BREAKTHROUGHS IN FIELD-SCALE BACTERIAL TRANSPORT
from: EOS, TRANSACTIONS, AMERICAN GEOPHYSICAL UNION, 82(38), September 18, 2001.

The site characterization for this project was done by prof. Rubin's group, and is described in detail in:

Hydrogeological characterization of the South Oyster bacterial transport site using geophysical data, by Susan Hubbard, Jinsong Chen, John Peterson, Ernest Majer, Kenneth Williams, Donald Swift, Brian Mailloux and Yoram Rubin, Water Resources Research, 37(10), 2431-2456, 2001

1. Introduction

Microbial transport in the subsurface environment has been of interest for decades due to concerns about contamination of water supplies by pathogenic bacteria or viruses. More recently, research has demonstrated that many bacteria can beneficially serve to degrade or immobilize other environmental contaminants. A research program sponsored by the U.S. Department of Energy is currently investigating the potential for enhancing and targeting bacterial transport to improve bioremediation efforts. This article summarizes a bioaugmentation research project undertaken by a multidisciplinary research team at a field site near Oyster, Virginia.

Bioaugmentation is a process in which naturally occurring microbes are added to the subsurface to augment the existing biomass for the purpose of improving the remediation efficiency of the system. Bioremediation in general, and bioaugmentation in particular, are considered viable techniques; however, microbial transport in the presence of natural hydrogeological and geochemical heterogeneities is not well understood.� Although the hydrogeological heterogeneity of subsurface sediments has been recognized as a key control on microbial transport at the field-scale, no study prior to this effort acquired a dense enough characterization data set in tandem with a field-scale bacterial transport experiment to enable investigation of the extent of the control. Of particular interest to the Department of Energy is the potential for bioremediation of metals and radionuclides that are common to many of their facility sites. Although bacteria cannot degrade metals and radionuclides, bacteria can either mobilize or immobilize these contaminants, facilitating remediation.� The iron-rich Atlantic coastal plain sediments at the uncontaminated study site near Oyster, Virginia, provide an unique opportunity to study enhanced and targeted bacterial transport under both oxic and suboxic conditions.� Because iron is precipitated and immobilized under the same conditions as uranium, this study site serves as an excellent surrogate for uranium-contaminated sites.

The overall purpose of the project is to evaluate the relative importance of hydrogeological and geochemical heterogeneities in controlling bacterial transport and to develop an approach for quantitative prediction of bacterial transport needed to design optimal bioremediation strategies. Our experiments were designed to investigate the influences of heterogeneities under varying degrees of complexity at two study sites (Focus Areas) within the Oyster Site.� Field-scale bacterial transport experiments have been performed at the oxic, relatively homogenous Narrow Channel (NC) Focus Area, and the anoxic, heterogeneous South Oyster Focus Area (SOFA).� Only the NC results will be discussed in this article.

Research at the NC Focus Area included multi-scale hydrogeological characterization, numerical modeling of bacterial transport, developing protocols for bacterial strain selection, designing and performing a field-scale tracer test, and developing novel methods for tracking bacterial transport.

2. Hydrogeological and Geochemical Characterization

Both hydrogeological and geochemical variations are expected to influence bacterial transport. In groundwater, the rate and extent of bacterial movement is controlled by pore water velocity and bacterial

attachment to and detachment from grain surfaces.◆◆ Attachment and detachment are commonly modeled as first-order kinetic processes.◆ The attachment rate constant can be represented using filtration theory as a product of two probabilities: the probability of the bacteria colliding with the sediment grains as they approach (collector efficiency), and the probability of bacterial attachment to the grains upon collision (collision efficiency).◆ Both the pore water velocity and collector efficiency are strongly influenced by grain size and porosity.◆ For loosely consolidated granular aquifers such as those present in the Oyster subsurface, these physical parameters often correlate with hydraulic conductivity.◆ Non-physical factors that influence bacterial transport are incorporated into the collision efficiency, which is associated with cell and grain surface properties including the sum of the electrostatic and van der Waals forces between the approaching bacteria and the sediment grain.◆ As bacteria and quartz-dominated sediments typically display an overall negative surface charge, the overall electrostatic repulsion between bacteria and sediment typically yields low collision efficiencies. However, some of the minor mineral phases in sediments such as iron oxides display positive surface charge, which tend to electrostatically attract the moving bacteria and thus yield higher collision efficiencies. Masking of positive surface charges by natural organic matter can lead to further complications.◆ Because bacterial transport mechanisms such as attachment and detachment are sensitive to hydrogeological and geochemical variations and because the variations in these parameters in natural geologic conditions are typically great, interpretation of field-scale bacterial transport data is often challenging.◆ In this study, it was important to characterize these variations with the highest possible spatial resolution in order to understand the influences of hydrogeological and geochemical heterogeneities on field-scale bacterial transport.

At the Oyster Site, conventional field methods for determining hydrogeological properties either lacked sufficient spatial resolution or were too invasive and would have disturbed the natural subsurface bacterial transport environment under investigation. To overcome these obstacles, surface and borehole geophysical methods, coupled with geologic confirmation, offered a means of providing minimally invasive multi-dimensional, high-resolution, hydrogeological characterization information necessary for investigating microbial transport in heterogeneous systems.

2.1 Hydrogeological and Geochemical Setting ◆

The Oyster Site is located on Virginia's Eastern Shore Peninsula (Figure 1).◆ The subsurface at the site consists of unconsolidated to weakly indurated, Late Pleistocene sediments of the Wachapreague Formation that were deposited in a marginal marine environment.◆ Below this formation is the Pliocene Yorktown Formation, an aquitard that serves as the lower boundary for our subsurface study volume.◆ The water table is located at approximately 2 to 3 m below ground surface.◆ The research conducted at the NC Focus Area focused primarily on a zone 3 to 9 m below ground surface (BGS), or 0 to 6 meters below mean sea level.

Characterization of the Oyster Site was performed using data collected across a range of spatial scales. More than 6 km of ground-penetrating radar◆ profiles were collected at subregional and site-specific scales. In addition, radar tomography, seismic tomography, cone penetrometer test profiles, borehole electromagnetic flowmeter measurements and individual core samples were collected. These data, together with hydrogeological, geochemical and microbial heterogeneity data obtained from a 20-m-long trench located◆ in the vadose zone adjacent to the NC Focus Area were used to guide the field operations, to provide constraints for numerical flow modelling, and to assist interpretation of the transport data.

The above measurements were used to infer the general hydrogeology of the two Focus Areas.◆ Both areas lie at the foot of the Mappsburg Scarp (Figure 1), which is a buried beach face that extends almost the entire length of Virginia's Eastern Shore Peninsula.◆ Groundwater close to the scarp, such as that at our NC Focus Area, is of local origin and is well oxygenated.◆ Further seaward and topographically lower from the scarp, the recharged groundwater mixes with an anaerobic component that upwells from a deeper,

confined aquifer.� This mixing produces the suboxic groundwater found in the SOFA aquifer. The NC Focus Area is underlain by well-sorted sand and pebbly sand The geology of the SOFA Focus Area is much more complex with a wider range of sediment grain sizes due to its juxtaposition with a buried lagoon.� The range of groundwater and geological conditions found at the two different Focus Areas is an important aspect of the Oyster Site, as many contaminated aquifers have varied geological and suboxic to anaerobic groundwater conditions.

2.2 Hydrogeological Characterization of the NC Focus Area

Eleven high-resolution seismic and radar tomographic data sets were acquired to provide a more detailed characterization of the NC Focus Area. Inversion of this geophysical data provided high-resolution information about interwell variations in geophysical attributes. The attribute information was then used together with wellbore flowmeter data within a stochastic approach to provide estimates of hydraulic conductivity probability distributions.� Figure 2a shows an example of the estimated means of these hydraulic conductivity distributions along the lower portion of a stratigraphic dip profile. This figure illustrates the horizontal to sub-horizontal hydrogeological units, which are typically <1 m thick and several meters in length. The units possess hydraulic conductivities ranging from 0.001 to 0.01 cm/s

Core samples from the NC Focus Area were subjected to laboratory measurements followed by statistical analysis to provide core-scale granulometric and hydrogeological information. Mean grain size, sorting, skewness and percent of gravel were used to cluster samples into four sediment types. Statistical analyses allowed the sediment types to be reduced to three key hydrofacies. Figure 3 illustrates a comparison of the core-scale hydraulic measurements, granulometric measurements and facies designations with the hydraulic conductivity estimates obtained using tomographic data as described above.� Establishing a link between granulometric and hydrogeological properties measured in the laboratory and the field-scale tomographic information using co-located data such as these enables us to interpret our field-scale estimates of hydraulic conductivity in terms of effective physical properties that are measured at the core scale.

2.3 Geochemical Characterization at the NC Focus Area

In addition to the oxygen content of the NC Focus Area groundwater (4 to 7 ppm), the electrochemical fields of mineral substrates are expected to exert an influence on microbial behavior.� Sediments at the NC Focus Area consist of a framework of quartz and feldspar detritus, with iron and aluminum grain coatings in the form of chlorite and smectite with minor oxyhydroxides, and to a lesser extent, as discrete phases such as goethite replacement of framboidal pyrite. Iron oxyhydroxides vary between 200 and 700 ppm. Total organic carbon ranges from 400 to 1,200 ppm, a large proportion occurring as organic carbon adsorbed to the surface of the grains. Within these narrow variations, the abundance of grain coatings and adsorbed organic carbon increases as grain size decreases. Groundwater pH ranges from 5.5 to 6.5, with anionic strength averaging 0.003 M dominated by Ca, Na, Mg, NO_3, SO_4 and inorganic carbon.

3. Design of the Bacterial Transport Experiment

One necessity for the transport experiment was selection of a adhesion-deficient bacterial strain from the indigenous population that would be optimal for injection. In the laboratory, repeated passage of isolated strains through the site sediments in mini-columns was performed to select a low-adhesion bacterial strain. Out of the close to 600 isolates analyzed, most field-derived strains adhered to site sediments at >90% in a simple adhesion assay, indicating little downgradient dispersion if these strains were injected in the field. The selected strain, a *Comamonas* sp. designated as DA001, exhibited low adhesion to site sediment (~30%) in the adhesion assay.� Up to 70% of injected DA001 cells were recovered from 0.5-m long intact sediment

cores, compared to less than 1% recovery for most other strains. DA001 also appeared in the effluent stream significantly before the Br, with an apparent mean velocity as much as seventy percent greater than that of the Br. This differential advection was attributed to size- and/or anion-exclusion of the colloid-sized bacteria.

A well-designed suite of quantitative models was needed to serve as a focal point for data integration, design and interpretation of experiments, quantitative hypothesis testing, data management and integration, understanding relationships among observations made at different scales, and enhancing interdisciplinary collaboration. To meet these objectives at the NC Focus Area, several levels of model complexity and various length scales were addressed through multiple linked numerical models ranging from 1-D core-scale models of laboratory experiments to high-resolution 3-D models of field-scale transport. A 1-D particle-tracking model was used to infer transport parameters describing solute dispersion and bacterial fate, based on laboratory transport experiments using intact sediment cores.� A geostatistical model of 3-D hydraulic conductivity variations was developed, conditioned on *in situ* hydraulic conductivity measurements as well as estimates available from geophysical data, and used to generate alternative descriptions of the experimental aquifer.� A regional-scale, 2-D flow model was used to design pumping rates of a forced-gradient hydraulic control system. Information from the models was then combined into a high-resolution, 3-D flow and transport model for the prediction of field-scale solute and bacterial transport. This model framework was used to generate estimates of bacterial and Br breakthrough times at each sampling port that were used to specify: (1) locations of multi-level samplers for monitoring transport; (2) frequency and timing of sample collection during bromide tracer injection experiments; and (3) frequency and timing of sample collection during a bacterial injection experiment. The models were applied sequentially, using information gained to update the models and to guide further experimentation.

4.� NC Focus Area Field-Scale Bacterial Transport Experiment

Bromide and bacterial tracers were injected into the subsurface within the NC Focus Area in late 1999.� A 720-liter solution of Br and bacteria was injected at concentrations of 85 ppm and 10^8 cells/ml, respectively, over a 0.5-m packed-off interval over 12 hours. A forced gradient was imposed to produce an average flow velocity of approximately 1.0 m/day across an array of 24 multi-level samplers (MLS) spread 5 m laterally and 7 m longitudinally. Bacteria and Br concentrations were monitored for one week at 192 sampling ports spaced over 2 vertical meters of the saturated zone located 0.5 to 7.0 m downgradient of the injection well.

A unique aspect of this experiment was the use of seven methods for detecting the injected bacteria. Each was developed to track the indigenous strain without affecting the metabolic activity or transport characteristics of the cells. The approaches developed included: enumeration of bacteria labeled with a vital fluorescent stain (5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester, CFDA/SE) using (1) direct epifluorescent microscopy, (2) microplate spectrofluorometry, (3) flow cytometry and (4) ferrographic (immunomagnetic) capture; (5) enumeration of highly ^{13}C-enriched bacteria using combustion-isotope ratio mass spectrometry (IRMS); and enumeration of cells using (6) quantitative polymerase chain reaction (qPCR), and (7) plate counts. Combustion IRMS and ferrographic enumeration had the lowest quantification limits (~2 and 20 cells/mL, respectively), followed by plate counts (100 cells/mL), flow cytometry (1000 cells/mL), qPCR (10^4 cells/mL), and microplate spectrofluorometry (10^5 cells/mL). The microplate spectrofluorometer was taken to the field site to provide real-time determination of the bacterial concentrations in groundwater samples from the MLS-array. The methods yielded comparable results, and the combination of methods with high and low detection limits allowed greater resolution of the bacterial plume distribution.

Using the various tracking technologies, bacteria were detected over the full length of the flow field and in the downgradient monitoring well 9.5 m from the injection well.� Figures 4a and 4b illustrate the

bromide and bacterial plume, respectively, in three dimensions at 48 hours after injection. The vertical distance separating sampling ports was only 25 cm, yet the concentration and velocity of bacterial breakthrough varied dramatically in adjacent ports. Researchers observed not only the transport of high concentrations of bacteria throughout the flow field, but in some subsurface areas the bacteria traveled faster than did the conservative Br tracer. Significant lateral dispersion did not occur. The plume was sharply defined and its shape conformed to the pattern of heterogeneity previously revealed by the hydrogeological characterization of the experimental cell described in Section 2.2. For example, the bacterial tracer breakthrough profiles at 12, 48 and 200 hours after injection along the injection centerline are shown in Figures 2b, 2c, and 2d, respectively.� Comparison with the hydraulic conductivity estimates obtained from geophysical data along the same traverse (shown in Figure 2a) reveals that at short times the tracer traveled quickly along the shallower and deeper high conductivity zones defined by the characterization data (Figures 2b, 2c), while at later times the tracer was retained in the low conductivity zone (Figure 2d). Although similar phenomena have been suggested based on previous field bacterial transport experiments (e.g., at Cape Cod), the detailed results of the transport observed at NC Focus Area can for the first time be directly related to the subtle, three-dimensional hydrogeological heterogeneities provided by the characterization data.

5.� Implications for Bioaugmentation

With our successful field-scale bacterial transport experiment within the oxic and relatively homogeneous NC Focus Area, we have demonstrated:

� Enhanced transport in the field by selecting for low-adhesion bacterial strains in the laboratory;

� The utility of numerical models for guiding the design of field-scale injection experiments, integrating disparate data sets, and for interpretation of the results;

� The utility of a suite of tracking methods that allow detection of viable, metabolically active bacteria essential for remediation;

� The utility of geophysical data for providing high-resolution hydrogeological characterization information in a minimally invasive manner.�

This experiment allowed us to investigate the relative effects of hydrogeological and geochemical heterogeneity on the transport of a low-adhesion bacterial strain through a simple but stratified aquifer.�

We are continuing to perform field-scale bacterial transport experiments, both at the oxic NC and the suboxic SOFA Focus Areas, using DA001 as well as a low-adhesion iron-reducing bacteria (IRB) strain for injection.� These additional experiments will allow us to further investigate the relative influences of hydrogeological and geochemical heterogeneities under varied subsurface conditions, the potential for targeted delivery of metal-reducing bacteria to the metals themselves, and the extent to which other factors, including attachment/detachment kinetics, nutrient effects and predation effects, influence bacterial transport at the Oyster Site.

To present and discuss several of the insights that have developed during the Oyster research project, a special session was convened at the 2000 AGU Fall Meeting on the subject of "Breakthroughs in Field-Scale Bacterial Transport." Eleven oral and ten poster presentations generated interest and lively discussion among the members of the audience. The presentations described research addressing investigations at Oyster and other field sites, including applications of bioaugmentation, methods of integrating laboratory and field experiments, bacterial tracking methods, surrogates for study of pathogenic organisms, subsurface biogeochemical characterization, and theoretical and numerical modeling approaches. It was clear from the discussions that field-scale bacterial transport studies, such as the one undertaken at the Oyster Site, are necessary in order to improve bioaugmentation strategy designs, to improve the predictive modeling capabilities of pathogen contamination in the groundwater, and to assess the impact of introduced organisms on subsurface microbial communities.

Authors
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Figure Captions
Figure 1. Location map of the Oyster Bacterial Transport Site in the Delmarva Peninsula of Virginia. Study areas at the Oyster Site include the NC Focus Area and the South Oyster Focus Area, or SOFA.
Figure 2. Comparison of (a) the log-conductivity values estimated using tomographic data at the NC Focus Area along the tracer injection centerline with relative bacterial concentrations at (b) 12 hours, (c) 48 hours, and (d) 200 hours after bacterial injection.
Figure 3. Relationship between laboratory physical and hydrogeological measurements on sediments from the NC Focus Area (grain size data, permeability data and facies, center and right) and estimates of hydraulic conductivity obtained from geophysical data at well NC-B2. The grain size in phi units is a dimensionless number, determined by dividing the negative log to the base 2 of the mean grain diameter in mm by a unit value.
Figure 4. A 3-D visualization of (a) bromide and (b) bacterial concentrations measured 48 hours after the start of the injection experiment. White spheres denote locations where concentrations were measured. Colored surfaces are isosurfaces of concentration interpolated from the point measurements and normalized by the injection concentration C_0. Those portions of the isosurfaces in front of the red plane have been cut away to allow viewing of the internal structure of the plume. Flow and transport are from lower left to upper right. The distance from the injection zone (near the center left edge of the red plane) to the most distant sample location is seven meters.
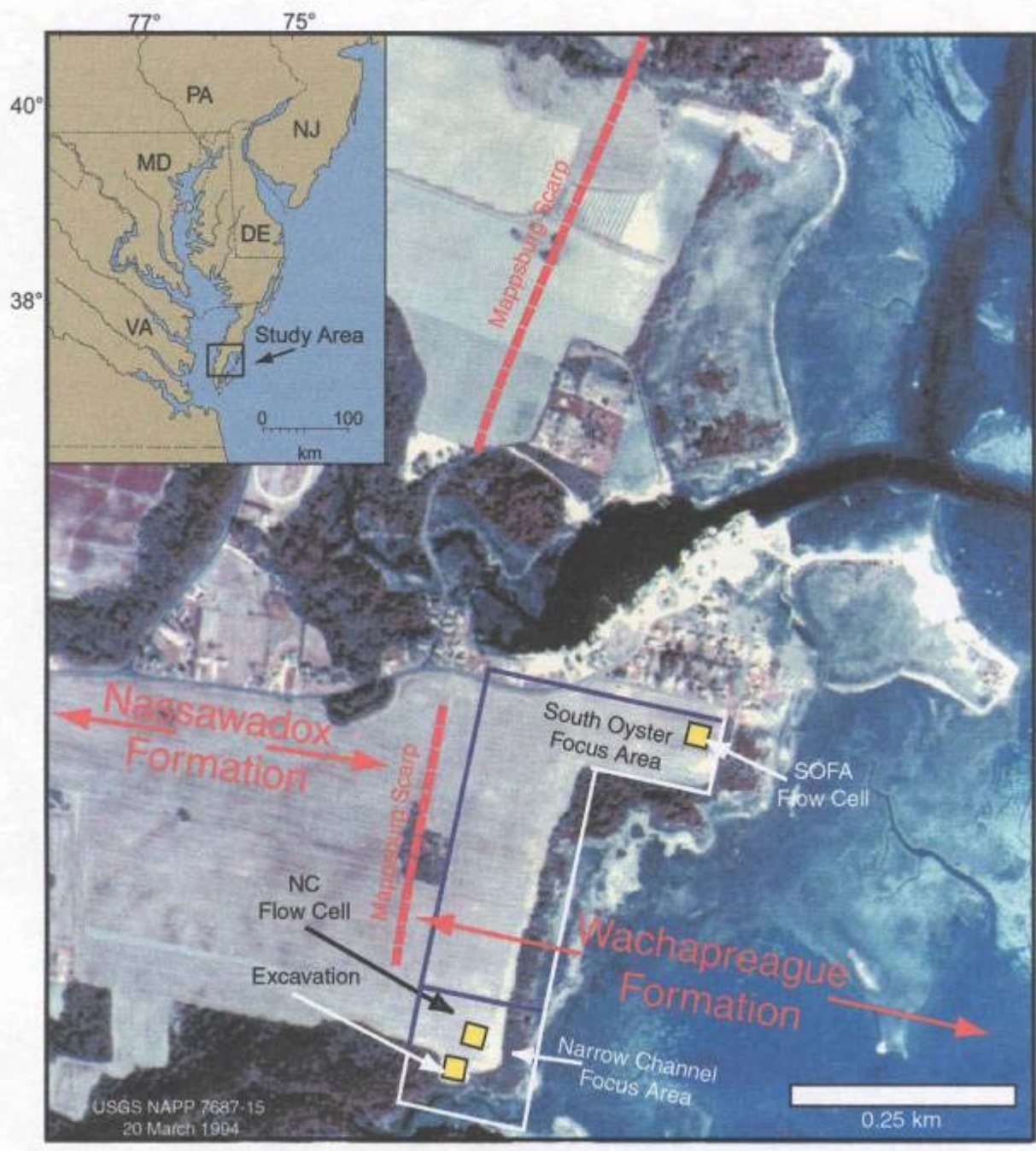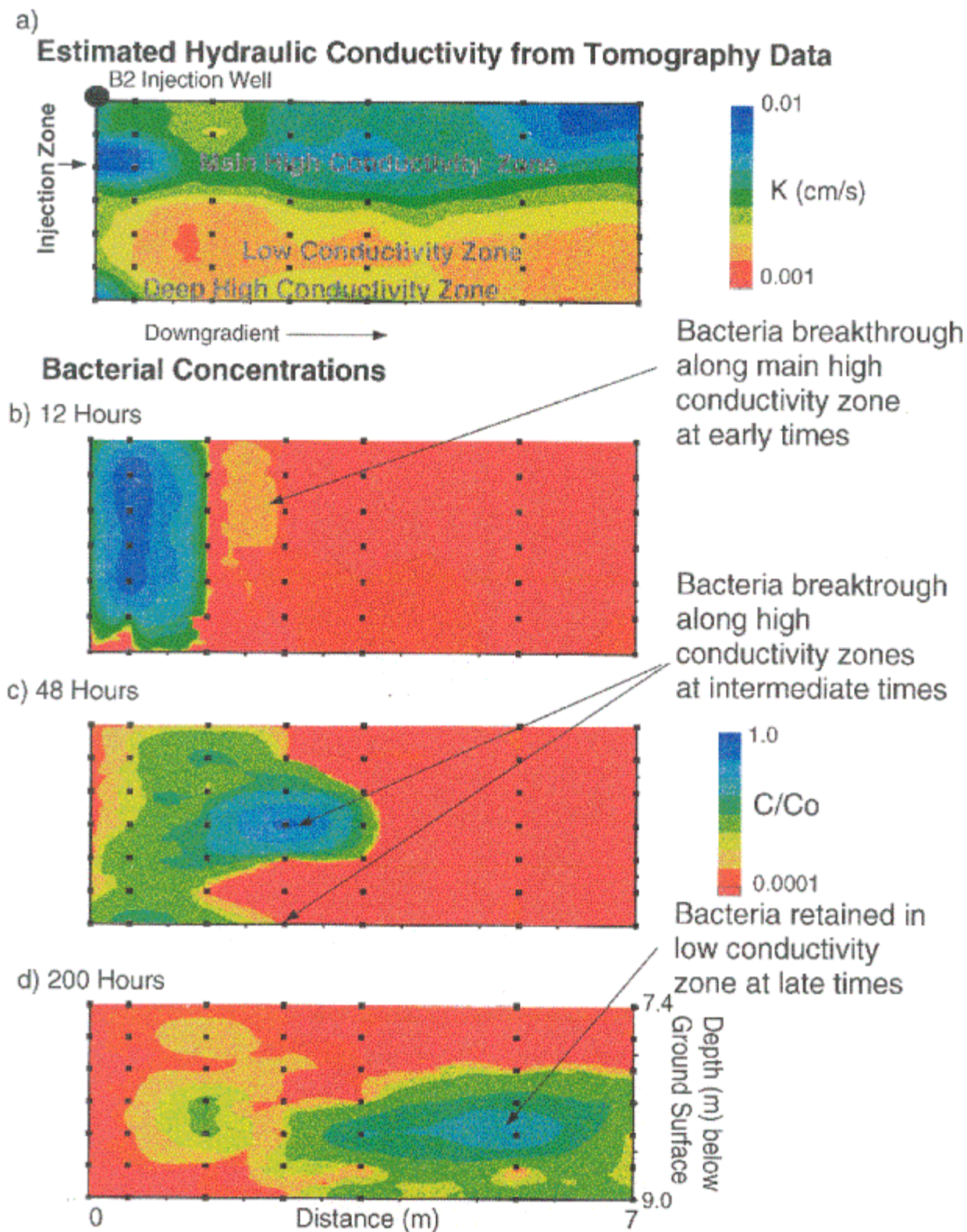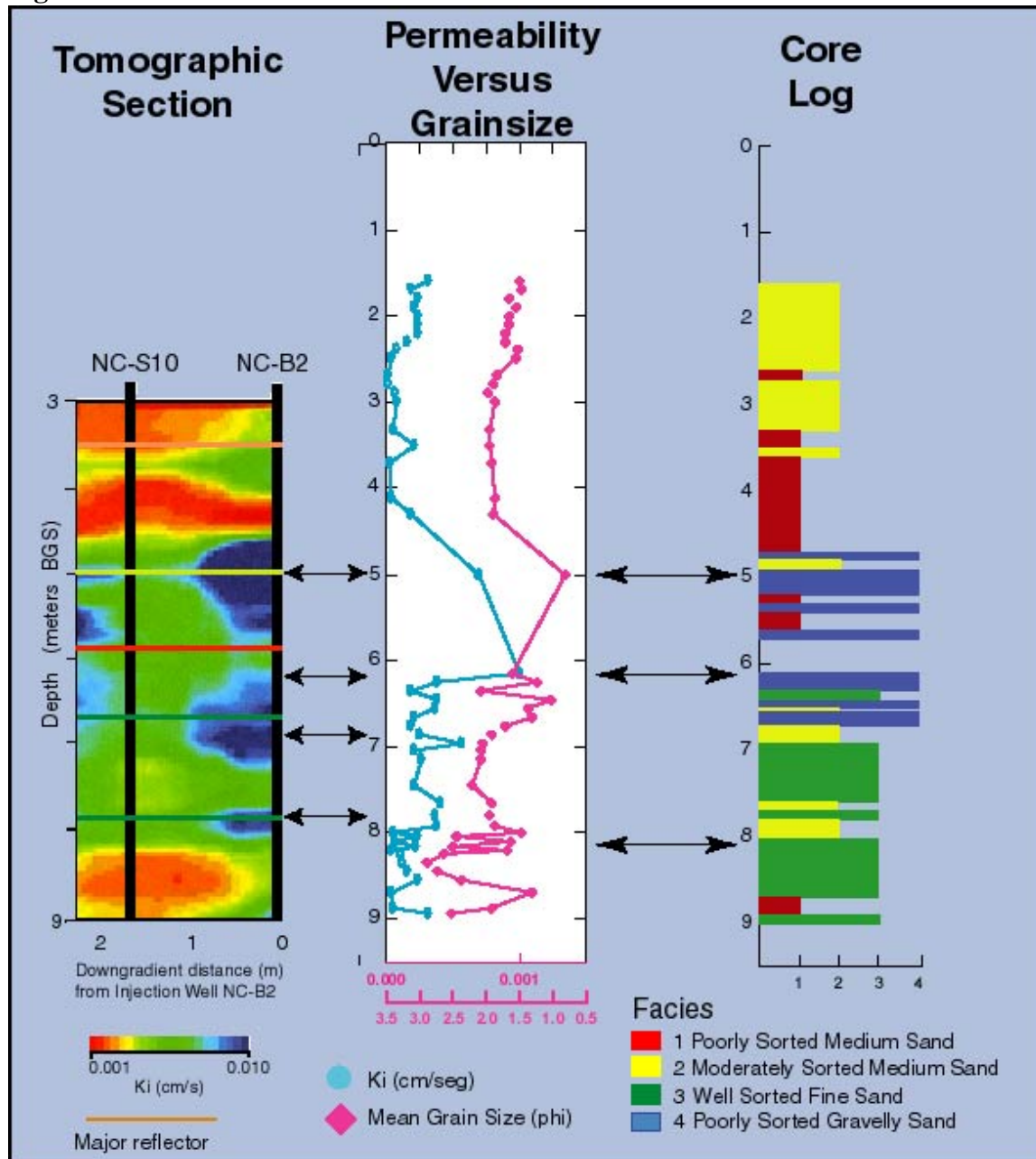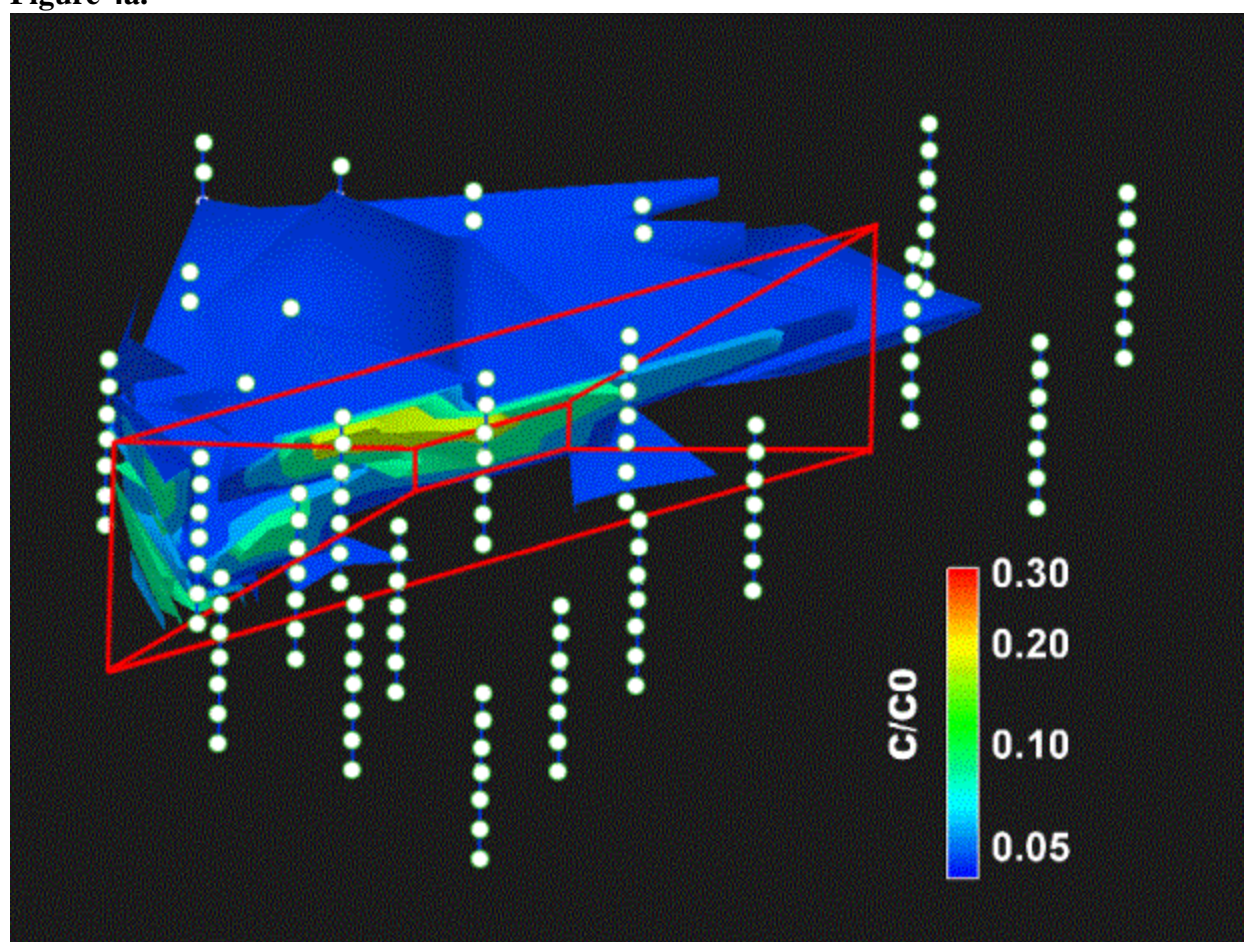
Figure 1.

Figure 2.

a) Estimated Hydraulic Conductivity from Tomography Data

Bacterial Concentrations

b) 12 Hours

Bacteria breakthrough along main high conductivity zone at early times

c) 48 Hours

Bacteria breaktrough along high conductivity zones at intermediate times

d) 200 Hours

Bacteria retained in low conductivity zone at late times

Figure 3.

Figure 4a.

Figure 4b