Preliminary observations on bacterial transport in a coastal plain aquifer

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Abstract

A multidisciplinary research team, funded by the U.S. Department of Energy (DOE) Subsurface Science Program, initiated a field-scale bacterial transport study in a sandy aquifer on the coastal plain of Virginia in 1994. The purpose of the study was to evaluate the relative importance of hydrogeological and geochemical heterogeneity in controlling bacterial transport. Extensive geophysical and geochemical characterization of the site was accomplished using intact cores obtained during the construction of the flow field and in a nearby sand pit exposure of the sedimentary facies found in the flow field. Geophysical techniques, including ground penetrating radar and cross borehole tomography, were used to relate the depositional environment of the sand pit to the flow field as well as to produce a 3-dimensional depiction of the flow field to be used in modeling the site and the results of the injection experiments. The 30 m long flow cell consists of ground water production and injection wells, a tracer injection well, and 10 multilevel samplers screened every half meter from 4.0 to 10.5 m below ground surface. The organization that owns the site required that only native microorganisms be introduced at the site, therefore, the injected bacterial strain was isolated from the indigenous community in the aquifer. Candidate strains were selected by a protocol that enriched for phenotypes of low adhesion and non-clinical antibiotic resistance which could be used to detect the organism on selective media. The bacteria were selected for low adhesion to site sediments so that they might be readily transported through the aquifer. For the field injection experiment detection and quantitation of the strain chosen by this screening process, PL2W31, was accomplished by isotopically enriching the cells with [13C]glucose. Forced gradient conservative (Br-) tracer tests were performed immediately prior to the bacterial injection experiment to provide a measure of non-reactive transport through the aquifer. The non-reactive tracer test indicated the presence of hydrogeological heterogeneities at the site that caused differential breakthrough of the tracer. Results from the bacterial transport experiment indicate that bacteria traveled the length of the flow field (4 m), but that the majority of the biomass injected was retained in the sediments between the injection well and the first multilevel sampler at 0.5 m. Preliminary bacterial transport models indicate that the observed behavior could be accounted for by the presence of two subpopulations within a single bacterial strain with differing transport properties.

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1. Introduction

The factors controlling transport of bacteria in the vadose zone and groundwater have taken on an increased importance with the field scale application of bioremediation technologies. Traditional engineering technologies such as pump and treat to remediate contaminated groundwater, or vacuum technologies to clean up the unsaturated zone, may be costly or ineffective. Bioremediation can complement existing clean up technologies or replace them. Bioaugmentation, and specifically the addition of natural or genetically modified bacteria to contaminated sites, requires that microorganisms move from a point of injection throughout the contaminated area. Microbial transport in the presence of complex subsurface geology and geochemical heterogeneity has proven to be a fundamental impediment to bioaugmentation. Research on microbial transport mechanisms is very limited, and this limitation hampers the implementation of a potentially successful technology.

A major difference between this and prior bacterial transport field experiments is the degree to which the site has been characterized and modeled to support the interpretation of the results. Previous field studies of bacterial transport have not had the benefit of the extensive site characterization that has taken place at the Oyster field site. In an in situ field study of transport of P. cepacia G4 [1], the data strongly suggest that movement of the bacteria was severely retarded by the aquifer material, since non-retarded organisms would have appeared in a recovery well at about the same time after injection as the conservative tracer, in this case within 8–10 h. The hydraulic flow of the aquifer was approximately 48 feet per day, but none of the injected bacteria were observed in a monitoring well 10 feet downgradient until 6 days after injection [1]. Because adequate characterization data were not available, the biological, physical and/or chemical causes of this retardation could not be determined in that study.

Harvey et al. [2–4] conducted a number of both forced- and natural-gradient transport studies of indigenous bacterial populations in a sandy aquifer on Cape Cod, MA. In these experiments the injected bacteria were concentrated from groundwater and stained with the fluorochrome 4',6-diamidino-2-phenylindole (DAPI) for detection by direct counts. It is known that this fluorochrome affects the metabolism of bacteria by DNA intercalation, but it is not known how staining affects the transport properties of bacteria. The effect of small-scale heterogeneities
on transport was observed in these studies. In an in situ multitracer study, Harvey et al. [2], report that the relative order of breakthrough of bacteria, microbial-sized microspheres and bromide differed for three sample locations within one meter in a single well. Modeling transport in these studies based on colloid filtration theory proved to be inadequate to account for differences in surface characteristics among bacteria, as well as the impact of physical heterogeneity on observed differences in bacterial transport at different locations in the same aquifer [4].

In order to address some of these issues, a research site on the Atlantic Coastal Plain of Virginia was selected (Table 1) for field scale injection experiments. The objectives of the study were to determine: (1) how the spatial heterogeneity of the physical and chemical properties of geologic deposits affect transport of bacteria; and (2) what physical, chemical and biological factors control the processes of deposition and entrainment of microbial cells in porous media under field conditions. The research site was established and characterized using geological, geochemical and geophysical methods by a multidisciplinary research team. Further information on the DOE Bacterial Transport Subprogram and the Oyster, VA site can be obtained in the Subprogram plan [5].

2. Site description

2.1. Geology

The bacterial transport site is located on the southern Delmarva peninsula about 19 km north of the Chesapeake Bay Bridge Tunnel, near the village of Oyster, VA (Fig. 1), on the eastern coast of the United States. A borrow pit located near the experimental plot (called the Oyster pit in this paper – see Figs. 1 and 2) exposes sediments similar to those in the bacterial transport flow cell. The Oyster pit lies several hundred meters south of the experimental site, but is located on stratigraphic strike with the experimental site. Extensive comparative studies of samples from both locations were undertaken to verify that the analogy between the bacterial transport flow cell and the Oyster pit location is reasonable (Wilson and Schafer, manuscript in preparation). Well-exposed surface analogies for subsurface reservoirs and aquifers are commonly used in petroleum geology and hydrogeology [6–9], because the limited information available from vertical boreholes does not permit an accurate assessment of the horizontal continuity of the physical and chemical properties that control the horizontal transport of subsurface fluids and particles. In the current study, modeling the transport of injected tracers (e.g. bromide and bacteria) in the subhorizontally bedded sediments required extensive information on the horizontal distribution of physical and geochemical properties. Therefore, in the site description and the description of characterization activities that follow, descriptions will be given of characterization efforts and information obtained both from vertical boreholes and geophysical characterization of the bacterial transport site, as well as from the Oyster pit.

The bacterial transport site and pit are located in a barrier-spit complex that prograded southward across the mouth of Chesapeake Bay during the last major sea-level highstand of 125 thousand years ago [10]. The sediments consist of unconsolidated to weakly cemented, well-sorted, medium- to fine-grained sand and pebbly sand, deposited by wave, wind and tidally driven currents on the shoreface of the spit. Stratification consists of thin to medium (10–100 cm thick) strata, strata sets and cross-strata
sets. Approximately 55% of the sedimentary beds in the measured sections are cross-stratified and some contain more than 10% pebbles [11].

Physical heterogeneity at the bacterial transport site occurs over a range of scales. Individual laminae (strata < 1 cm thick) are tabular, with great length and width relative to thickness. They reflect grain size variation at the centimeter scale and generate relatively small scale changes in hydraulic conductivity (< 1 order of magnitude). Laminae are organized into beds (strata > 1 cm thick), bed sets and cosets. These elements are also tabular. Their upper and lower boundaries are erosional in origin and constitute grain-size discontinuities, resulting in hydraulic conductivity contrasts of 1–2 orders of magnitude [12]. Laminae, beds, bed sets and bed co-sets dip 5° to the south-southeast, creating flow anisotropy. The cross strata sets are not tabular, but are elongate lenses in plan view, and are plano-convex (lateral) lenses in cross section. Cross-strata sets are oriented northeast-southwest, and may cause a marked anisotropy in hydraulic conductivity which affects transport within the aquifer.

2.2. Mineralogy and sediment geochemistry

The sediments are dominantly quartz sand, with minor amounts of plagioclase and potassic feldspars, plus mafic and lithic grains. The bulk organic matter content is low in sediments from the site. Sand samples collected from an exposed face in the nearby Oyster pit yielded total organic carbon contents of less than 0.5% for six samples. Gravelly layers may contain abundant lithic fragments (> 30%) as well as calcium carbonate shell material, especially shells or fragments of the surf clam, Spisula solidissima. The calcium carbonate of the clam shells has been completely replaced with hydrus iron oxides. Clay minerals are not abundant in the sediments at the site, most of which is well-sorted medium sand.

Heavy mineral laminae are present and stratal boundaries, strata set boundaries and cross strata set boundaries are often outlined by orange-brown ferric oxyhydroxide pigment (Fig. 2). The most important chemical heterogeneity present at the site is the distribution of ferric-oxyhydroxide coatings on sediment grains. Analysis of cores from the sediments that underlie the study site suggest that the distribution of most ferric-oxyhydroxide coatings is highly correlated with physical heterogeneities; there are, however, some feldspar patterns which cut across primary sedimentary structures. The chemical heterogeneities occur on a relatively smaller scale, and the correlation length for the heterogeneities is much greater horizontally than vertically. For example, horizontally extensive thin layers enriched in ferric oxides and hydroxides (1–2 cm thick) occur at some bed boundaries separating thicker layers that contain minor disseminated iron oxides. Data from laboratory column studies [13] suggest that these iron oxyhydroxide bands have the potential to exert a strong influence on bacterial transport in the aquifer.

2.3. Groundwater geology and geochemistry

Three hydrogeologic facies have been identified at the site, including a horizontally bedded sand facies, a cross-bedded sand facies, and a cross-bedded shelly, gravelly sand facies which is a subpopulation of the cross-bedded sand facies. The permeability distributions of all three facies exhibit some overlap, with the highest median permeabilities measured in the shelly, gravelly sand, and the lowest in the horizontally bedded sand (Fig. 2, bottom). Based on preliminary analysis of data from the flow cell, groundwater flow in the aquifer appears to be controlled by the distribution of the cross-bedded sand and the shelly gravelly sand facies, which have a median permeability more than double that of the horizontally bedded sand facies.

The groundwater at the injection site is oxygen-
Fig. 2. Top: Photograph of the Oyster pit, showing sediments exposed in three benches during recent excavations of the site. The studies in the pit provide excellent data on the horizontal continuity of geological and hydrogeological properties in the subsurface sediments at the site. Bottom: Detailed view of heterogeneity present in the sediments. The cross-bedded sands in the center of the photo have higher permeability than horizontally bedded sediments in the lower portion of the photo. The sweeping cross-bed sets are highlighted and outlined by variations in the amount of metal oxyhydroxides present in the individual laminae and near the edges of the sets.

ated (6–8 mg l\(^{-1}\) dissolved oxygen), and has pH values ranging from 5.4 to 6.0. The dissolved organic carbon ranges from 1 to 4 mg l\(^{-1}\). No ammonium has been detected, and the dissolved iron concentration is low, ranging from 0.1 to 1.0 mg l\(^{-1}\).
3. Site characterization and modeling

Detailed site characterization and high-resolution three-dimensional numerical simulation are being performed in order to interpret the bacterial tracer injection experiments at the Oyster site. The purpose of the simulation effort is to integrate the multidisciplinary characterization data being collected into a quantitative framework for synthesis of bacterial transport processes, that can subsequently be used for hypothesis testing and interpretation of the field transport experiments. A secondary purpose is to quantify the incremental value of the various types of characterization data in terms of their impact on the predictability of field scale transport of bacteria.

3.1. Site characterization

A total of 14 boreholes were continuously cored to a depth of 10.6 m below ground surface (bgs) at the site, including a 20 m × 30 m flow cell grid of nine boreholes for well installations, and an array of five boreholes for tomography surveys in the center of the flow cell. The diameter of the boreholes was 14.1 cm and water levels measured in these boreholes ranged between 5 and 6 m bgs. An additional array of ten boreholes was drilled directly down-gradient of the middle injection well (UC-B-2) for the installation of multilevel samplers (MLS). The flow cell was oriented in such a manner to ensure that the MLS would be predominantly downgradient of the central injection well over the full range of seasonal fluctuations in groundwater flow direction (Fig. 1). All of the boreholes in the flow cell were continuously cored utilizing ‘roto-sonic’ coring technology that is capable of collecting continuous core in unconsolidated, collapsing (or ‘flowing’) sands such as those found in the subsurface at the Oyster injection site.

Detailed characterization was performed on the cores from the flow cell. Except for the boreholes in which the MLS were installed, all boreholes were continuously cored, and complete geologic descriptions of sedimentary texture and structures were made for each borehole. Core recovery using the roto-sonic coring technique was excellent, with only minor gaps due to recovery problems in the unconsolidated sand. Closely spaced samples were taken from the cores and analyzed for grain size, surface area, falling head conductivity, radiolabeled sulfate adsorption and extractable iron and aluminum concentrations.

Geophysical methods were also used to characterize the Oyster site. The primary goal of the geophysical work is to develop and apply high resolution methods for determining the physical parameters that affect fluid and bacterial transport properties. The approach employs seismic and radar methods to define multiple scales of physical heterogeneity. Seismic crosshole tomography and radar tomography were applied in the flow cell (for location of tomography boreholes see Fig. 1), and using high frequencies, resolution on the order of 10 cm was obtained. Tomographic plots of seismic wave velocity attenuation and radar velocity were then derived for a number of cross sections through the flow field.

In addition to the field measurements, laboratory measurements are being made using a unique apparatus designed to duplicate in situ conditions of triaxial pressure. These measurements, along with measurements of porosity and grain size from core studies, are being used in conjunction with the geophysical field data to determine the distribution of porosity, grain size, lithology and permeability. This will then be used to constrain flow models and bacterial transport models.

Because vertical boreholes do not provide sufficient information on the horizontal distribution of important geological, hydrogeological and geochemical properties, extensive characterization was also performed in the Oyster pit (Fig. 2). Three vertical faces were excavated in the pit, each approximately 1.5 m high and 20 m wide, and separated from one another by horizontal benches approximately 1.5 m deep (Fig. 2, top). Characterization of the exposed faces included complete geologic mapping of the sedimentary facies. A detailed air-minipermeameter survey encompassing over 1000 samples was based on grid sampling designed to capture multiple scales of heterogeneity. At several hundred of the air-permeability sample locations, sediment samples were taken from the pit and then analyzed for grain size analysis, falling head conductivity, and the measurement of extractable iron and aluminum concentrations.

In addition to the geological, geophysical and geo-
chemical surveys, efforts have also been made to characterize the site microbiologically. Much of that effort focused on analysis of microorganisms in the site groundwater for the selection and characterization of candidate microorganisms suitable for the bacterial transport experiments. Bacterial strains isolated from both groundwater and sediment from the aquifer were characterized based on their metabolic capabilities (Biolog), DNA fingerprints (AP-PCR) and percent adhesion to site sediments (DeFlaun and Holben, in preparation). Spatial and temporal variations of microbial abundance, activity and community-level physiological profiles were examined for both aerobic and anaerobic populations. The process used to select the injected bacterial strain is described below.

3.2. Modeling

In order to provide support in guiding experimental design and characterization efforts, the modeling activity is sequential by design. This approach allows the integration of new information on bacterial transport processes and sediment properties at the injection site as it becomes available and allows the quantitative evaluation of the relative importance of diverse data. Four stages of aquifer model refinement have been defined as follows: (1) unconditional
simulations based only on Oyster pit data; (2) simulations conditioned on borehole data from the injection site; (3) simulations conditioned on geophysical data from the injection site; and (4) simulations conditioned on non-reactive tracer observations. Unconditional simulations (stage 1) were performed prior to emplacement of the boreholes at the injection site, for premodeling and experimental design. An initial suite of stage 2 simulations has been performed and applied to the non-reactive bromide injection experiment. An example realization is shown in Fig. 3. The stage 2 suite will be revised following further analysis of the pit and borehole data. Geo-

physical data for stage 3, and bromide tracer data for stage 4, are available and are currently being incorporated into the models.

Data available from the different characterization efforts that will be incorporated in the modeling include information on hydrogeologic (physical and chemical) and microbiologic properties controlling transport and microbial attachment. Geologic heterogeneity in the numerical model of the Oyster aquifer system is represented by a two-scale geostatistical simulation approach that incorporates large scale variability in geological facies using an indicator simulation method and represents local variability (with-
in facies) using a Gaussian simulation method [15]. The two-scale approach facilitates the representation of the two major scales of heterogeneity observed in outcrop studies. The stochastic approach allows explicit representation of model uncertainty by generating multiple, equally likely alternative aquifer model scenarios (termed ‘realizations’), each of which varies in detail where no local data are available, while honoring those data that are available. Each alternative description of the spatial distribution of aquifer properties is input to a flow and reactive transport simulation model [16] to derive a distribution of predicted outcomes.

Simultaneously, aquifer characteristics and mechanisms controlling bacterial transport are being defined through intact core experiments. Replicate intact columns selected from each of the primary hydrofacies types and obtained from the outcrop are being used for characterization of bacterial attachment/detachment processes within each hydrofacies. Additional cores from facies transition zones will be used to identify attachment mechanisms at such zones where large gradients in permeability and metal oxyhydroxide coatings occur. Kinetic and/or equilibrium biomass partitioning relations will be constructed for each hydrofacies for use in the aquifer models. Based on preliminary column study results, an initial bacterial transport simulation has been performed using a single stage 2 realization to test hypotheses regarding subpopulations within the selected bacterial strain.

4. Experimental protocol

4.1. Construction of the flow field

Wells installed in the nine boreholes of the flow cell grid were screened in the 4.6–10.6 m bgs interval, with the exception of the middle three injection wells (UC-A-2, UC-B-2, UC-C-2) which were screened between 3 and 9.1 m bgs. The wells were constructed of 7.6 cm ID PVC pipe and 0.025 cm slot screen with bottom end-caps and locking surface caps. The annulus of each well was backfilled with silica sand to a point approximately 1–1.5 m above the top of the screen, then to the surface with a combination of silica sand and bentonite chips. The five central tomography boreholes, arrayed at 4 m spacing around injection well UC-B-2 (Fig. 1), were constructed of 10.2 cm ID solid PVC pipe with bottom end-caps and locking surface caps. The multi-level samplers were constructed of 2.5 cm OD PVC tubing to which were attached 14 individual 0.63 cm OD sampling tubes screened with braided veil and spaced at 0.5 m intervals between 4.0 and 10.5 m bgs. The MLS were installed inside 5.1 cm ID, 5.9 cm OD drill pipe which was subsequently extracted to leave the samplers in place.

4.2. Bromide tracer test

To determine the hydrologic characteristics of the flow cell, a conservative tracer test was conducted immediately prior to the first bacterial injection (Herman et al., in preparation). Background bromide (Br\textsuperscript{−}) concentrations in the injection well and all ten multilevel samplers were measured (using a Br\textsuperscript{−}-selective electrode) during the initial pumping of the flow cell. Background concentrations ranged from 0.6 mg Br\textsuperscript{−} l\textsuperscript{−1} to 3.24 mg Br\textsuperscript{−} l\textsuperscript{−1}.

The flow cell was operated by pumping water from the groundwater production well (UC-B-3; Fig. 1) at a rate of 75 l min\textsuperscript{−1} and injecting it into the ground water injection well (UC-B-1). Pumping at this rate yielded a gradient in the multilevel-sampler field of approximately 0.05 (based on potentiometric surface plots of the entire well field). After 48 h, a solution of NaBr was injected into the tracer injection well (UC-B-2) to yield a Br\textsuperscript{−} concentration in the injection well of approximately 100 mg l\textsuperscript{−1}. The pumping rate was approximately 76 ml min\textsuperscript{−1}, and the tracer was injected for a total of 46 h (i.e., ~210 l). An additional pump and tubing was used to mix the solution in the injection well to prevent stratification of the injection solution. Samples were withdrawn from the injection well immediately on initiation of the injection and every 30 min thereafter for the first 4 h of the injection. A peristaltic pump was attached to the outlet port of the multilevel sampler and pumped to extract water from the specific depth interval. The first 50 ml of sample rinsed the sampler tubing, and a 20 ml sample was then collected in a clean glass vial. At the end of the 4-h period, the injection well and the first multilevel sampler (at all depths) were sampled. Samples were taken from all
sampling locations every 4 h after that for the duration of the injection, then every 6 h for the duration of the tracer experiment. Bromide measurements were made for a total of 6 days.

4.3. Bacterial strain selection

The Virginia Nature Conservancy that owns the Oyster site required that the organism chosen for the injection experiment be indigenous to the aquifer, non-pathogenic and sensitive to antibiotics of clinical importance. Candidate strains were identified by a protocol that selected for bacteria with relatively low adhesion, resistant to either kanamycin, nalidixic acid, or rifampicin (non-clinical antibiotics). Enrichments containing 10 ml Oyster site groundwater, 90 ml 10% PTYG medium (peptone 0.5 g l⁻¹, tryptone 0.5 g l⁻¹, yeast extract 1.0 g l⁻¹, glucose 1 g l⁻¹, magnesium sulfate 0.6 g l⁻¹ and calcium chloride 70 mg l⁻¹) and 50 µg ml⁻¹ of one of the three antibiotics were incubated at room temperature until growth was visible. The cells were then harvested, washed and suspended in Oyster artificial groundwater (OAGW), the composition of which is based on the Oyster site groundwater chemistry. After 48 h in OAGW the cell suspensions were passed over a series of three columns containing Oyster sediment in a modification of a protocol developed by DeFlaun et al. [17] to enrich for non-adhesive organisms. Individual isolates resulting from this protocol were then screened on plates for sensitivity to eight different antibiotics: rifampicin (rif; 50 µg ml⁻¹), streptomycin (str; 50 µg ml⁻¹), ampicillin (amp; 50 µg ml⁻¹), tetracycline (tet; 12.5 µg ml⁻¹), erythromycin (ery; 30 µg ml⁻¹), nalidixic acid (nal; 50 µg ml⁻¹), kanamycin (kan 50 µg ml⁻¹) and gentamycin (gen; 30 µg ml⁻¹). Only a very small percentage of the strains resistant to one of the non-clinical antibiotics were sensitive to all five of the clinical antibiotics tested, severely limiting the number of strains that could be considered for injection.

Candidate bacterial strains were then tested individually in the adhesion assay [17] to verify the low-adhesion phenotype. Of 293 strains tested in the adhesion assay, 67% of these adhered to Oyster sediment at > 90%. Several candidates that had lower adhesion values were tested for survival and limit of detection in aquifer microcosm studies. These studies involved adding a range of concentrations of bacteria to Oyster groundwater and sediment and incubating the microcosms at site groundwater temperature (15°C). Replicate microcosms sacrificed over a period of 104 days were used to assess viability and numbers of cells, both in the groundwater and attached to the sediment, by selective plate counts [18].

PL2W31 was identified as a strain resistant to 50 µg ml⁻¹ nalidixic acid and sensitive to all of the other antibiotics tested. This strain has relatively low adhesion (~70%) and survived well at all concentrations tested (10⁴, 10⁵ and 10⁶) for more than 100 days in microcosm experiments. Even at the highest concentration tested, the concentration of PL2W31 only decreased from 2.2×10⁸ to 7.3×10⁷ cells ml⁻¹ over 104 days. The surface of this strain is relatively hydrophilic as measured by contact angle (~25°) and electrophoretic mobility measurements indicate that it is neutrally charged (−0.4×10⁻⁸ m² V⁻¹).

The requirement to use indigenous bacteria posed a major problem because the injected bacteria must be distinguishable in some way from the in situ population, which includes the bacterial strain to be injected. Resistance to high levels of non-clinical antibiotics was found to be very useful for detecting organisms in non-sterile microcosm studies [18], however, the limit of detection by this method was highly dependent on incubation time of the plates, a factor that could not be well-controlled with the number of samples to be processed in the field experiment. Since radioisotopes and selectable engineered mutations could not be used for tracking injected bacteria at this site, a new detection technology based on stable isotope labeling was developed to monitor the injected bacteria. For these experiments PL2W31 was cultured at 30°C in OAGW. The glucose provided as the sole source of carbon and energy for this culture was highly (>99%) ¹³C-enriched to provide a unique isotopic signature to facilitate tracking these bacteria in situ (Holben and Ostrom, manuscript in preparation). Following growth on this substrate, the cells were washed extensively and then starved for 72 h in OAGW with no carbon source. The isotopically enriched bacteria could then be detected by mass spectrometric analysis of groundwater samples from the
injection well and the array of MLS downgradient from the injection well.

4.4. Bacterial tracer experiment

Immediately following the cessation of measurements in the Br⁻ tracer experiment, the bacterial tracer experiment was begun. The isotopically enriched bacteria were introduced into the flow field via injection well UC-B-2 (Fig. 1) over a 3.5-m depth interval spanning several depositional layers in the aquifer (5.5–9.0 m bgs). One liter of PL2W31 cells grown on [¹³C]glucose by the protocol described above was diluted in 175 l of site groundwater to a final concentration of 1.26×10⁷ cells ml⁻¹. The bacterial suspension was held in a collapsible bladder that was placed in a wooden tank and kept covered with flowing groundwater to serve as a temperature control. The suspension was pumped from the bladder into the injection well (UC-B-2) at 86 ml min⁻¹ for 34 h. As for the bromide, the circulating pump mixed the cells throughout the injection well volume during the injection. Samples were taken at 1-m intervals (6 m, 7 m, 8 m and 9 m) in the injection well and at 0.5-m spacing in each of the 10 MLS every 4 h for the first 60 h, then every 6 h for the next 72 h, then every 12 h until day 14.

Samples were taken as described for the bromide tracer test except that 10 ml was collected for plating purposes and a 50 ml sample was collected for analysis of [¹³C]. Samples for isotope analysis were placed on ice and shipped to the University of Montana every 2 or 3 days. Samples for plating were taken to the field laboratory and dilutions were plated on R2A plates containing 50 μg nalidixic acid ml⁻¹. Plates were incubated at 32°C and counted at the field lab.

5. Preliminary results and discussion

The first detection of Br⁻ occurred in the first MLS (#1, 0.5 m from the injection point) at port 7.5 (m bgs) 12 h after the beginning of the injection. The travel times (flow velocities) for the half meter depth intervals were computed on the basis of arrival of Br⁻ at the first MLS (#1 at 0.5 m) to yield a concentration equal to 50% of the maximum as follows: 5.5 m bgs, 24 h; 6.0 m bgs, 20 h; 6.5 m bgs, no Br⁻; 7.0 m bgs, 10 h; 7.5 m bgs, 14 h; 8.0 m bgs, 34 h; 8.5 m bgs, 12 h; 9.0 m bgs, 10 m bgs and 10.5 m bgs, no Br⁻. The differences in the bromide breakthrough time at the various depths highlights the hydrogeological heterogeneity of the formation.

The Br⁻ tracer generally followed a straight path through the MLS array, although a small amount of Br⁻ was observed in sampler 3 but not 4, and a small amount was observed in sampler 10 but not 8 (Fig 1). Furthermore, Br⁻ was seen at sampler 9 (4 m downgradient) at concentrations above 10 mg l⁻¹ (background concentrations are approximately 3.5 mg l⁻¹) only at 9.5 and 10 m depths. The low concentrations of Br⁻ seen in the far field samplers suggests that the Br⁻ did not sink in the flow cell due to density differences, if this were going to occur it would have been apparent at the higher concentrations of bromide measured in the near field samplers. The measurement of the bromide in the deeper samplers farther from the injection well is more likely a result of a slight downward flow of water in that area of the sampler array. An earlier Br⁻ tracer study conducted in the fall of 1995 produced nearly identical results.

The results of selective (nal⁻¹⁰⁵) plate counts for the injection experiment indicated that the number of cells that reached MLS #1 was too low to be detected by this method; the [¹³C] method, however, is much more sensitive, and data obtained with that method have been used to interpret the results of the bacterial injection experiment. Results to date indicate that heterogeneities in the depositional layers in the flow field affect both the hydrological and bacterial transport characteristics of the aquifer. These effects are manifested as differences in non-reactive tracer (bromide) and bacterial breakthrough at the various sampling points in the flow field. While a full interpretation of results obtained to date is still in progress, there are several salient points regarding these heterogeneities and their effects on transport. For example, at sampler 2, which is 1 meter downgradient of the injection well, the approximate time of appearance, center of mass, magnitude and duration for bromide breakthrough was different at essentially every sampling depth interval. Values ranged from low magnitude (~25 mg l⁻¹), rapid (~10 h) appearance of the bromide pulse...
which lasted 40 h at depth interval 6.5 m, to high magnitude (100 mg l⁻¹) breakthrough appearing at about 20 h at depth intervals 7.5 and 8.5 m and lasting 40 h. Interestingly, the intervening depth interval (8.0 m) had 60 mg l⁻¹ peak breakthrough beginning at about 80 h and lasting approximately 75 h. Thus, just in this 2-m depth interval, at a single sampling point 1 m from the injection well, there was four-fold difference in peak magnitude, eight-fold difference in time of appearance and two-fold difference in duration of the bromide pulse.

Preliminary modeling of the bromide break-through using stage 2 aquifer simulations conditioned on borehole data from the injection site (Fig. 3) were able to reproduce the general fingering character of the bromide tracer pulse as it moved through the aquifer (Fig. 3B). The ability of the model to make detailed predictions of bromide breakthrough at individual sampler ports was variable. For some MLS ports, the bromide breakthrough from the field experiment fell well within the predicted interval from the model simulation runs (Fig. 4, top). However, the experimental results at other ports was outside the predicted range (Fig. 4, bottom). The variability in predictive power of the modeling is due in large part to the uncertainty in the distribution of aquifer properties caused by the small amount of conditioning borehole data available at the injection site. It is anticipated that incorporation of the geophysical and tracer data in the stage 3 and 4 modeling effort should narrow that uncertainty considerably, and improve the predictive capability of the transport model.

Results for bacterial breakthrough are currently less complete but several general observations can be made. While calculating a mass balance for injected bacteria will be difficult, if not impossible, it is apparent that large numbers (> 99%) of bacteria have been retained by the solid phase immediately adjacent to the injection well, because the peak concentrations of bacteria at MLS 1 (0.5 m downgradient of injection) were 2–3 orders of magnitude lower than the bacterial concentration in the injection well. However, those microbes that were transported appear to be able to travel far in the flow field as evidenced by the arrival of bacteria (indicated by measurement of ¹³C isotopic signature) at several of the deeper MLS ports at sampler 9 which is 4 m downgradient of the injection well.

A laboratory bacterial transport experiment was conducted on a single intact core from the Oyster pit using the ¹³C label counting method. This experiment indicated that the large majority of bacteria were rapidly and irreversibly attached on the time scale of the experiment. However, a small fraction of the bacteria (detectable because of the sensitivity of the ¹³C method) were observed in the core effluent. The observed breakthrough curve could be well described using a simple equilibrium retardation model, with a retardation coefficient of \( r = 1.4 \).
proportion of bacteria observed in the effluent was only 0.002% of the initial injected mass. The high propensity for rapid attachment is consistent with the results of small-scale (2 cm) column experiments (MARK assays) conducted at the University of Arizona. Based on the intact core experiment, it was hypothesized that bacterial strain PL2W31 is composed of two or more subpopulations, and that one of these subpopulations is much more mobile than the overall population. This hypothesis was tested by simulating the above scenario in one of the realizations from the stage 2 aquifer model suite. The individual realization was selected on the basis that it provided the best overall match to the bromide tracer observations from the field experiment. By simulating the corresponding fraction (0.002%) of the field injected bacterial source as a mobile retarded tracer with \( r = 1.4 \), a reasonably good agreement with observations from the field bacterial tracer experiment was obtained, lending support to this hypothesis.

Another interesting feature of the bacterial breakthrough curves is the character or shape of the breakthrough profiles. This was especially apparent at sampler 2 where, at several sampling depths, the bacteria appear to break through in two peaks separated by approximately 20 h but largely within a window of about 40 h. While there was a general tendency for bacteria to begin to appear at approximately the same time that bromide did at a given sampling point, the overall breakthrough curves tend to be broader than was seen for bromide, and in many cases have long trailing edges. This general character was observed at the 7.5, 8.5 and 9.0 m depth intervals at this sampler, indicating that it is a real phenomenon whose cause and importance is not yet clear. This first injection experiment has already served to generate a new series of questions related to bacterial transport including: (i) the importance of sorbed phase bacteria which represent the majority of those injected; (ii) the role of biological heterogeneity, in addition to physical and chemical heterogeneity, in governing the transport of microbes; (iii) the nature of the interaction(s) between microbes and the solid phase in this system; and (iv) the ultimate fate of the microbes added to this system.

Acknowledgments

The investigators would like to acknowledge the financial support of the U.S. Department of Energy, Office of Energy Research, Subsurface Science Program (SSP) and the leadership of our Program Manager, Dr. Frank Wobber. In addition to the authors, the principal investigators involved in this study are: Drs. Arnold, Baygents and Ogden (University of Arizona); Dr. Fletcher (University of South Carolina); Drs. Herman and Hornberger (University of Virginia); Dr. Ostrom (Michigan State University); Dr. Palumbo (Oak Ridge National Laboratory); Drs. Smith and Schafer (Idaho National Engineering Laboratory) and Dr. Swift (Old Dominion University). The research at the Oyster site is also supported by numerous associates including PIs from other subprograms in the SSP, graduate students, post-doctoral fellows and research associates. Access to the field site was granted by the Virginia Coast Reserve, a project of The Nature Conservancy. Logistical assistance was provided to the investigators by the University of Virginia's Long Term Ecological Research Program and by Old Dominion University.

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