size (μ)	151-423	89-304

Approach

At the flow cell sites, characterization is based on intact vertical cores, cross borehole radar, and seismic tomography. The accuracy of these geophysical approaches is optimized when calibrated against the chemical and physical characteristics of the core material.

From the tomographic data and grain size analyses of the cores, the sedimentary bed structure and permeability structure of the flow cell can be estimated. The modeled structure can be refined, however, from observations of bed geometry and permeability variation at the excavation site. This structural basis is pivotal to the development of a new fluid flow/transport modeling approach.

The following analyses on the vertical cores and horizontal cores from the excavation will be required to achieve the stated goals:

- Permeability and seismic velocity measurements (Figure 6; Swift and Majer).
- Grain size and porosity determinations for initial conditioning of permeability variation in hydrological models (Swift).
- Mineralogical characterization and pore size variation by scanning electron microscopy (Onstott).
- Fe, Mn, and Al oxyhydroxide concentrations (Onstott).

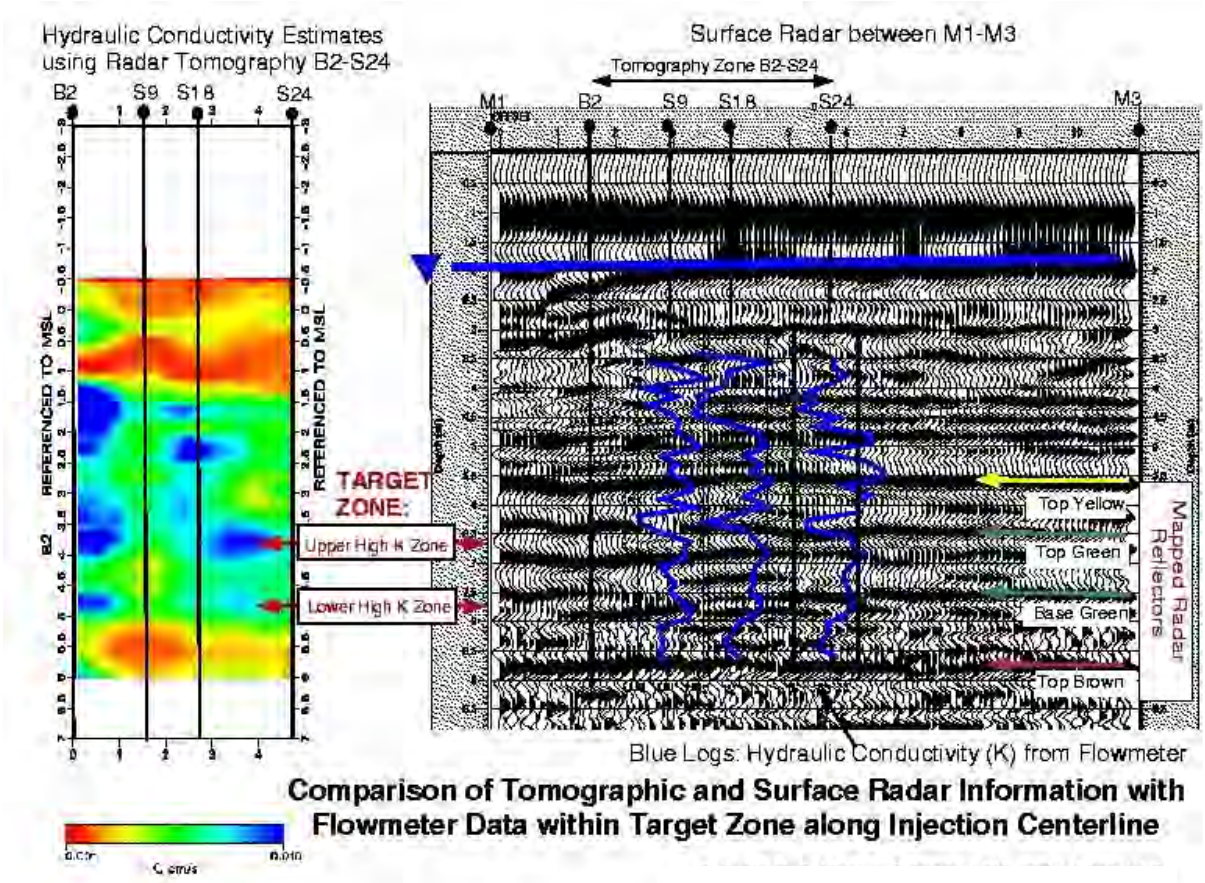

Figure 6. *Cross Borehole Radar and Seismic Tomographic Profiles Along the Axis of the Narrow Channel Flow Cell (from Borehole B2 to NC-S24 in Figure 12). High velocities and attenuation correlate with high hydraulic conductivity.*

- Measurement of bed geometry and orientation and grain size variation within the bedded structures at the excavation site (Swift).

- Infrared, air permeability, and multispectral measurements on vertical cores to establish joint relationship between physical/geochemical heterogeneities (Long, Wilson, and Onstott).

1.4 Excavation Site

Task Leader and Collaborators

Griffin, DeFlaun, Onstott, Swift, Majer, McInerny, Long, and Wilson

Goals

- To determine the 3D spatial geometry of sedimentary beds that correlate with those that constitute the flow-cell target zone.

- To compare physical, chemical, and mineralogical heterogeneities and bed geometry to geophysical tomography data.

- To collect intact cores for bacterial transport experiments, permeability measurements, and microbial analyses.

Approach

A limited excavation was made in the Narrow Channel Focus Area where the water table dips down in elevation close to a stream incision. This permits access to subsurface formations that are present below the water table in the flow cell. A plan for stabilization/reclamation was drafted in cooperation with TNC to minimize visual and environmental impact. An excavation took place in August 1998 along the east-west scarp on the north side of Narrow Channel Branch (Figure 7) and was approximately 20 m long x 1 m deep. This task involved the following activities:

- Draft of field sampling plan circulated to the PI's. Annual, DOE-sponsored meetings (including external reviewers) with PI's were also used to formulate a field sampling plan. A formal draft was circulated to all PI's before the date of excavation (Griffin).

- Detailed ground-penetrating radar and cross borehole tomography were used to characterize the subsurface at the excavation site prior to excavating (Majer and Griffin).

Figure 7. *Photographic IR Imaging of the Excavation in the Narrow Channel Focus Area in August 1998. Subsequent to opening and smoothing the face of the excavation, an IR camera was used to obtain high-resolution images in conjunction with air minipermeameter measurements. Research by Dr. Phil Long (in collaboration with Griffin and Swift) has shown that IR response can be correlated with permeability, potentially obviating the need for exhaustive small-scale sampling. His work at the South Oyster Site will further verify this technique as a means of obtaining quantitative representations of the heterogeneous physical and geochemical properties of the subsurface.*

- After excavating down to the water table, mapping and sediment facies descriptions, infrared (IR) digital photography, and mini-air permeability measurements were made across the smoothed surface of the excavation (Figures 7 and 8; Swift, Long, and Wilson).

- Samples were collected from the excavated face for falling head permeability, permeability anisotropy, grain size, porosity, microbial activity, microbial biomass, geochemistry, and mineralogy. Oriented intact cores were collected for bacterial transport and for core manipulation experiments (Figure 9). Bulk sediment samples were collected for microbial reduction experiments and microbial adhesion assays. All sampling points on the excavation face were surveyed and analyzed to provide an accurate 2D representation of all data (DeFlaun, Swift, Onstott, McInerney, and Griffin).

- Oriented intact cores (Figure 10) were collected from below the water table by trenching down with the back hoe and quickly extracting core before the trench collapsed (DeFlaun, Onstott, and Griffin).

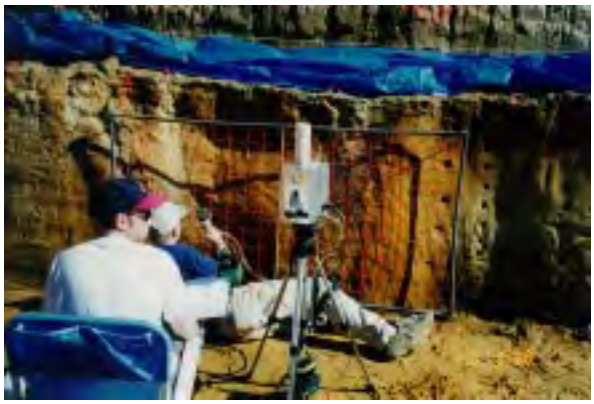

Figure 8. *Collection of Air Mini-Permeametry Data at the Excavation Site in the Narrow Channel Focus Area in August 1998. Mini-air permeametry is a technique developed by Dr. John Wilson at the New Mexico Institute of Mining and Technology to measure the permeability of geological formations in the field.*

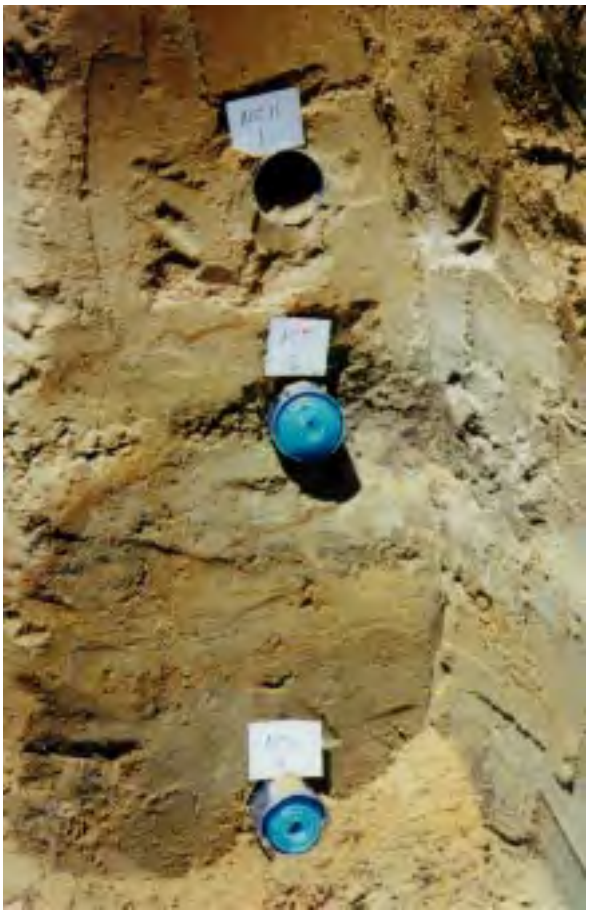

Figure 9. *End View of Three Intact Cores Collected in the Vadose Zone During Excavation of the Narrow Channel Focus Area in August 1998. Vadose zone intact cores were collected from each of three visually distinct facies in the vadose zone during excavation at Narrow Channel. Samples collected during the excavation will be used to characterize each of these facies. The blue foam end pieces were placed into the shelby tube to keep the sediments from slumping as the cores were being driven into the face of the excavation. Each core was driven into the face in the orientation of the local groundwater flow.*

- Finally, the excavation site was buried and reclaimed (Griffin).

Excavation was done in collaboration with another NABIR project ("Heterogeneity of Sedimentary Aquifers: Role in Microbial Dynamics Assessed by Radar Imaging and Acoustic and Radar Tomography"). Principal investigators on this project are Drs. Swift and Majer, who will characterize the pit by

Figure 10. *Collection of Horizontal Intact Cores from Below the Water Table (saturated zone) During the Excavation in the Narrow Channel Focus Area in August 1998. The excavator was used to create a smooth surface below the water table and aluminum shelby tubes were driven into the face of the excavation in the orientation of local groundwater flow. These intact cores will be used for transport studies that will be used to design the field bacterial transport experiments.*

geological and geophysical techniques. Dr. McInerney will conduct the microbial characterization, and Dr. Murray will integrate all these characterization activities with geostatistical techniques. They will share the field work, samples, and data with the investigators in this project.

1.5 Microbial Analyses of Site Materials

Task Leader and Collaborators

Balkwill, DeFlaun, Holben, Fredrickson, White, Dobbs, and Marsh

Goals

- To quantify and characterize the indigenous microbial communities in both groundwater and sediment samples.
- To identify candidate strains for injection.
- To provide a baseline for assessing the impact of transport experiments on the indigenous microbial community.

Approach

The methods outlined below are designed to determine the size and composition of the microbial community and microbial distributions (heterogeneity). Culturable heterotrophs and IRB, and (depending on the needs/requirements of TNC) non-culturable microorganisms will be characterized using the following approaches:

- Phospholipid fatty acid analyses of sediment and water samples to determine biomass and general community structure. PCR-DGGE (Denaturing Gradient Gel Electrophoresis) of PCR-amplified eubacterial rDNA to provide a diversity fingerprint will be performed on the same samples as the phospholipid fatty acids (White).
- Viable counts (plate counts) on several media will be used to enumerate culturable aerobes and facultative heterotrophs and to provide a source of isolates for screening (Balkwill).
- Enrichment of sediment and groundwater samples for IRB, including facultative IRB reducers, will be conducted. Isolation of cultures from positive enrichments and physiological characterization to assess utilization of electron donors/acceptors (Balkwill and Fredrickson).
- Colony morphology and diversity analyses (done on plates used for viable counts) will be undertaken to estimate the number of distinct culturable types in each sample and to facilitate comparison of samples from different depths, boreholes, and time points e.g., before and after transport experiments (Balkwill and DeFlaun).
- Representative colony types will be isolated and deposited in the DOE Subsurface Microbial Culture Collection (Balkwill).

- Analysis of 16S ribosomal RNA sequences for selected isolates will be done to provide genus-level identification of numerically predominant culturable forms, if required by TNC (Balkwill and Holben).

- Isolates will be screened for phenotypes of interest for injection. Low adhesion to site sediments, non-pathogenicity, and sensitivity to clinical antibiotics will be the selection criteria (Table 2; DeFlaun and Balkwill).

- Restriction Fragment Length Profile (RFLP) analyses of water samples will be performed to detect the presence of previously characterized Oyster isolates at the new site (Holben and Balkwill).

- Characterization of nonculturable microbial communities will be undertaken by direct extraction of DNA, followed by PCR amplification, PCR-DGGE of eubacterial rDNA to provide a diversity fingerprint on the same samples as the phospholipid fatty acids, cloning, and sequencing of 16S rRNA genes (Balkwill and White).

- Direct counts of protozoans in groundwater and sediments will be made (Dobbs).

- Microbial community phylogenetic analysis by terminal restriction fragment length polymorphisms of PCR-amplified 16S rRNA genes (Marsh).

1.6 Flow Cell Design

Task Leader and Collaborators

Scheibe, Onstott, DeFlaun, Ginn, Griffin, Majer, Murray, and Hallett

Goal

- To design flow cells based on integration of the site characterization data to provide useful information on bacterial transport.

Table 2. Strain Characteristics

Strain	ID	Antibiotic Resistance	HIC/ESIC	Electrophoretic (10^{-8} $m^2V^{-1}s^{-1}$)	Zeta Potential	Size	Lowest % Adhesion	Recent % Adhesion
PL2W31	*Arthrobacter* sp.	Nal 50	Weakly hydrophilic/ high negative	-1.82 ± 0.04		0.9 x 0.9 µm	55%	99%
Mach 1	*Arthrobacter* sp.	Nal 50	Strongly hydrophilic/ negative	-1.16 ± 0.02		1.5 x 0.5 µm	20%	20%
OYS50	*Erwinia herbicola*	Sensitive	Weakly hydrophilic/ weak negative	-0.45 ± 0.05	0-2	1.8 x 0.5 µm	64%	64%
OYS2-A	*Erwinia herbicola*	Sensitive	Strongly hydrophilic/ neutral	-0.37 ± 0.04	0-2	1.6 x 0.5 µm	14%	54%
DA001	*Comamonas* sp.	Rif 50	Strongly hydrophilic/ neutral	-0.45 ± 0.01	0-2	1.2 x 0.6 µm	0%	11%
DA001-R	*Comamonas* sp.	Rif 150	Strongly hydrophilic				17%	35%
B2-4	*Comamonas* sp.	Rif 50	Very weak/negative	-1.60 ± 0.08	0-2		95%	95%
FER-02	*Paenibacillus polymyxa*	Sensitive	Very weak hydrophilic/neutral				93%	99%

(a) Electrophoretic mobility was measured in Narrow Channel artificial groundwater of ionic strength 0.0034 M.

Approach

The South Oyster Site will include both suboxic and aerobic flow cells. The flow cells will be circumscribed by nine injection/extraction wells in a 20-m x 30-m grid (Figure 11). Six to eight additional wells will be installed in the center of the flow cells for borehole tomography, along with a series of approximately 25 MLS's. In addition, four monitoring wells will be positioned around the MLS's to monitor any release of bacteria or tracer beyond the sampling array (Figure 12). Such a release would require remedial action designed to confine the bacterial or tracer plume to within the flow cell.

A baseline groundwater characterization, monitoring, and contingency plan will be prepared and implemented to ensure that pre-existing groundwater quality at the site is maintained in the vicinity of the flow cells.

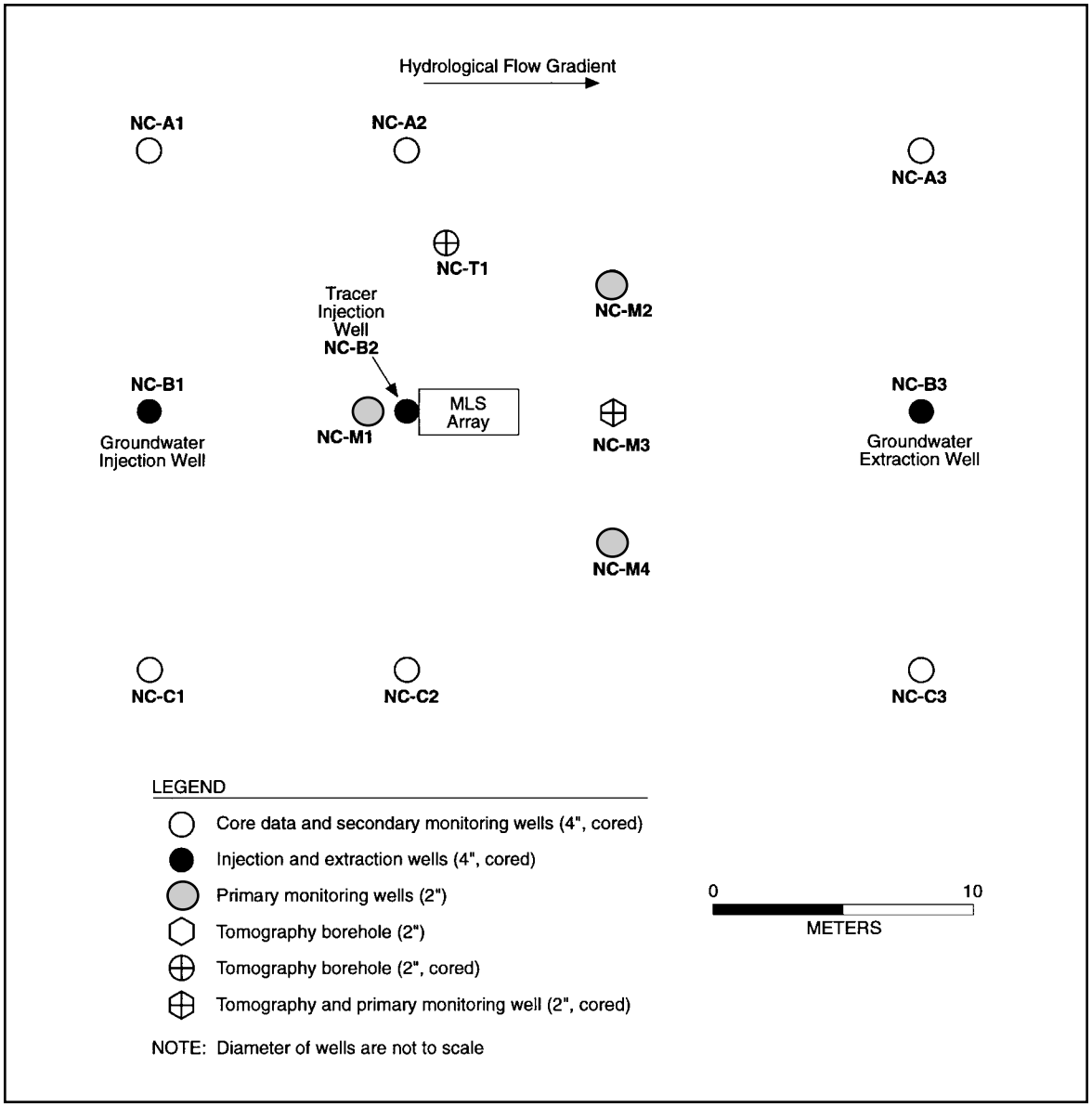

Figure 11. *Flow Cell Configuration for Narrow Channel and South Oyster Focus*

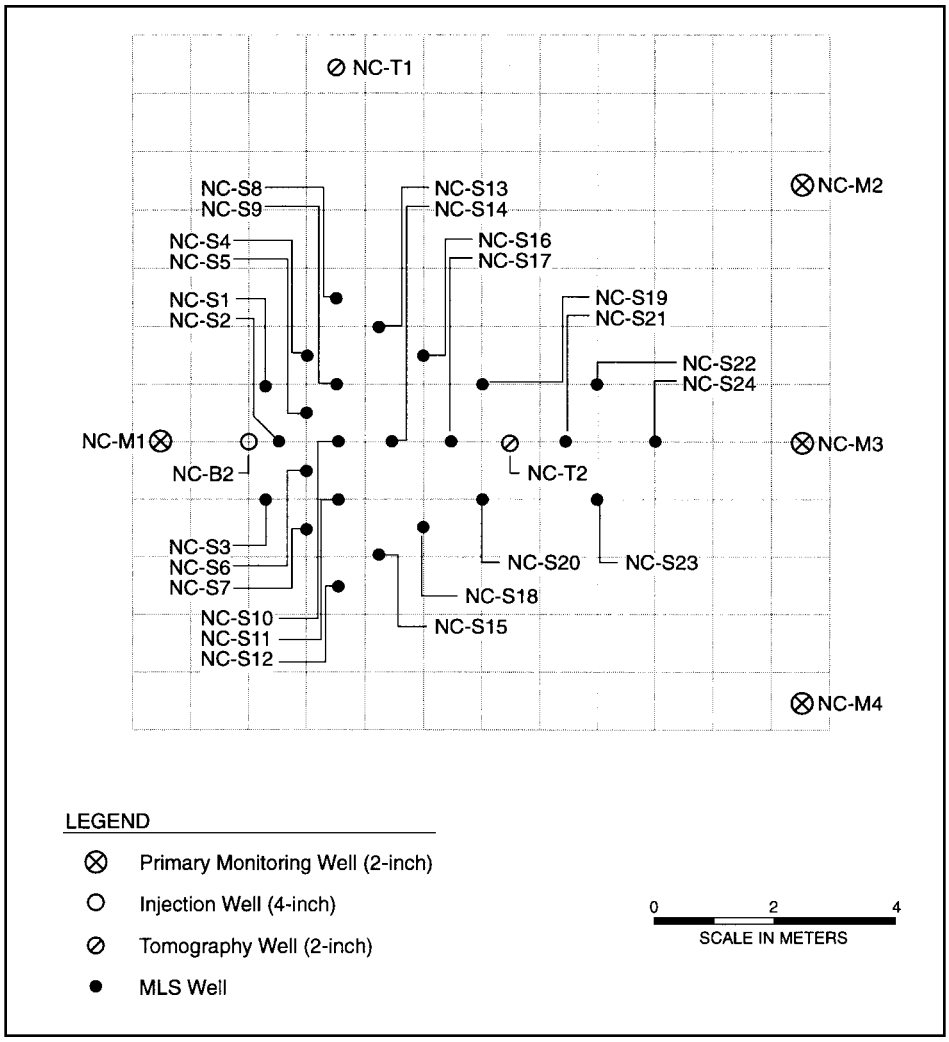

Figure 12. *Multi-level Sample Array for Narrow Channel Flow Cell. Groundwater monitoring system consists of tranduces placed in NC-B1, M2, and M4 and a probe for monitoring dissolved O_2, pH, conductivity, and temperature in M3.*

This task involves the following steps:

- Install the injection/extraction wells and tomography boreholes and collect cores from boreholes and wells (Griffin).
- Collect groundwater for geochemical and microbial analyses (Onstott, DeFlaun, and Griffin).
- Collect bulk groundwater for laboratory bacterial transport experiments (DeFlaun and Holben).
- Perform down-hole seismic and radar tomography on between six to eight tomography holes (Majer).
- Perform pump/slug tests and analyze field data to determine vertical variation in hydraulic conductivity using a flow meter; perform water level measurements over a period of days to test for tidal influences (Griffin and Hallett).
- Perform geological description and IR imaging of split cores on site (Swift and Long).

- Collect samples from split cores for microbial and geochemical analyses and for falling head permeability measurements (Swift, DeFlaun, Holben, White, Balkwill, and Onstott).

- Archive remaining cores, and perform grain size analyses (Swift).

- Correlate geophysical tomography data with grain size analyses, falling head permeability, flow meter measurements, and geochemical analyses (Figure 6; Majer, Swift, Onstott, and Griffin).

- Develop a preliminary 3D model of the flow cell using geophysical, grain size, pump test, flow meter, and permeability data (Scheibe, Murray, and Griffin).

- Determine the optimum horizontal distribution of MLS's within the flow cells based on this 3D model and on bacterial transport intact core experiments (Scheibe and Ginn).

1.7 Design, Construction, and Installation of Multi-Level Samplers

Task Leader and Collaborators

Griffin, Onstott, Phelps, and Scheibe

Goal

- To design, install, and field test semi-automated sample collectors for aerobic and suboxic flow cells.

Approach

Multi-level samplers were designed based on data obtained in previous CPT campaigns that allowed us to obtain samples for grain size analysis and estimate the conductivity of the aquifer with depth. The MLS's will have 10-cm-long samplers with a 0.01-in. slot size. These samples will be distributed across an interval from 6 to 9 mbls. The MLS's will be connected to the surface by a 3/8-in. OD reinforced PVC tubing attached to a central PVC rod for stability. Peristaltic pumps will be used to obtain samples from various depths.

The flow cell for the Narrow Channel Focus Area was installed during Fall 1998, and the flow cell for the South Oyster Focus Area was installed during Spring 1999. These activities entailed the following:

- Research, design, and construction of samplers and shipment to site (Phelps and Griffin).

- Installation of samplers into cased well with sampler depths established using the geophysical data (Griffin, Hallett, and Phelps).

- Accurately surveying and measuring elevation of MLS's and depths to samplers (Griffin and Hallett).

1.8 Scaling-Up From Laboratory to Field

Task Leader and Collaborator

Scheibe and Long

Goal

- To develop and use parametric models generated at the core and field scales to determine whether an understanding of heterogeneity at the core scale can be used to predict field-scale transport.

Parametric models of laboratory-scale bacterial transport studies will be developed using intact cores that yield cellular attachment/detachment parameters for sub-facies-scale physical and chemical heterogeneity. These parameterizations can be combined with facies-scale characterization of physical heterogeneity to develop quantitative models

for field-scale bacterial transport. Establishing the relationship between model accuracy/precision and the level of characterization detail will be one aim of the field experiment.

Approach

The approach will be to assess the research value of intact core experiments according to the following hypotheses:

- The use of intact core experiments to test bacterial transport enables the design of field-scale bacterial transport experiments.
- Intact core experiments test the veracity of bacterial tracking methodologies.
- Intact core experiments provide a test bed for developing deployment methodologies to facilitate transport.

Intact core experiments relating the amount of bacterial breakthrough to the concentration and duration of inoculation and velocity of the groundwater will lay the groundwork for a successful field experiment. New deployment strategies, such as development of the vibrational approach of Dr. Phelps or the co-injection of humics, may improve the dispersion of bacteria.

Research using intact cores will test whether centimeter-sized physical and chemical heterogeneity control bacterial transport at the meter scale. The diameter of these cores (7.3 cm) captures considerable variation in the sedimentary fabric across a distance of 50 cm. Because this scale is directly comparable to the field experiment, the core experiments provide a direct test of scaling up. Larger diameter cores can be collected if necessary.

Cores from the intact core experiments will be included in the IR and multispectral imaging experiments of Long/Scheibe/Wilson to establish the relationship between physical and geochemical heterogeneity and to include this information in appropriate scale-up models (Long, Onstott, and Wilson).

2.0 Tracking Bacteria in Porous Media

Expected Result

- Development of new field methods for tracking indigenous subsurface organisms that are candidates for *in situ* bioremediation by means of bioaugmentation.

2.1 Detection Strategies, New Methods Development, and Bacterial Survival

Task Leader and Collaborators

Holben, Onstott, White, Johnson, and Fuller

Goal

- To develop and implement appropriate, sensitive, and reliable methods to track injected bacteria in field and laboratory transport experiments. These methods will be used to determine transport as well as survival of injected strains.

Approach

- Further develop the ^{13}C stable isotope tracking method of Holben et al. (in preparation) to determine stability of label in the injected cells and for streamlining the method for quick and efficient analysis of a large number of samples (Holben).

- Establish dual stable-isotope labeling capabilities for co-injection and unambiguous detection and monitoring of different strains. This incorporates statistical tools to determine precision and detection limits for labeled strains in natural groundwater (Holben, Onstott, White, and Fuller).

- Assess the suitability of specific isotopes and growth substrates for isotopic enrichment of bacteria to maximize specific activity (hence sensitivity) and minimize label turnover, exchange, or other compromising label factors (Fuller).

- Develop and optimize isotopic enrichment strategies for the candidate strains (DeFlaun, Holben, and Fuller).

- Develop a more specific labeling strategy for the injected bacteria that uses gas chromatography-chemical reaction interface mass spectrometry (GC-CRIMS), gas-chromatography-isotope ratio mass spectrometry (GC-IRMS), and high-performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS).

  By this method, ^{13}C-labeled and ^{15}N-labeled cellular components, such as fatty acids, proteins, or nucleic cells are extracted, purified, and separated via GC and converted in the chemical reaction interface to oxidized products that then are measured in the mass spectrometer.

  By comparing the isotopic enrichment of specific cellular components of the target cells before injection with those of downgradient samples, the presence and quantity of the target cells in the post-injection samples can be determined. The major advantage of this approach is that the

^{13}C-labeled and ^{15}N-labeled cellular components establish a "signature" for the target cells. Variations in this signature indicate changes in the target cells themselves, cell death, or incorporation of labeled cellular materials by other microbes, all of which would allow for adjustments to be made in the calculated number of injected microorganisms (Onstott, White, and Fuller).

- Assess the use of newly developed fluorescent dyes for tracking organisms in the subsurface. These dyes may allow cells to be stained without loss of activity, viability, or changes in transport properties. Some newer dyes specifically stain cell membranes while others cross the membrane and covalently bond to intracellular proteins. In either case, some dyes have been shown to be retained in cells for up to 3 to 4 weeks, without loss of cell viability or alterations in cell function or adhesion.

  These stains will be evaluated for their applicability for use in the bacterial transport studies. Their longevity in cells, retention of cell viability, and retention of adhesion/transport properties will all be tested in the laboratory before being transitioned to the field. Adhesion properties will be tested in the sand column adhesion assay, and transport properties will be tested in intact cores. Equally important is their lack of toxicity and suitability for field use (Fuller).

- Explore and develop alternative molecular detection strategies, such as quantitative PCR, that may provide additional and independent monitoring capabilities in support of field and laboratory transport experiments (Holben).

2.2 Development of Fermentation, Storage, and Transportation Protocols

Task Leader and Collaborators

DeFlaun, Holben, and Griffin

Goal

- To develop protocols for growth, labeling, storage, and transportation for organisms selected for injection that will result in viable, labeled cells on arrival at the field site.

Approach

The bacterial strain selected for injection will be grown in a manner that maximizes the amount of the label to be used in tracking the organism in the field. Protocols will then be developed to deliver the maximum number of viable organisms to the site.

3.0 Processes Controlling Bacterial Transport in Porous Media

Expected Results

- Quantification of the relative impacts of physical and chemical heterogeneity on microbial transport.
- Evaluation of the incremental value of characterization data in terms of the predictive ability of the resulting model.
- Preliminary understanding of the potential impacts of grazing by protozoa.
- Determination of how core-scale bacterial transport experiments can be scaled-up to field-scale transport experiments.
- Quantification of the effect of aerobic bacterial adhesion on transport through aquifer sediments.

3.1 Intact Core Bacterial Transport Studies

Task Leader and Collaborators

DeFlaun, Onstott, Ginn, Scheibe, Holben, Long, and Dobbs

Goals

- To determine kinetic adsorption/desorption parameters for bacterial strains in intact cores from the South Oyster excavation to constrain the horizontal spacing of the MLS's for the new flow cells.
- To determine the effect of grain size, mineralogy, texture, and porosity on bacterial transport.
- To quantify protozoan bactivory of injected bacteria in South Oyster intact cores.

Approach

The activities for intact core experiments are as follow:

- Radiolabeled bacteria are introduced from the bottom of the core, with water flow in the upward direction. A pressure transducer measures the pressure difference between the influent and effluent ends. A conservative tracer (chloride or Br) is injected to assess water flow dynamics. The cores are run in an environmental chamber at 15°C, corresponding to the average groundwater temperature of the South Oyster Site. Water flow rates vary from 0.5 m to 2 m/day, bracketing the range of the *in situ* groundwater velocities measured at the South Oyster Site during the forced gradient transport experiments (Figure 13). Bacterial breakthrough is monitored by measuring both plate counts and the radiolabel in the effluent fractions, which are collected at 20-min intervals throughout the experiment (DeFlaun, Onstott, and Holben).
- For protozoan bactivory studies, both bacteria and cultured protozoan grazers will be injected simultaneously. A time

Figure 13. *Bacterial Transport Experiments are Performed Using Intact Cores Collected in Shelby Tubes from the Excavation and Groundwater Collected from the Flow Cell Site. Experiments are run in a cold room maintained at 15°C, the ambient groundwater temperature at the site. Groundwater and radiolabeled bacteria enter the core from the bottom and move upward. Effluent is collected with fraction collectors and the radioactivity measured with a liquid scintillation counter. At the end of the experiment, the core is split, the sediment subsampled and thin sectioned to determine distribution of adsorbed bacteria.*

course of population dynamics for both predator and prey will be determined. Bacteria will not be radiolabeled in these studies. Protozoan population dynamics will be assessed by direct epifluorescence techniques. Bactivory will be directly assessed by enumerating fluorescent bacteria in protozoan vacuoles (Dobbs).

- At the completion of the experiments, the cores are split and the distribution of attached bacteria measured on the 2D face with phosphorimage screens and in 3D with liquid scintillation counting of the subsampled core (Onstott and DeFlaun).

- For selected cores, IR and multispectral imaging will be performed on the non-epoxied half of the core (Long, DeFlaun, and Onstott).

3.2 Modeling Bacterial Transport in Intact Cores

Task Leader and Collaborators

Scheibe, Ginn, and Onstott

Goal

- To derive kinetics of bacterial adsorption/desorption for incorporation into a predictive field-scale transport model.

Approach

- Data from breakthrough curves and the cells retained by the intact cores will be simulated by a 1D advection/dispersion model (Onstott, Ginn, and Scheibe).

- The kinetic adsorption/desorption terms derived from multiple core experiments will be used to develop distribution functions of these parameters for each facies and/or empirical functions relating these parameters to physical, chemical, and mineralogical properties (Onstott, Ginn, Scheibe, Swift, and Holben).

- The kinetic adsorption/desorption terms for intact core experiments will be compared with kinetic parameters derived from intermediate-scale experiments containing representations of the facies chemical and physical heterogeneities (Murphy, Ginn, and Scheibe).

- These kinetic parameter distributions or empirical functions will be used to condition the kinetic parameters for the 3D model by stochastic analyses (see Task 3.5; Scheibe and Majer).

- Inject conservative tracer and reactive tracers into select intact cores and monitor breakthrough curves. Split core, subsample, and determine concentration of adsorbed reactive tracer. Determine distribution of reactive tracer with respect to the Fe, Mn, and Al oxyhydroxides (Smith and Onstott).

- Develop 1D advective/dispersion model for reactive tracers. Explore 2D and 3D models if reactive tracer concentrations in core are heterogenous. Use these data and models to select an appropriate reactive tracer for field injection that will be accepted by TNC and regulatory agencies (Smith).

3.3 Conservative/Reactive Tracer Transport Experiments in Aerobic/Suboxic Flow Fields

Task Leader and Collaborators

Scheibe, Onstott, and Smith

Goals

- To assess the effects of physical/chemical heterogeneity on the movement of water and reactive constituents within the flow field.

- To establish the relationships between heterogeneity in permeability and heterogeneity in biogeochemical reactivity.

Approach

- Inject a conservative tracer (e.g., Br^-) at different depths, and monitor tracer breakthrough in multilevel samplers to track groundwater flow across facies boundaries (Griffin and Scheibe).

- Model conservative tracer experiments conditioned by measured permeability in intact cores, and the orientation and size of sedimentological structures (Figure 14). Measurements obtained by conducting intermediate-scale (tens of centimeters) permeability measurements (slug tests) at different intervals in the injection borehole are used to obtain conditioning data for the physical model (Griffin Scheibe, Swift, and Majer).

- Conduct pump tests and inject reactive tracers (e.g., F^-, Sr^{2+} or others) simultaneously with a conservative tracer at different depths and monitor tracer breakthrough in multilevel samplers to track reactive transport across facies boundaries. Model predictions will be compared to field observations to evaluate the predictability of field-scale advection transport (Griffin, Smith, and Scheibe).

- Model reactive tracer experiments conditioned by the measured spatial distributions of Fe and Al oxyhydroxides and the results of the conservative tracer experiments to establish the integrated biogeochemical reactivity along flow paths. Based on the results of the tracer experiments, select vertical intervals and times for sampling during the bacterial injection experiment (see Task 3.4; Smith, Scheibe, and Onstott).

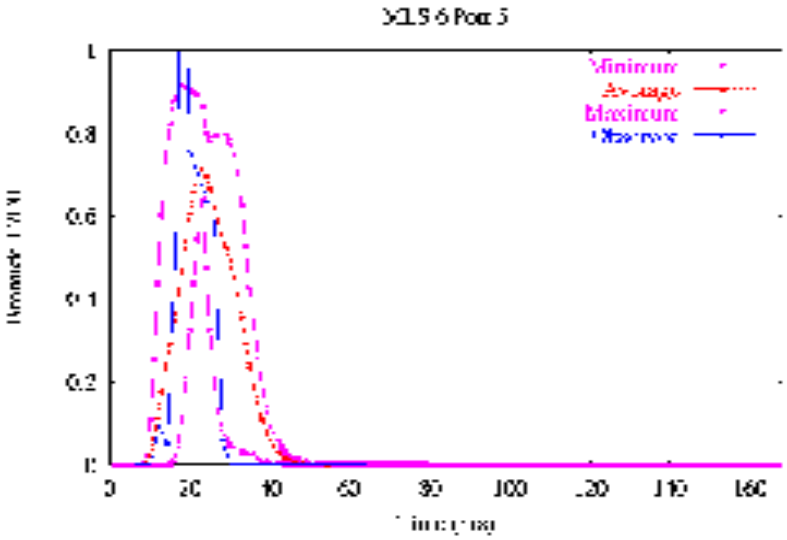

Figure 14. *Comparison of Br Breakthrough at One of the MLS's During a Tracer Injection Experiment at Narrow Channel. The minimum, average, and maximum curves represent the range of predicted Br breakthroughs from a 3D stochastic model of the Narrow Channel flow cell.*

3.4 Field Injection Experiments Planned for the Narrow Channel Aerobic Flow Cell

Task Leader and Collaborators

DeFlaun, Griffin, Onstott, Swift, Majer, Holben, Balkwill, Dobbs, Ginn, Scheibe, Johnson, and White

Goals

- To determine the effect of grain size and hydraulic conductivity distribution and the spatial distribution and type (composition and crystallinity) of Fe, Mn, and Al oxyhydroxide phases on bacterial adhesion and transport.

- To understand the role of microbial adhesion to Fe, Mn, and Al oxyhydroxide surfaces on bacterial transport.

- To understand the influence of Fe(II) adsorption to Fe, Mn, and Al oxyhydroxide and cell surfaces on microbial adhesion.

- To develop rapid and sensitive methods for quantifying bacteria introduced to the subsurface in groundwater and sediment samples.

- To develop efficient scaling-up methodologies that yield accurate predictions while economizing on data requirements.

- To assess the importance of bacterial predation on the reduction of microbial populations introduced as part of bioaugmentation strategies.

The Narrow Channel Focus Area offers significant opportunities for addressing these objectives, because 1) it contains sediments that have a range of Fe(III), Al and to a lesser extent Mn oxyhydroxide contents, grain size and hydraulic conductivity distributions; 2) the groundwater is aerobic and has a pH 5.5 to 6, low DOC, and low ionic strength; and 3) anaerobic groundwater with elevated DOC and Fe(II) is available nearby at the South Oyster Focus Area (see Table 1 in Section 1.2).

Approach

A series of field injection experiments have been planned that collectively will contribute to the scientific goals identified in this task (see Table A.1).

- The first experiment at the Narrow Channel Focus Area will involve injection of an adhesion-deficient, aerobic, hydrophilic, bacterium with neutral surface charge (Comamonas DA001 in Table 2). Multiple bacterial tracking tools[7] will be used to quantify the transport of the microorganism in the field (see Section 2). The resiliency, the detection limit, the rapidity of analyses, and the economics of implementation for each tracking technique will be compared in this experiment to determine which subset of techniques will be used in future field injections. The variations in the grain size and hydraulic conductivity distribution at this site will provide insight into the influence of these properties on bacterial transport relative to that of a conservative solute tracer, Br.

  This experiment will also be the first evaluation of model predictions of field transport based on results of core-scale bacterial transport experiments complemented by field imaging of physical heterogeneity using radar and seismic tomography. The number and type of indigenous protozoa will be determined before, during and after the injection to ascertain the potential impact of bactivory on the breakthrough profiles of the introduced microorganism. If protozoan predators are present, a subsequent field experiment (see below) will be designed to ascertain the rates at which the protist communities respond to the injected microorganisms.

- The second injection at the Narrow Channel Focus Area will include the simultaneous injection of strain DA001 and a negatively charged, facultative IRB, that preferentially adheres to Fe-oxyhydroxides (see Table 2), into the aerobic flow cell. Multiple tracking tools will be applied to delineate the transport behavior of the two injected bacterial strains.

  The specific methods for labeling and independently tracking each bacterial strain will be selected on the basis of their performance during the first field injection experiment. This experiment is also designed to determine the relative importance of the spatial distribution and type (composition and crystallinity) of Fe, Mn, and Al oxyhydroxide phases versus the sediment grain size and hydraulic conductivity distribution on bacterial transport.

  Depending on the results of intact core experiments with the facultative IRB, additional measures may be explored to facilitate the transport of the IRB. These measures include, for example, increasing the pH of the injectate water to 8 by equilibrating groundwater with air, reducing the ionic strength by dilution with distilled water, or co-injection with colloids filtered and concentrated from the groundwater. The type and number of indigenous protozoan communities will be monitored before, during, and after the injection to further assess the potential for bactivory.

- The third injection experiment at the Narrow Channel Focus Area will be designed to evaluate coupled bioaugmentation and biostimulation approaches for potential bioremediation strategies. Specifically, a facultative IRB enriched from SOFA will be injected into the aerobic flow cell. The oxidation potential of a portion of the flow cell will then be progressively lowered by injecting a narrow plume of suboxic groundwater from the South Oyster Focus Area.

(7) The tracking tools will include IRMS, GC-IRMS, and HPLC-ESI-MS analyses of ^{13}C labelled bacterial cells, plate reader or cytometer counts of CFDA-stained cells, quantitative PCR, plate counts and ferrographic separation immunoassay.

By reducing the injection rate relative to the extraction rate, the dispersion of the plume will be less than that of the bacterial injection. A portion of the flow cell, therefore, will remain aerobic as a control. The transport of the facultative IRB, the concentration of DOC, Fe(II), pH and trace metals will be monitored as the oxidation potential declines. The high concentration of DOC (i.e., carboxylic acid portion) will act as an additional electron donor promoting activity and growth of the indigenous bacterial populations. Growth of the IRB may enhance its transport.

These experiments will also determine if the DOC (i.e., humic acids portion) adsorbed to the positively charged Fe and Al oxyhydroxides enhances the desorption and transport of the IRB. The concentration of Fe(II) will be monitored during these manipulations as a proxy for IRB activity. Select trace metals will be monitored to determine if they are being immobilized as precipitates by the biogenically produced Fe(II). Since growth of the indigenous bacterial populations may stimulate growth of the protozoan community feeding upon them, predation rates may increase for the injected IRB as well. Consequently, the numbers of protozoa will be monitored to determine if their increase corresponds to any decreased in injected IRB.

- The fourth injection experiment at the Narrow Channel Focus Area will entail coinjection of the facultative IRB and anionic complexes. If the results of previous injections indicate that the adsorption of the facultative IRB to Fe-oxyhydroxides strongly inhibits the bacterium's transport, then alternative means may be required to reduce the adsorption of the IRB.

  Strongly binding anionic complexes compete with negatively charged bacteria for the positively charged sites on the surfaces of the Fe and Al oxyhydroxides. Injection of such a complex prior to and during the injection of the facultative IRB strain into the aerobic flow cell should reduce the adsorption of IRB.

  Hypothetically, the anionic complex should have little effect on the transport of neutrally charged DA001. Coinjection of DA001 and the facultative IRB with the anionic complex should enhance the transport of the IRB relative to that of DA001 and Br. Na-pyrophosphate may represent one candidate complex, but because is solublizes Fe(III) phases, its impact on sediment mineralogy has to be evaluated.

  Any anionic complex will be tested in intact core experiments prior to proposing their use at the Oyster site to the VaDEQ. The bacterial injection will be followed by injection of suboxic South Oyster groundwater to lower the oxidation potential into the Fe(III) reduction regime. The total IRB activity will be determined to assess the net affect of adding the anionic complexes to the system. As before, a portion of the flow cell will be kept aerobic as a control.

- The fifth injection experiment at the Narrow Channel Focus Area will use the observations on protozoan populations during the previous injections and a sequential injection strategy for DA001 that will test models of bactivory. This experiment will monitor how rapidly the indigenous protozoan population responds to the introduction of DA001 and how quickly the population diminishes after the departure of DA001.

  The rise and decline of the protozoan population during the subsequent injection will be compared to the first injection to determine the optimal frequency of bacterial injections. These results should also assess the impact of the protozoan bactivory on the cost versus potential benefit arising from multiple injections.

- The sixth injection experiment at the Narrow Channel Focus Area will examine the importance of bacterial size in enhancing bacterial transport. The higher velocity of DA001 with respect to Br observed in the intact core experiments is hypothesized to be caused by the preferential exclusion of DA001 from fine-grained zones within the sedimentary fabric. As this exclusion is a size-dependent phenomena, decreasing the size of DA001 should reduce its average velocity. Colloid filtration theory also predicts that the amount of adsorption is size dependent. DA001 will be injected simultaneously with a dwarf strain of DA001 into the aerobic flow cell to monitor their relative velocities and amounts of adsorption.

3.5 Integration of Field and Laboratory Data into a High-Resolution 3D Numerical Simulation of Field Data

Task Leader and Collaborators

Scheibe, DeFlaun, Onstott, Ginn, Holben, Majer, Swift, and Murray

Goals

- To construct a high-resolution 3D stochastic numerical model, representing bacterial transport in the experimental flow cells, based on integrated laboratory- and field-scale data.
- To use the model system in conjunction with the field experimental observations to evaluate the effects of field-scale physical and chemical heterogeneity and in particular the existence of preferential flow paths on field-scale bacterial transport.
- To test the predictability of field-scale bacterial transport based on laboratory-scale parameterization of attachment/detachment models combined with facies-scale characterization of physical heterogeneity (scaling-up).
- To provide a quantitative framework to support interpretation of field experiments and for testing detailed hypotheses.

Approach

A 3D numerical framework representing non-reactive (Br) tracer transport and simulation tools has been developed. This framework has been adapted to the South Oyster Site conditions and configuration. Laboratory results from intact core experiments have been used to condition biogeochemical parameterization of the aquifer model and to expand its predictive capabilities to bacterial (reactive) and non-reactive tracer transport.

Specific tasks are as follow:

- Use the model framework to assist in experimental design of the MLS array (Scheibe, Majer, and Deflaun).
- Following emplacement of the flow cells and geological and geophysical characterization, update the model to reflect site-specific conditions (Scheibe, Swift, Majer, and Murray).
- Use the updated model to predict Br breakthrough at sampler locations and compare to field observations to validate model performance (Scheibe, DeFlaun, and Onstott).
- Develop a method based on intact core experimental results for assigning bacterial transport properties to grid cells of the numerical model (scaling-up; DeFlaun, Holben, Scheibe, and Onstott).
- Use the numerical model to predict bacterial breakthrough at sampler locations and evaluate experimental hypotheses by comparison with field observations (DeFlaun, Holben, Scheibe, and Onstott).

4.0 Environmental Factors Controlling Bacterial Transport

Expected Results

- Development of new concepts for facilitating bacterial transport in the subsurface.
- Quantification of the effect of bacterial adhesion on transport through aquifer sediments.
- Determination of the influence of reductive dissolution of Fe oxyhydroxides by iron-reducing bacteria (IRB) on bacterial transport in the field.
- Determination of the effect of adsorbed metals on bacterial adhesion and transport.

4.1 Screening for IRB/DIRB

Task Leader and Collaborators

Balkwill, Fredrickson, and White

Goal

- Enrichment, isolation, and characterization of facultative IRB or dissimilatory IRB (DIRB) that can be injected into both the aerobic and suboxic flow cells.

Approach

- Isolate (by enrichment culture) DIRBs for use in field experiments (Fredrickson).
- Screen existing Oyster culture collection and new isolates (from Task 1.5) for IRB using a soft agar indicator medium (Balkwill).
- Screen candidate IRB for the ability to reduce South Oyster sediment Fe and Mn oxyhydroxides (Fredrickson and Zachara).
- Screen existing Oyster collection and new isolates for presence of *Shewanella* strains, using a genus-specific 16S rRNA-based gene probe (Balkwill).
- Identify IRB strains that meet TNC requirements for antibiotic resistance by screening candidate strains for resistance to eight different antibiotics: ampicillin, nalidixic acid, rifampicin, streptomycin, and kanamycin (50 mg/mL); gentamicin and erythromycin (30 mg/mL); and tetracycline (12.5 mg/mL). Those resistant to the clinical antibiotics ampicillin, streptomycin, gentamicin, erythromycin, and tetracycline will be eliminated from further consideration (Balkwill).
- Develop alternative methods to enhance transport including genetic engineering to reduce cell size.[8]

4.2 Characterization of Facultative IRB

Task Leader and Collaborators

DeFlaun, Balkwill, Fredrickson, Zachara, and Fletcher

(8) This task is associated with the South Oyster Site but is not an integral part of the Bacterial Transport project.

Goal

- To identify and select strains with acceptable antibiotic resistance profiles that have reduced adhesion for facilitated transport.

Approach

Adhesion screening and selection for adhesion variants are performed in a sand column assay (DeFlaun et al. 1990). Percent adhesion as determined in this assay is generally high for soil isolates (>90%). This assay selects for strains with <50% adhesion to an Ottawa sand standard. Candidates from this screening are tested further in the same assay with South Oyster Site sediment and selection for a less adhesive variant is performed. This selection involves use of the same sand column assay, except that the variants are selected by repeated passage of the effluent from the column over subsequent columns. This enriches for cells that pass through the column i.e., the non-adhesive variants. The stability of the non-adhesive phenotype is tested by growing strains of interest for more than 100 generations in a non-selective medium and retesting for percent adhesion.

Sand column assays run under both aerobic and suboxic conditions will be used to determine percent adhesion values for these strains. Percent adhesion will be compared in sediments with and without ferrous iron. Adsorption of Fe to cells will be tested as a means of facilitating transport. Other means of facilitating transport, (i.e., humics, colloids) will also be tested.

4.3 Survival of Facultative IRB in South Oyster Sediment Microcosms

Task Leader and Collaborators

Balkwill, Fredrickson, Holben, Matin, and White

Goals

- To determine the longevity of candidate IRB at various population densities in site sediment and groundwater.

- To determine the limit of detection by several methods (viable plate counts, stable isotopes, PCR).

Approach

Microcosm survival studies will be conducted under both aerobic and suboxic conditions and will include studies with DIRB strains. Studies will assess the survival of strains that are candidates for injection at high concentrations under *in situ* conditions in the presence of the indigenous microbial community. These microcosms will also test bacterial tracking methods. PCR-DGGE of eubacterial rDNA will provide a diversity fingerprint on the same samples as the phospholipid fatty acid analysis.

4.4 Bacterial Transport Under Suboxic Conditions

Task Leader and Collaborators

DeFlaun, Onstott, Holben, Ginn, Scheibe, Zachara, and Fredrickson

Goals

- To relate mineralogical and chemical properties of South Oyster sediments (heterogeneity) to transport of IRB.

- To obtain retardation coefficients of IRB in aerobic and microbially reduced South Oyster sediments.

Approach

The primary purpose of these experiments is to obtain adsorption/desorption kinetic parameters for candidate bacteria being

transported through aerobic South Oyster sediment and sediment in which the Fe, Mn oxyhydroxide phases have been partially or quantitatively reduced.

Experiments will be conducted with facultative IRB under both aerobic and suboxic conditions. The Fe, Mn oxyhydroxide phases in South Oyster sediments will be characterized before and after biogenic reduction. The biogenically reduced sediments will then be used to assess the impact of Fe(III) reduction on bacterial transport in repacked sediment columns.

Future studies will include evaluating the impact of biogenic reduction in the presence of natural humic acids on the transport of bacteria in repacked and intact South Oyster sediment cores. Humic acids can facilitate the reduction of Fe, Mn oxyhydroxides by functioning as electron shuttles between the bacterial and oxide surfaces and by complexing Fe(II), preventing its absorption to oxyhydroxides and bacterial cells.

Experimental systems and methods include:

- Kontes Chromaflex Chromatography columns (4.8 cm x 15 cm) are filled with 410 gm of air-dried, pre-homogenized Oyster sediment. The columns are run in an upflow manner with groundwater pumped through with a peristaltic pump. Flow rates analogous to those in the intact cores and in previous field experiments will be used (0.5 m to 2.0 m/day). These experiments will be performed at average South Oyster groundwater temperature (15°C).

- Groundwater will be pumped through the columns for at least 24 h before proceeding with conservative tracer tests. The conservative tracer will be used to determine the exact groundwater flow rate through the column.

- Radiolabeled bacteria will be injected into the influent flow and collected at the effluent in a fraction collector. The number of cells in the effluent will be determined by scintillation counting.

4.5 Evaluation of Cell Surface Characteristics

Task Leader and Collaborators

Fletcher, DeFlaun, Onstott, and Holben

Goal

- To determine physiological conditions that modify surface properties related to bacterial adhesiveness.

Approach

- Relative adhesiveness of test strains will be established by simple *in vitro* adhesion assays (DeFlaun).

- Relative surface hydrophobicity and surface charge of test strains will be determined by hydrophobic interaction chromatography and electrostatic interaction chromatography, respectively. Any relationships between adhesiveness and surface property evaluations will be identified (DeFlaun).

- Changes in adhesiveness and relative surface properties that alter relevant physiological responses will be assessed. These include differences in strains that a) have been equilibrated to aerobic or suboxic conditions or b) have been prepared for stable isotope incorporation and according to the injection protocol (and are therefore adapted to low nutrient conditions) and then are subjected to higher nutrient levels, stimulating metabolic activity (DeFlaun, Holben, and Fletcher).

- Compare surface charge of candidate aerobic and facultative IRB strains to the surface charge of fine-grained diagenetic Fe, Mn, and Al oxyhydroxides that are present in the South Oyster sediments. This will be performed with Zetameter streaming potential measurements and capillary electrophoresis using South Oyster groundwater. The data will be used to calculate the double-layer thickness for reversible absorption to oxyhydroxides versus quartz (Onstott, DeFlaun, and Fletcher).

4.6 Role of Humics in Bacterial Transport

Task Leader and Collaborator

McCarthy and Fuller

Goals

- Determine if humics alter the retention of bacteria to mineral surfaces through effects on surface potentials or blocking of sorption sites.

- Evaluate the redox behavior of humics and their potential effects on bacterial transport through humic-enhanced reduction of mineral oxides.

Approach

- Humics in groundwater at South Oyster will be characterized with respect to their chemical composition to evaluate differences in organic matter between the aerobic and microaerophilic conditions.

- The composition of the South Oyster humics will be compared with that of the NABIR reference humics to facilitate transfer of information from investigators working with the reference humics to observations at South Oyster.

- The transport and retention of humics in South Oyster sediments will be measured in batch and column experiments to evaluate the extent of humic interactions with sediments and the potential of using humics to facilitate bacterial transport. Measurements of streaming potential or electrophoretic mobility will be used to determine the effect of humics on surface charge of the sediments.

- Effects of humics on bacterial transport will be evaluated first by screening humic-coated sediments in repacked column adhesion assays and then by core-scale experiments similar to those described in Task 3.1. Results will relate the extent of humic retention and transport to the mobility of bacteria.

- The redox behavior of humics will be evaluated in batch potentiostat experiments to evaluate the reduction capacity of humics under different Eh potentials. These data will provide an initial indication of humic-bacteria-iron oxide interactions under varying redox conditions during experiments with IRBs.

4.7 Intact Core Studies with Facultative IRB

Task Leader and Collaborators

DeFlaun, Onstott, Holben, Zachara, Ginn, Scheibe, and Fredrickson

Goal

- To compare transport of the same strain of facultative IRB in intact cores collected from the aerobic and suboxic flow cells under controlled laboratory conditions.

Approach

- Intact cores will be collected in both the aerobic and suboxic sites at South Oyster during construction of the flow cells. Care will be taken to preserve the redox conditions in the cores during collection and transport to the laboratory (DeFlaun, Onstott, Holben, and Griffin).

- A strain of facultative IRB will be used in transport experiments in these cores to compare transport in the aerobic and suboxic sediments with the same microorganism. These experiments will be performed under conditions that most closely mimic field conditions (DeFlaun, Onstott, and Holben).

- Intact core experiments will be performed as described in Task 3.1. Groundwater from the two flow cells will be used in these experiments. The intact core from the suboxic flow cell will be kept at low redox values throughout the experiments (DeFlaun, Onstott, and Holben).

4.8 Field Injection Experiments Planned for the South Oyster Suboxic Flow Cell

Task Leader and Collaborators

DeFlaun, Griffin, Onstott, Swift, Majer, Holben, Balkwill, Dobbs, Ginn, Scheibe, Johnson, and White

Goals

- To determine the effect of grain size and hydraulic conductivity distribution and the amount and type of Fe, Mn, and Al oxyhydroxide on bacterial transport.

- To understand the role of IRB adhesion to Fe, Mn, and Al oxyhydroxide surfaces on IRB transport and activity.

- To determine the effect of IRB activity and bacterially produced Fe(II) on the mobilization and precipitation of trace metals.

- To determine the effect of bacterially produced Fe(II) on IRB transport.

- To adapt and refine the bacterial tracking tools for suboxic environments.

- To assess the importance of bactivory in suboxic environments and designing effective bioaugmentation strategies that minimize the detrimental impact of bactivory.

The South Oyster Focus Area (SOFA) offers an excellent opportunity for fulfilling these objectives, because 1) it contains Fe-oxyhydroxide coated sediments of varying Fe content, grain size and hydraulic conductivity distribution; 2) the Fe(II)/Fe(III) of the sediments is variable within the flow cell; 3) the groundwater has varying, but low O_2 concentrations and oxidation potentials; 4) the groundwater has a subneutral pH, high DOC, and low ionic strength (see Table 1 in Section 1.2); and 5) the concentrations of certain trace metals, Ni, Co, and Zn, are correlated with Fe(II), whereas those of other trace metals, e.g., Sn, are inversely correlated with Fe(II).

Approach

A series of field injection experiments have been identified that collectively will contribute to these scientific goals (see Table A.1).

- The first field injection at SOFA will employ an adhesion deficient, facultative IRB that will be co-injected with Br into the suboxic flow cell under suboxic conditions. This bacterial injection experiment will provide the first field test of multiple bacterial tracking tools under suboxic conditions. It will also be the first field injection of the IRB and will seek to verify numerical model predictions based

upon core-scale, suboxic bacterial transport experiments in conjunction with field-scale physical heterogeneity determined by radar and seismic tomography. Intact core experiments performed using SOFA sediments and the candidate IRB may predict that detectable breakthrough of the IRB at the MLS's of the flow cell cannot be achieved without employing additional measures. These measures include increasing pH to 8 by equilibrating groundwater with air, reducing the ionic strength by dilution with distilled water, or co-injection with colloids filtered and concentrated from the groundwater. The intact core experiments will be used to evaluate which of these measures will be implemented in the field. The number and type of indigenous protozoa will be measured before, during, and after the injection to assess, for the first time, the field rate of bactivory in suboxic conditions.

- During the second field injection at SOFA the facultative IRB will be co-injected with DA001. As DA001 is most sensitive to physical heterogeneity (i.e., grain size and hydraulic conductivity distribution), this injection is designed to determine the relative importance of spatial distribution and type (composition and crystallinity) of Fe, Mn, and Al oxyhydroxide phases versus the sediment grain size and hydraulic conductivity distribution on IRB transport.

  This experiment will also be compared to the results of a similar experiment performed at the Narrow Channel flow cell where the chemical and physical heterogeneity of the Narrow Channel sediments differ somewhat from those of SOFA (see Table 1). The number and type of indigenous protozoa will be quantified before, during and after the injection to further constrain the field rate of bactivory.

- The third experiment at SOFA will be designed to simulate potential bioremediation approaches that combine bioaugmentation with biostimulation strategies. The facultative IRB strain used in the previous field experiments will be injected into the suboxic flow cell. The more oxic portions of the SOFA flow cell will be targeted with an injection of groundwater with the highest DOC from the flow cell to lower the oxidation potential of the targeted zone.

  The portions of the flow cell untouched by the low O_2, low Eh plume will be monitored as controls. The transport of the facultative IRB, the concentration of DOC, Fe(II), pH and trace metals will be monitored as the oxidation potential declines. These experiments will determine if the DOC enhances IRB activity. The concentration of Fe(II) will be monitored during these manipulations as a proxy for IRB activity. The high concentration of DOC (i.e., carboxylic acid portion) will also promote growth of the indigenous bacterial populations. Growth of the IRB may enhance its transport.

  Select trace metals will be monitored to determine if they are being immobilized as precipitates by the biogenically produced Fe(II). These experiments will also determine if the DOC (i.e., humic acids portion) adsorbed to the positively charged Fe and Al oxyhydroxides enhances the desorption and transport of the IRB. Since growth of the indigenous bacterial populations may stimulate growth of the protozoan community feeding upon them, predation rates may increase for the injected IRB as well. Consequently, the number and type of protozoa will be monitored to determine if their increase corresponds to any decrease in the injected IRB.

- The fourth experiment at SOFA is designed to investigate the effect of enhanced IRB transport on total IRB activity. If the results of previous injections indicate that the adsorption of the facultative IRB to Fe, Mn and Al oxyhydroxides under suboxic conditions strongly inhibits the broad dissemination of the IRB throughout the flow cell, then alternative means may be required to reduce the adsorption of the IRB. Strongly binding anionic complexes compete with negatively charged bacteria for the positively charged sites on the surfaces of the Fe, Mn, and Al oxyhydroxides. Injection of such a complex (see Section 3.4) before and during the injection of the facultative IRB strain into the aerobic flow cell should reduce the adsorption of IRB. Hypothetically, the anionic complex will have little effect on the transport of neutrally charged DA001. Coinjection of DA001 and the facultative IRB with the anionic complex should enhance the transport of the IRB relative to that of DA001 and Br. The effect of the anionic complex on net IRB activity (Fe[III] reduction) will also be monitored.

- The fifth injection experiment at SOFA will use the observations on protozoan populations during the previous injections, the experiments performed at Narrow Channel and a sequential injection strategy for the IRB to determine whether the conceptual models of bactivory in oxic settings apply to suboxic environments. This experiment will determine how rapidly the indigenous protozoan population responds to the introduction of the IRB and how quickly the population diminishes after the departure of the IRB.

  The rise and decline of the protozoan population during the subsequent injection will be compared to that of the first injection. A frequency for bacterial injections will be derived which minimizes the impact of bactivory and enhances IRB total activity. The rates of bactivory derived from this experiment will be used to assess the impact of protozoan bactivory on the cost versus potential benefit arising from multiple injections.

- The sixth injection experiment at SOFA will examine how the relative size of IRB to the pore size affects IRB transport and activity. Petrographic observations have revealed that the Fe, Mn, and Al oxyhydroxides are concentrated in the finer grained portions of the sediment. If these fine-grained layers are physically less accessible to the normal-size, facultative IRB than to a dwarf strain of IRB, and the natural humic acids are not acting as electron shuttles, then the total IRB activity may be greater following the injection of dwarf cell IRB. To determine whether size is important, the facultative IRB and the dwarf strain will be coinjected and the relative rates of migration monitored. IRB activity will be evaluated by measurements of Fe(II) in the groundwater and related to the distribution of the facultative IRB and dwarf IRB adsorbed to the sediment.

4.9 Intermediate Flow Cell Experiments

Task Leader and Collaborators

Murphy, Ginn, Scheibe, and Zachara

Goals

- To determine the effect of geochemical heterogeneity on bacterial transport.

- Intermediate-scale experiments will be used to test specific theories for scaling

up process-based modules. The stochastic convective reaction model works well with nonlinear reactions that are characteristic of biological systems. The ISE provides a complete data set to rigorously test this theory before applying it to a less-controlled field environment.

Approach

IRB cause dynamic concentrations of aqueous Fe(II). This bioreaction may produce transient transport events to occur in the IRB population through adsorption of the Fe(II) with IRB extracellular polysaccharides or membrane components (e.g., polar lipids) and/or reaction of Fe(II) with mineralogical components of the solid-phase. These processes may facilitate or retard IRB transport in porous media in the short term. In the long term, IRB activity should decrease the Fe(III) mineral content and enhance IRB transport. To test these hypotheses in an intermediate-scale experiment, information is needed to characterize the reactions of Fe(II) with IRB and mineralogical surfaces:

- How does Fe(II) reaction with IRB change the surface charge properties of the cells? One experimental approach may be to monitor electrophoretic mobility of IRB with increasing concentrations of Fe(II).
- What are the transport properties (attachment/detachment kinetics) of IRB in selected mineralogies when surface sites on the IRB are saturated with Fe(II) compared to IRB where Fe(II) is absent?
- What are the transport properties (attachment/detachment kinetics) of IRB when the Al and Fe(III) oxyhydroxide contain adsorbed Fe(II)?

Pacific Northwest National Laboratory has established an intermediate-scale flow cell facility for collaborative research at EMSL. This facility is being used to perform experiments in support of this and other NABIR projects.

- Prior to the design of the intermediate-scale flow cell experiment for this project, a column packed with a homogeneous mineralogy of quartz, Al oxyhydroxide, or Fe(III) oxyhydroxide-coated sand will be used to monitor the breakthrough of an IRB pulse under different Fe(II) treatments.
- An IRB strain either from the Oyster site or an existing strain of IRB (e.g., *Shewanella putrifaciens* CN32) will be chosen for these column experiments and the experiments completed.
- The effect of Fe(II) on electrophoretic mobility of the IRB will be determined to examine the mechanisms responsible for the behavior observed in the columns.
- Kinetic parameters from these column experiments will then be used to design the intermediate-scale flow cell and refine the experimental hypothesis.
- Intermediate-scale flow cell transport experiments will also be used to test the effect of spatial heterogeneity on bacterial transport and the veracity of the intact core experiments.

Affiliated NABIR Task: Spatial Heterogeneity of Microbial Fe Reduction Potential

Task Leader and Collaborators

Murray, Roden, Swift, Majer, Hubbard, Gorby, and Brockman

Goal

- Develop the ability to predict microbial Fe reduction potential (MIRP) at unsampled locations using geostatistical methods that integrate the spatial model for MIRP and geological/geophysical information on the distribution of sedimentary facies.

Approach

- Identify a batch measurement that can be used as a proxy to characterize the spatial distribution of *in situ* MIRP.

- Employ geostatistical methods to characterize the spatial heterogeneity of MIRP and relate it to the spatial distribution of environmental properties.

- Use a combination of batch and intact core microcosm incubations to develop an inexpensive measure to assess the potential for MIRP in sediments. The incubations would be performed using a medium consisting of electron donors, inorganic nutrients, vitamins, and trace minerals designed to selectively promote microbial Fe reduction. The batch measurements will be performed on samples from vertical boreholes at the Oyster or Abbott Site.

  Geostatistical techniques will then be used to characterize the spatial heterogeneity of the MIRP and its relationship to sedimentary facies. Assuming that a relationship exists, geological and geophysical data on facies distributions and the spatial heterogeneity models will be used to predict the distribution of MIRP at unsampled locations.

Affiliated NABIR Task: Vibration-Accelerated Transport of Microbes in Subsurface Media

Task Leader

Phelps

Goal

Develop and demonstrate a conceptual framework for vibration-facilitated microbial transport through subsurface porous media by examining the effects of vibrational energies on particulate and microbial transport using intact core columns and by field testing hypotheses for accelerated transport in porous media.

Approach

- Examine effects of vibration energies on particulate and microbial transport using intact sediment cores recovered from the Eastern Shore.

- Determine processes and variables controlling vibration-facilitated microbial transport in laboratory-based column studies.

- Identify the impacts of frequency, power, sediment structure, and vibration duration on transport processes using intact cores.

- Using similar sites, organisms, and procedures to those used by the South Oyster Field Experimentation Program, conduct field applicability test of vibration-accelerated microbial transport.

5.0 Technology Transfer Opportunities

Goal

- To promote transfer of new concepts, information, and research tools to DOE groups responsible for site remediation and to industry.

Approach

It is anticipated that new, field-relevant information, research methods, and novel technologies will emerge from research at the South Oyster Site. Experiments at the site are being conducted under aerobic and suboxic conditions that are relevant to the environments commonly associated with contaminated groundwater. Spinoffs from these experiments will benefit remedial action programs at contaminated sites under DOE stewardship.

Intrinsic bioremediation offers a viable option at a number of DOE sites, but *in situ* microbial populations at some locations are limited in numbers, especially in thick vadose zones and deep aquifers in the western United States. Under these conditions, bioaugmentation will be needed to accelerate *in situ* bioremediation activity by increasing microbial numbers.

Even at sites where microbial numbers are generally elevated, it is known that microbial spatial distribution (microbial heterogeneity) in the subsurface is controlled by natural hydrogeologic variations such that injection of bacteria may be needed to ensure that isolated zones of contamination, including poorly permeable regions and pockets of "dead end" pore space can be treated. Given national concern regarding the release of genetically engineered bacteria to the environment, the reintroduction of strains obtained from indigenous microbial communities may be an economically and environmentally viable approach. This conceptual approach underpins the field experiments being conducted at the South Oyster Site.

5.1 Seek Opportunities for Research Transfer

The most significant opportunities for technology transfer are likely to emerge following analysis of results from field campaigns. As research proceeds, and especially during the conduct of annual investigator "stock-taking meetings," progress will be assessed with the goal of identifying research results that may contribute to ongoing and anticipated DOE remediation efforts.

Idaho National Engineering and Environmental Laboratory-Test Area North (INEEL-TAN) represents a potential opportunity for transfer of research results (Smith and Colwell 1998). At INEEL-TAN, intrinsic bioremediation is targeted for a zone of sewage-metal-TCE contamination, with the goal of reducing cleanup costs in a fractured bedrock aquifer. The Record of Decision permits the use of new and emerging research approaches at the site. INEEL and University of Idaho scientists are seeking to exploit *in situ* microbial communities that may prove to be insufficient in distribution, numbers, and/or activity to remediate the contaminant plume. Another opportunity exists at the Hanford Site, where injection of indigenous, iron-reducing microorganisms and a simple electron donor might serve as a cost-effective, less disruptive,

and safer alternative to chemical injection, which has been shown to modify redox conditions and attenuate the movement of chromium in an initially aerobic aquifer. The applicability of this microbially based approach is being explored as a means of attentuating chromium, uranium, and technetium mobility at the Hanford 100-H Area where these elements are impinging on salmon redds in the Columbia River.

Opportunities for research transfer also exist at other sites at which intrinsic bioremediation is planned in zones with complex geology and where a need may exist to supplement indigenous populations with bacterial amendments.

5.1.1 Facilitate General Site Remediation Problem Solving

Field-tested methods and technologies with broad applications to remediation across the DOE site complex potentially include the following:

- Field protocols for core and groundwater sampling and analysis under aerobic and suboxic conditions, which also retain the microbiological integrity of samples.
- Core experimentation and modeling approaches for scaling mechanistic information, such as microbial attachment/detachment, to the field.
- A suite of bacterial markers to tag and track bacteria, and for examination of *in situ* microbial ecology, such as changes in community structure and dynamics that result from manipulation.
- Field-tested, low-cost multi-level samplers for mid- to long-term groundwater monitoring.
- Three-dimensional models for design and analysis of field injection experiments.

5.1.2 Complete Transfers Relevant to Bioaugmentation at DOE Sites

At DOE sites where bioaugmentation is anticipated, examples of research transfer from field research experiments at South Oyster may include any or all of the following:

- Innovative protocols for identifying adhesion-resistant strains from among *in situ* populations at laboratory (core) and field scales.
- High throughput methods for screening and culturing indigenous bacterial variants for bioaugmentation via aquifer injection.
- Combined (tracer, bacterial marker) approaches for tracking cells in the subsurface and monitoring locations and rates of movement within contaminated zones.
- Predictive, field-scale bacterial transport models that address natural physical and geochemical heterogeneity and are applicable across a range of sedimentological and geochemical regimes.

The South Oyster Site also provides an opportunity to study the microbial ecology of a natural redox gradient in which N and Fe cycling occurs under suboxic conditions. As a result, this site could provide field validation of laboratory studies and source materials for the "Biogeochemical Dynamics Element" and other elements of the NABIR program.

5.1.2 Complete Transfers Relevant to *In Situ* Remediation Technologies

Remedial action programs at DOE and other sites include establishing, maintaining, and/or enhancing the longevity of in-ground, permeable biotic or abiotic (e.g., zero-valence

Fe) barriers to contaminant migration. Spinoffs may lead to new or improved barrier technology that may be useful at DOE sites. Opportunities include the following:

- Field-tested methods for conducting forced-gradient injections under aerobic and suboxic groundwater conditions.
- New discrete-interval tracer injection technologies to predict bacterial, and possibly chemical, dispersion within aquifers and for monitoring effectiveness of installed permeable barriers.
- New evaluation methods that predict the adhesion of metal-reducing bacteria to Fe mineral surfaces—information useful to predict the potential for transport of these organisms through Fe oxide-containing sediments, the microbial reductive dissolution of Fe oxide-contaminant coprecipitates, and the microbial reduction of structural Fe for contaminant attenuation.

5.2 Design and Test Methods for Information Transfer

A future mechanism of technology transfer is likely to be through the NABIR Field Research Centers at DOE sites where bioaugmentation may be a necessary part of *in situ* remediation. As the research proceeds at the South Oyster site, results will also be transferred as informational briefs to all DOE sites. Other transfer vehicles that have been shown to be suitable include onsite workshops and training of DOE site personnel at the South Oyster Site itself.

5.3 Public/Community Outreach Program

Insights into public concerns about bioremediation may be gained as a result of interactions with local environmental groups, state and county officials, other local organizations, and the citizens of coastal Virginia. These insights may range from determining the public acceptance of *in situ* bioremediation as a cleanup tool to improved methods for effective communication of field research activities.

This experience will be useful to those involved in public outreach at DOE sites. Although no formal program of public participation or outreach is planned, any experience that is generated at the South Oyster site will be transferred to NABIR's BASIC Program.

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Appendix A

Table A.1. Calendar of Field Experiments at Oyster Site

Dates	Location	Experiment
1999	Narrow Channel	First field injection of DA001.
2000	South Oyster	First field injection of facultative IRB.
2000	Narrow Channel	Dual injection of DA001 and facultative IRB.
2001	South Oyster	Dual injection of DA001 and facultative IRB.
2001	Narrow Channel	Co-injection of facultative IRB and high DOC SOFA water.
2002	South Oyster	Co-injection of facultative IRB and high DOC SOFA water.
2002	Narrow Channel	Co-injection of facultative IRB, anionic complexes and high DOC SOFA water.
2003	South Oyster	Co-injection of facultative IRB, anionic complexes and high DOC SOFA water.
2003	Narrow Channel	Sequential injections of DA001 to test bactivory rates.
2004	South Oyster	Sequential injections of the facultative IRB to test bactivory rates.
2004	Narrow Channel	Dual injection of DA001 and a dwarf cell.
2005	South Oyster	Dual injection of facultative IRB and a dwarf cell