Influence of Flow Rate on Transport of Bacteriophage in Shale Saprolite

L. D. McKay,* A. D. Harton, and G. V. Wilson

ABSTRACT

The objective of this study was to investigate the influence of flow rate on transport and retention of bacteriophage tracers in a fractured shale saprolite, which is a highly weathered, fine-grained subsoil that retains much of the fabric of the parent bedrock. Synthetic ground water containing PRD-1, MS-2, and bromide was passed through a saturated column of undisturbed shale saprolite at rates ranging from 0.0075 to 0.96 m d⁻¹. First arrival of the bacteriophage tracers in effluent samples in each of the experiments occurred within 0.01 to 0.04 pore volumes (PV) of the start of injection, indicating that bacteriophage were advectively transported mainly through fractures or macropores. Bacteriophage transport velocities, based on first arrival in the effluent, were very similar to fracture flow velocities calculated using the cubic law for flow in a fractured material. For MS-2, maximum concentration and mass of tracer recovered both increased steadily as flow rate increased. For PRD-1, these values initially increased, but were nearly constant at flow rates above 0.039 m d⁻¹, indicating that approximately 50% of the observed losses were independent of flow rate. Evaluation of the data indicates that physical straining and electrostatic or hydrophobic attachment to fracture or macropore walls were the dominant retention processes. Inactivation and gravitational settling playing secondary roles, except at the slowest flow rates. The study suggests that microbial contamination from sources such as septic fields and sewage ponds may pose a threat to the quality of ground water and surface water in areas with saprolic subsoils.

SAPROLITE, which is a decomposed fine-grained material that retains much of the fabric of the parent bedrock, is the dominant subsoil over large areas on the eastern and western flanks of the Appalachian Mountains. Recent field and laboratory tracer experiments carried out in saprolite derived from sedimentary rock in east Tennessee show that microorganisms, and synthetic microspheres used as surrogates for microorganisms, can be transported through the saprolite at very high rates, with widely variable retention or concentration loss (Haun, 1998; Cumbie and McKay, 1999; McKay et al., 2000). In a field tracer experiment carried out in 1994 in the upper 6 m of a shale saprolite at Oak Ridge National Laboratory (ORNL) in eastern Tennessee, four particle tracers (PRD-1, MS-2, 0.100- μm-diameter fluorescent latex microspheres, and killed Pseudomonas syringae) were observed to travel, under natural flow conditions, at rates of 11 to 200 m d⁻¹. Peak tracer concentrations in the sampling wells tended to be much lower than injection concentrations, but the particles were still sufficiently stable to be detected for distances of 13.5 to 35 m from the injection point. The measured particle transport rates are many hundreds of times faster than typically observed for field-scale particle tracer experiments in granular aquifers (Harvey et al., 1989; Bales et al., 1995). The high flow velocities responsible for the rapid particle transport in the saprolite occur because flow is concentrated in the fractures, which represent only a small portion of the total porosity (Wilson et al., 1993; Cropper, 1998; Driese et al., 2001). By comparison, flow in granular aquifers tends to be distributed throughout a much larger fraction of the pores, resulting in much slower pore water velocities. The field-scale particle tracer experiment described above and another field-scale study by McCarthy (1998) suggest that particulate contaminants, such as microbial pathogens from septic fields, may adversely influence water quality in aquifers or in streams or ponds fed by ground water discharge from saprolitic soils. Since saprolite is widely distributed in the southeastern United States, this may represent a regional rather than a local problem.

Since 1994, a series of laboratory-scale tracer experiments have been carried out in undisturbed columns of shale saprolite collected from ORNL to investigate particle retention mechanisms and factors influencing retention. One study, by Cumbie and McKay (1999), examined the influence of particle diameter on transport of fluorescent latex microspheres. They found that there was an optimum particle size of about 0.5 to 1.0 μm for transport, with larger than optimum size particles experiencing greater losses due to gravitational settling and/or physical straining, and smaller than optimum size particles experiencing greater losses due to attachment to fracture or macropore walls. This is consistent with previous theoretical or experimental investigations, mainly in granular materials, where an optimum particle size was also predicted or observed (Yao et al., 1971; McDowell-Boy et al., 1986; Elimelech and O’Melia, 1990; Fontes et al., 1991; Harvey, 1991). Tracer experiments described in Haun (1998) investigated the influence of ground water chemistry on transport of latex microspheres in shale saprolite from ORNL and found that particle retention was strongly influenced by ionic strength and by the valence state of the dominant cations.

Abbreviations: BTC, breakthrough curve; C/C₀, concentration of a tracer in effluent from the column relative to its injection concentration; MS-2 and PRD-1, strains of bacteriophage; ORNL, Oak Ridge National Laboratory; PV, pore volume of the soil column used in the tracer experiments.
in the ground water. For solutions with low ionic strength, or containing mainly monovalent cations, there tended to be much lower retention of tracer particles. This was believed to be due to expansion of the electric double layer and increased repulsive interactions between the microspheres and the walls of the fractures or macropores. Again, this is consistent with previous theoretical and experimental studies of particle transport in granular media (Hunter, 1986; Fontes et al., 1991; Bales et al., 1991, 1995; Amirbahman and Olsen, 1993).

Flow rate is also expected to be a dominant factor in controlling particle transport in shale saprolite. Previous studies in granular materials (Wollum and Cassel, 1978; Wang et al., 1981; Tan et al., 1994) show that flow rate can strongly influence particle retention and, given the very high flow rates indicated by the field experiments at ORNL (Wilson et al., 1993; McCarthy, 1998; McKay et al., 2000), it is likely that flow rate will prove to be a critical factor in transport of colloids in shale saprolite. However, the only previous studies of the influence of flow rate in fractured or macroporous fine-grained materials (Smith et al., 1985; Kretzschmar et al., 1995) give conflicting results. Smith et al. (1985) observed transport of the bacteria, *Escherichia coli*, through partially saturated undisturbed soil columns of six different types of silt-loam soils. They concluded that flow rate had a major influence on transport of *E. coli*, with higher losses experienced at lower flow rates. Five of the soils in the Smith et al. (1985) study had visible evidence of macropores, which in some cases included cracks, roots, and disturbance by soil fauna. Transport experiments in uniform repacked samples of the same soil material showed much higher *E. coli* losses than in the undisturbed samples, apparently due to the smaller pore sizes and lower velocities in the repacked samples. A more recent study by Kretzschmar et al. (1995) in undisturbed saturated columns of saprolite derived from weathering of a granite gneiss showed that flow rate had relatively little influence on the shape of breakthrough curves or loss rates for the colloidal Fe-oxide and clay tracers. The difference in findings between Smith et al. (1985) and Kretzschmar et al. (1995) may be a function of differences between the range of flow rates, the types of particle tracers, or the characteristics of the geologic materials. For example, the soil used in the study by Kretzschmar et al. (1995) had an unusually high proportion, relative to many fine-grained geologic materials, of macroporosity (9% of pores >50 μm) and mesoporosity (16% of pores 5–50 μm). Neither of the geologic materials in the above two studies is a close analog to the shale saprolite, in which combined fracture and macroporosity values are estimated as less than 2% (Cumbie and McKay, 1999; Cropper, 1998; Driese et al., 2001).

The main objectives of the research described in this paper are to determine the influence of flow rate on transport of the bacteriophage tracers MS-2 and PRD-1 over the range of flow rates typical of field conditions in a shale saprolite, and to examine the relative significance of different loss mechanisms, such as inactivation, physical straining, gravitational settling, and electrostatic or hydrophobic attraction to the walls of fractures and macropores. Secondary objectives include determining the effective porosity through which bacteriophage transport occurs and determining whether flow velocities calculated using the cubic law (Snow, 1969) can be used to predict bacteriophage transport velocities.

**HYDROGEOLOGIC SETTING**

An undisturbed saprolite column used for the experiments was excavated from a depth of 1.4 m at a research site in a proposed solid waste storage area (SWSA-7) on the Oak Ridge Reservation in eastern Tennessee. The site is underlain by soils and saprolite derived from bedrock of the upper unit of the Dismal Gap Formation (formerly Maryville Limestone), which is part of the Conasauga Group (Rothchild et al., 1984; Hatcher et al., 1992). The Cambrian-age Dismal Gap Formation ranges from 95 to 158 m in thickness on the Oak Ridge Reservation and consists of intraclastic, oolitic, and wavy laminated limestone interbedded with dark gray shale (Hatcher et al., 1992). The soil at the site is a thin, loamy-skeletal, mixed, thermic Typic Dystrochrept to Dystric Entochorept (Driese et al., 2001). Combined thickness of the A and B horizons is typically in the range of 15 to 30 cm. The underlying C horizon has undergone extensive chemical weathering in situ, but retains structural features of the parent bedrock, and is referred to as a shale saprolite. The saprolite grades in color from brown near the surface, to gray at the base of the weathered zone (at about a depth of 10 m), and still retains bedding features and many of the fractures from the underlying bedrock. Numerous macropores formed by plant roots and fauna are typically present in the upper 1 m of the soil and saprolite which is part of the Conasauga Group (Rothchild et al., 1984; Solomon et al., 1992; Driese et al., 2001). Bedding generally dips toward the southeast at about 17 to 72° due to regional thrust faulting (Hatcher et al., 1992), but because of local folding at the SWSA-7 site, values of dip can change from 0 to 90° over a distance of just a few meters. Fracture mapping in the upper 2 m of the saprolite shows that there are typically three sets of fractures: one set parallel to bedding and two sets perpendicular to bedding, with fracture spacing ranging from 0.5 to 5 cm (Cumbie and McKay, 1999; Driese et al., 2001).

Fractures, rootholes, and other weathering-related macropores play a major role in controlling subsurface flow and solute transport at the site (Wilson et al., 1993; Driese et al., 2001). Hydraulic conductivity values measured at 39 locations on the surface and in the shallow subsurface (at a depth of approximately 1 m) of the subcatchment by Wilson et al. (1989) had a geometric mean of 8.3 and 0.17 m d⁻¹, respectively. These measurements are several orders of magnitude higher than typical values measured in the underlying, unweathered shale (Solomon et al., 1992). Field-scale solute tracer experiments on the steep slope of the subcatchment showed that perched water table zones can quickly develop in the upper 2 m of these deposits during storm events, resulting in very rapid transport (up to 65 m in 3 h) of solutes through fractures or macropores (Wilson et al., 1993).

**INVESTIGATIVE METHODS**

**Column Excavation and Setup**

Excavation of the saprolite column and setup for the miscible displacement experiments generally followed methods developed by previous researchers at the field site (Jardine et al., 1993; Reedy et al., 1996). The column of undisturbed fractured saprolite was collected from a hand-excavated trench at a depth of 1.4 m with the axis of the column oriented along...
the dip of bedding, which was approximately 45° from vertical. The fracture spacing ranged from 0.5 to 2.5 cm, and the most prominent fractures were along the bedding planes. Initially, a 60-× 60-cm pedestal of saprolite was isolated from the surrounding material and coated with paraffin wax to prevent drying or crumbling. The pedestal was then trimmed to the desired columnar shape and dimensions (approximately 21 cm in diameter by 33 cm long) by repeatedly cutting through the wax and removing thin layers of saprolite and then recoating the exposed areas with wax. The base of the column was then cut free of the underlying saprolite, a precut piece of 25.4-cm-i.d. PVC sewer pipe was placed around the column, and the annulus was filled with paraffin wax. After the wax hardened, the saprolite column was transported to the laboratory. The total porosity of subsamples of the saprolite collected from the excavation was approximately 0.40 (Cumbie, 1997) and the total pore volume (PV) of the column, calculated based on its total porosity and volume, was 4.2 L.

The ends of the column were cut off to expose an undisturbed surface of the saprolite, where bedding planes and fractures were clearly visible (Fig. 1A). A 0.4-cm-thick layer of medium-grained (<1 cm in diameter), well-sorted quartz sand was placed on the saprolite and a PVC cap, containing four influent–effluent ports covered with layers of fine nylon mesh, was glued onto each end. The column was inverted, with influent entering the base of the column (Fig. 1B), and standpipe manometers were installed on fittings drilled and tapped into the sides of the column near the top, midpoint, and base. After the miscible displacement experiments were complete, an additional manometer was connected to the influent line just before it entered the column, so that head drop across the entire column and end caps could be measured.

The column was saturated from the bottom with a 0.005 M CaCl₂ carrier solution, which was chemically similar to pore fluids in the field (Jardine et al., 1993). The column was slowly saturated by raising the influent head at a rate of approximately 4.5 cm per day to allow air to escape through the top and to ensure that the saprolite matrix was completely saturated. After initial saturation, a constant flux of the 0.005 M CaCl₂ carrier solution was maintained through the column for approximately 15 d to remove entrapped air. The column was kept at room temperature (approximately 22°C) throughout the flow and miscible displacement experiments.

Flow Tests

Flow tests were performed on the column prior to and after the miscible displacement experiments to measure spatial variability in hydraulic conductivity of the saprolite and to determine if there were any changes in hydraulic conductivity due to the experiments. The tests involved pumping the 0.005 M CaCl₂ carrier solution through the column with a peristaltic pump at flow rates ranging from 3.6 to 29.8 mL min⁻¹. Hydraulic head values were monitored in the standpipe manometers until values were constant for each flow rate.

Miscible Displacement Experiments

Four miscible displacement tracer experiments were carried out, each at a constant volumetric flow rate per unit area of soil, with flow rates ranging from 0.0075 to 0.96 m d⁻¹ (Table 1). Measured hydraulic gradients for these flow rates ranged from 0.001 to 0.1, and were within the range of typical field-measured values for saprolite at ORNL (McKay et al., 1997, 2000). For each experiment, approximately 8.4 L, or 2.0 pore volumes (PV), of tracer solution was pumped through the soil column. The tracer injections consisted of an approximately 0.005 M CaBr₂ (800 mg L⁻¹ of Br⁻) solution spiked with two strains of bacteriophage (MS-2 and PRD-1) to achieve a concentration of between 10⁶ and 10⁷ plaque forming units per milliliter (PFU mL⁻¹), as shown in Table 1. The CaBr₂ tracer solution was prepared at least 24 h prior to the injection and the bacteriophage were mixed with the CaBr₂ solution a few minutes before the injection. At the start of each tracer injection, the influent lines containing the CaCl₂ carrier solu-
tion were clamped shut and the tracer-filled lines were immediately opened. During the injection, a sample of the tracer solution was collected and analyzed daily to record any change in the bacteriophage concentration in the reservoir.

Discharge from the four effluent ports at the top of the column was collected in a single tube that led to an ISCO (Lincoln, NE) automatic sampling fraction collector. Samples (usually 10 mL) were collected in sterile polypropylene centrifuge vials and placed in a refrigerator at 4°C within a few hours of collection to prevent die-off of the bacteriophage. After each tracer injection, 0.005 M CaCl₂ carrier solution was flushed through the column at the same flow rate as the tracer injection and samples of the effluent were collected and analyzed. All of the effluent samples were collected and weighed to detect any variations in flow rate and hydraulic gradient was monitored periodically during the experiments. Between experiments, which were up to a month apart, the 0.005 M CaCl₂ carrier solution was continuously flushed through the column at a slow rate to help remove the remaining tracers.

Tracers and Analytical Methods

Two types of bacteriophage, MS-2 and PRD-1, were used in the tracer solution as colloidal tracers. MS-2 is an icosahedral bacteriophage with a diameter of 0.026 μm and an isoelectric point of 3.9 (Powelson et al., 1993). *Escherichia coli* (ATCC 15597) was the host bacteria for MS-2. PRD-1 is an icosahedral lipid bacteriophage with a diameter of 0.062 μm and an isoelectric point of <4.5 (Powelson et al., 1993). The protein coat of PRD-1 contains lipids that cause it to be slightly more hydrophobic than MS-2. *Salmonella typhimurium* LT-2 was the host bacteria for PRD-1. Both bacteriophage were obtained from the Microbiology and Immunology Laboratory at the University of Arizona, Tucson.

The samples were assayed using the plaque forming technique described by Adams (1959) and Bales et al. (1991). The samples were diluted using a TRIS buffered saline solution. Three dilutions were plated for each sample, and plates containing 30 to 300 plaques were counted and recorded. Most samples were analyzed within 24 h of collection and samples with anomalously low values were retested the following day. The solute tracer, bromide, was analyzed on an Orion (Beverly, MA) Model EA 940 pH/ISE meter using a Corning (Corning, NY) Br⁻ specific electrode. The electrode was calibrated using a minimum of three prepared solutions with concentrations ranging from 0 to 800 mg L⁻¹ of bromide. To ensure consistency of the bromide data, all samples from one experiment were measured the same day using the same calibrated probe.

**RESULTS**

Miscible Displacement Experiments

Physical conditions and influent concentrations for the miscible displacement experiments are shown in Table 1. For the high flow rate experiments (3 and 4), both of which had injection periods lasting less than 2 d, the influent concentration of all the tracers remained essentially constant. For the two slower flow rate experiments (1 and 2), which had injection periods lasting 32 and 6 d, respectively, the bromide influent concentrations remained constant, but there were measurable declines in bacteriophage concentrations, especially for MS-2. The concentrations declined at rates ranging from 0.005 to 0.06 log cycles per day for PRD-1 and 0.04 to 0.12 log cycles per day for MS-2, and the declines were probably due to inactivation of the bacteriophage. These rates are within the ranges previously measured for PRD-1 and MS-2 in ground water (Yates et al., 1986, 1987; Schijven et al., 2000).

Breakthrough curves (BTC) for the tracers are shown on Fig. 2. The tracer concentrations are presented in terms of relative concentration, C/Cᵢ, where C is the measured concentration in the effluent and Cᵢ is the influent concentration. Background concentrations, which were typically 0.3 to 1% for the bromide tracer and 0.001 to 0.01% for PRD-1 and MS-2, were subtracted from both the measured and the influent concentrations prior to calculation of C/Cᵢ. For the cases in which influent concentrations declined with time (as described above), the Cᵢ value used for each effluent data point was determined from a linear regression of the log of the measured influent concentrations. This was extended into the recovery portion of the BTC, and acts as a correction for inactivation of the bacteriophage tracers during the experiment. Actual inactivation rates in the saprolite column may differ from rates measured in the influent reservoir, but this is a reasonable approximation.

First arrival of MS-2 and PRD-1 tracers in the effluent occurred within 0.014 to 0.037 PV of the start of each tracer injection, which corresponds to travel times ranging from 2 to 850 min and transport velocities ranging from 0.024 to 210 m d⁻¹ (Tables 2 and 3). In some cases, there were low background concentrations from previous experiments (see above), in which case first arrival was defined as the point at which the effluent concentration trend changed from declining to increasing for each tracer. Concentrations of MS-2 and PRD-1 tracers in the effluent continued to increase sharply for about the first 0.25 to 0.5 PV of injection and then either leveled off at a roughly constant “plateau” concentration (for flow rates of 0.039, 0.19, and 0.96 m d⁻¹ as shown on Fig. 2B–D) or continued to rise, but at a much slower rate (for flow rate of 0.0075 m d⁻¹, as shown on Fig. 2A). In the latter case, MS-2 and PRD-1 did

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**Table 1. Summary of miscible displacement experiments.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Flow rate (m d⁻¹)</th>
<th>Hydraulic gradient, /</th>
<th>MS-2 (PFU mL⁻¹)</th>
<th>PRD-1 (PFU mL⁻¹)</th>
<th>Bromide (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0075</td>
<td>0.001</td>
<td>3.66 x 10⁹</td>
<td>5.4 x 10⁹</td>
<td>724</td>
</tr>
<tr>
<td>2</td>
<td>0.039</td>
<td>0.005</td>
<td>4.9 x 10⁷</td>
<td>2.6 x 10⁷</td>
<td>750</td>
</tr>
<tr>
<td>3</td>
<td>0.19</td>
<td>0.03</td>
<td>4.3 x 10⁷</td>
<td>6.6 x 10⁷</td>
<td>950</td>
</tr>
<tr>
<td>4</td>
<td>0.96</td>
<td>0.1</td>
<td>4.0 x 10⁶</td>
<td>3.5 x 10⁶</td>
<td>764</td>
</tr>
</tbody>
</table>

† Concentrations were constant for all cases except for MS-2 and PRD-1 in Experiments 1 and 2, where values declined due to inactivation at rates of 0.005 to 0.12 log cycles per day (see text). For these cases the initial concentration values are shown.
eventually approach plateau concentrations after approximately 1.0 to 1.5 PV of injection, as shown in Table 2. Generally, the relative concentration of the plateaus for both PRD-1 and MS-2 tended to increase with flow rate. Concentrations of bromide increased throughout the injection portion of each experiment and although

Table 2. Summary of breakthrough times and concentrations.

<table>
<thead>
<tr>
<th>Experiment and tracers</th>
<th>First arrival of tracer</th>
<th>Arrival at plateau concentration</th>
<th>Approximate plateau concentrations†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pore volumes</td>
<td>C/C₀</td>
<td></td>
</tr>
<tr>
<td>Experiment 1, flow = 0.0075 m d⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-2</td>
<td>0.037</td>
<td>1.5</td>
<td>0.03</td>
</tr>
<tr>
<td>PRD-1</td>
<td>0.037</td>
<td>1.5</td>
<td>0.10</td>
</tr>
<tr>
<td>Bromide</td>
<td>0.11</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Experiment 2, flow = 0.039 m d⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-2</td>
<td>0.016</td>
<td>0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>PRD-1</td>
<td>0.014</td>
<td>0.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Bromide</td>
<td>0.046</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Experiment 3, flow = 0.19 m d⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-2</td>
<td>0.022</td>
<td>0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>PRD-1</td>
<td>0.024</td>
<td>0.3</td>
<td>0.70</td>
</tr>
<tr>
<td>Bromide</td>
<td>0.065</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Experiment 4, flow = 0.96 m d⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-2</td>
<td>0.03</td>
<td>0.5</td>
<td>0.60</td>
</tr>
<tr>
<td>PRD-1</td>
<td>0.03</td>
<td>0.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Bromide</td>
<td>&lt;0.11</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

† Bromide did not reach a plateau or constant concentration during any of the tracer injections.
they appeared to asymptotically approach a relative concentration of one, this value was not reached in any of the experiments.

During the flushing (or elution) portion of each BTC, there was a rapid initial decline in concentration of both PRD-1 and MS-2 (Fig. 2). The rapid initial drop in bacteriophage concentration was followed by a period of slowly declining values. Bromide concentrations also declined during the flush portions of the experiments, but at much slower rates than observed for the bacteriophage tracers. Following the lowest flow rate experiment, the elution rate was abruptly increased from 0.0075 to 0.96 m d\(^{-1}\) and the effluent monitored for recovery of bacteriophage (Fig. 3). As a result of this sharp increase in flow rate, the concentration of PRD-1 increased by a factor of 50, and then slowly declined. In contrast, the concentration of MS-2 appeared to only increase slightly, and then continue to decline.

The proportion of tracers recovered from each experiment was calculated by dividing the mass of tracer recovered during the first 4 PV of each experiment divided by the mass of tracer injected during the 2 PV injection period (Fig. 4). Recovery of PRD-1 and MS-2 during the portion of the flush beyond 4 PV was expected to be minimal because their concentrations were both very low (\(C/C_0\) of 0.000002 to 0.0007) by 4 PV. For Experiments 1 and 2, where there was evidence of significant inactivation of PRD-1 and MS-2 during the experiment, the recovery values were also calculated from the relative concentration BTCs (Fig. 2A,B), which include a correction for inactivation in both the influent and flushing portions of the experiments. This method yielded PRD-1 and MS-2 recovery values at the two slowest flow rates that were slightly higher (<1 to 15%) than the values that were calculated directly from the concentration measurements, but still showed the same trend toward increasing recovery with increasing flow rate. Recovery of MS-2 increased steadily, from <1 to 58%, as flow rate increased (Fig. 4). For PRD-1 there was a sharp initial increase in recovery as flow rate increased, but at the two highest flow rates the recovery was constant at approximately 58%.

Recovery values for bromide were much higher than the bacteriophage tracers and increased slightly, from 86 to 91%, as the flow rate for each experiment increased (Fig. 4). The bromide recovery values are incomplete because at the end of the detailed monitoring period of each experiment.
period (at 4 PV) the relative concentrations were still relatively high (2–14%). These concentrations were less than 1% when monitoring was restarted just before each new injection, so it is expected that almost all of the bromide was eventually recovered.

**Hydraulic Conductivity**

Local hydraulic conductivity values were calculated using Darcy’s law for each pair of piezometer pairs in the wall of the column, and for each of the three flow tests carried out before the tracer tests. For individual piezometer pairs, the calculated value of hydraulic conductivity remained relatively constant (within a factor of three) as flow rate increased. Comparison of values from different piezometer pairs at the same flow rate shows that hydraulic conductivity varied spatially within the sample by a factor of up to six, with most of the values varying by a factor of less than two. This relatively narrow range of variation indicates a high degree of interconnectedness of fractures in the saprolite column. The mean and geometric mean of all the local hydraulic conductivity values were 15.2 and 13.5 m d$^{-1}$, respectively. These values were approximately 2.5 times higher than the bulk hydraulic conductivity, which was measured based on the hydraulic gradient between the inflow and outflow of the column after completion of the tracer experiments. The differences may be due to a decline in hydraulic conductivity over the period of the tracer experiments, but could also be due to low hydraulic conductivity regions within the interior of the sample or head losses in the end caps. Subsequent tests, in which local and bulk hydraulic conductivity were measured at the same time, also showed values differenced by a factor of 1.3 to 2.4 times, suggesting that the locally measured values were not entirely representative of the bulk hydraulic conductivity of the sample.

**DISCUSSION**

**Bacteriophage and Bromide Transport**

The rapid arrival of all the tracers, at pore volumes of much less than one, clearly shows that flow was occurring through only a small portion of the total porosity (Table 2). First arrival of PRD-1 and MS-2 occurred earlier than first arrival of the bromide by a factor of three to six times, but this is at least partly due to differences in detection limits. The rapid first arrival of the bacteriophage tracers, followed by attainment of a relatively constant plateau concentration, which usually occurs at substantially less than one pore volume, is consistent with a conceptual model of advective transport occurring mainly through the fractures and macropores. This is similar to what was observed in other colloid tracer experiments in columns of shale saprolite (Haun, 1998; Cumbie and McKay, 1999) and fractured glacial clays (Hinsby et al., 1996). By comparison, saturated flow transport experiments in columns of granular soils typically show first arrival of colloidal tracers occurring at pore volumes of 0.2 to 0.8 and peak (for short pulse experiments) or plateau concentrations (for constant concentration influent) being reached within 1 to 1.5 PV (Wollum and Cassel, 1978; Tan et al., 1994; Kretzschmar et al., 1997).

Bromide was also initially transported primarily through the fractures, as indicated by its rapid first arrival. However, unlike the bacteriophage tracers, concentrations of bromide in the effluent continued to slowly increase throughout each injection and asymptotically approached the injection concentration. Also, the bromide concentrations tended to decline much more slowly than the bacteriophage during the flush portion of each BTC. The slower rise and decline of bromide concentrations is believed to be due to diffusion-controlled transfer between the rapidly moving water in the fractures and the relatively immobile pore water in the fine-grained matrix between the fractures. Previous field, laboratory, and modeling investigations have shown that this mechanism, which is often referred to as either interregion diffusion or matrix diffusion, plays an important role in controlling solute transport in fractured, clay-rich materials (Grisak et al., 1980; Harrison et al., 1992; Reedy et al., 1996; McKay et al., 1997). Interregion diffusion is expected to be much less significant for microorganisms and other colloidal particles, because of their larger size and slower diffusion coefficients (Bales et al., 1989; McKay et al., 1993a; Harvey, 1997). This was demonstrated in an experiment by Cumbie and McKay (1999), where they observed that the vast majority of microspheres retained in shale saprolite were found along the walls of fractures, with only a few found in the matrix.

The trends toward increasing relative plateau concentration and higher percentage of tracer recovered for MS-2 as flow rate increased were consistent with the results of transport studies by Wollum and Cassel (1978), Wang et al. (1981), Tan et al. (1994) and Kretzschmar et al. (1997), which used a variety of mineral and microbial colloids in granular soils. These findings were also consistent with the study of *Escherichia coli* transport through fractured or macropore-dominated silt loam soils by Smith et al. (1985), which showed higher losses at lower flow rates. The behavior of PRD-1 was in general agreement with the above studies, but also indicated that particle losses were largely independent of flow rate above a flow rate of about 0.039 m d$^{-1}$. This could be responsible for the lack of a strong correlation between flow rate and colloid loss observed by Kretzschmar et al. (1995) in their study of transport of Fe and Al colloids in a macroporous saprolite derived from crystalline bedrock.

**Fracture Aperture, Effective Porosity, and Flow Velocity**

Effective porosity values, $n_e$, which represent the porosity of the fractures and macropores accessible to the bacteriophage, were calculated by dividing the volume of flow at which the bacteriophage first arrived in the effluent, or at which it reached a plateau concentration, by the total volume of the column (Table 3). Effective porosity values calculated from the first arrival of the
bacteriophage ranged from 0.0067 to 0.013, which were 30 to 60 times lower than the total porosity (0.4) of the column. The effective porosity values calculated for the plateau concentration ranged from 0.11 to 0.40. This confirms that the bacteriophage traveled mainly through a relatively small portion of the total porosity of the material.

It was assumed that the time (and effluent volume) of the initial arrival of the bacteriophage in the effluent, and the time at which the bacteriophage reached a plateau concentration, represent the approximate travel times in the fastest and slowest pathways, respectively, for advective transport of bacteriophage through the saprolite. Using the initial arrival and plateau times for each bacteriophage (MS-2 and PRD-1) and the length of the column (29 cm), transport velocities were calculated for the tracer experiments at each flow rate (Table 3). Transport velocities for the bacteriophage tracers ranged from 0.49 to 210 m d\(^{-1}\), based on the initial arrival time, and from 0.02 to 4.6 m d\(^{-1}\), based on the time to reach plateau concentration. The first arrival velocities are comparable with measured values of 11 to 56 m d\(^{-1}\) for first arrival of MS-2 and PRD-1 from a natural gradient field tracer experiment in lithologically similar saprolite at a nearby site at ORNL (McKay et al., 2000).

Fracture aperture values and flow velocities, based on the cubic law (Snow, 1969) for flow through smooth-walled fractures with uniform aperture, were also calculated for comparison with the measured bacteriophage transport velocities. These calculations assume that all fractures can be represented as an equivalent system of smooth-walled fractures, and since many of the casts, which are the dominant macropores, occur along fracture surfaces, it is reasonable to lump them together. Fracture aperture, \(2b\), was calculated assuming that flow occurred through both the bedding plane fractures and the fractures cutting across bedding and that fracture spacing was the same (approximately 0.02 m) in both directions. These assumptions are supported by a recent study (Cumbie and McKay, 1999) in which fluorescent microspheres were injected through a column of saprolite from the same site. The column was dismantled and examined under UV light, and the microspheres were found on approximately half of the bedding plane fractures and nearly all of the cross-bedding fractures. This resulted in a nearly equal spacing of “active” fractures in both directions. The value of fracture aperture, \(2b\), for a system of two sets of equally spaced vertical parallel fractures, oriented orthogonal to one another, was calculated using:

\[
(2b)^3 = (K_b - K_m) \frac{6\mu b}{\rho_w g} \]  

[1]

where \(K_b\) is the bulk vertical hydraulic conductivity of the column (13.5 m d\(^{-1}\), based on the geometric mean of the local hydraulic conductivity measurements), \(K_m\) is the matrix hydraulic conductivity (assumed to be zero), \(2B\) is the fracture spacing (0.02 m), \(\mu\) is the kinematic viscosity of water, \(\rho_w\) is the density of water, and \(g\) is the gravitational constant (McKay et al., 1993b). Values for \(\mu\) and \(\rho_w\) were taken from published values for pure water (Daugherty and Franzini, 1977). Using this equation, the calculated fracture aperture for the saprolite column was 1.3 \(\times\) 10\(^{-4}\) m (130 \(\mu\)m).

The fracture flow velocity was calculated using:

\[
v_i = \frac{i (2b)^2 \rho_w g}{12\mu} \]  

[2]

where \(v_i\) is the calculated fracture flow velocity and \(i\) is the average measured hydraulic gradient for each experiment. Calculated fracture flow velocities based on the cubic law ranged from 0.9 to 90 m d\(^{-1}\) and were within a factor of 0.4 to 1.8 of the transport velocities calculated based on first arrival of the bacteriophage (Table 3). This suggests that the cubic law may be appropriate for predicting first arrival of colloid tracers, at least in this fractured saprolite. The calculated fracture flow velocities were substantially faster (12 to 45 times) than transport velocities based on time or number of pore volumes to reach plateau concentration. This is reasonable, because the time to reach a plateau concentration is influenced by transport through the smaller, slower flow rate fractures and macropores.

### Bacteriophage Loss Mechanisms

Possible causes of the bacteriophage losses include inactivation, straining, gravitational settlement, and electrostatic or hydrophobic attachment to mineral surfaces or soil organic matter. It is not possible to fully evaluate the causes of all of the losses of MS-2 and PRD-1 within the limitations of this set of experiments and the scope of this paper, but several factors are discussed in the following section.

The influence of inactivation on transport of MS-2 and PRD-1 was examined by comparing bacteriophage residence times within the column to the measured decay rates for these bacteriophage strains. Calculated residence times of the bacteriophage in the sample for Experiment 1 (flow rate of 0.0075 m d\(^{-1}\)) ranged from 0.6 to 49 d, based on first arrival and time to reach plateau concentration, respectively. Residence times for Experiments 2, 3, and 4 were each less than 1, 0.2, and 0.06 d, respectively. For PRD-1 and MS-2 deactivation rates measured in this study, and in other studies in ground water by Yates et al. (1986, 1987), these residence times were too small to have any significant effect on concentrations, except for the experiment at the slowest flow rate.

Straining, meaning losses due to physical lodging of particles in fractures or pore throats that are smaller than the diameter of the particles, is not expected to be influenced by flow rate (Harvey, 1991). Experiments 3 and 4, which had the two highest flow rates, had about the same plateau concentration, 50 to 70% of the influent concentration, and the same recovery, 60%, for PRD-1. MS-2 also showed losses of 40 to 50% at the highest flow rate. These values indicate that about 30 to 50% of the bacteriophage losses were independent of flow rate, and hence could potentially be due to
straining. However, this value appears to be unreason-
ablelly high, given the calculated hydraulic fracture aperture
of 130 μm, which is 2000 to 5000 times larger than the diameter of the bacteriophage tracers, and suggests that factors other than straining in fracture or pore throat constrictions might contribute substantially to retention at high flow rates. These could include factors related to roughness of fracture or macropore surfaces.

Gravitational settling is expected to be related to resi-
dence time of bacteriophage in the column, and hence would be influenced by flow rate. This was evaluated by calculating the distance the bacteriophage tracers could have settled during their residence times in the column. The overall direction of flow in the column was upward, which should reduce the importance of settling, but there may have been enough tortuosity in the actual flow paths, or enough asperities along the fracture walls, to allow for significant losses due to settling. Settling rates were calculated for MS-2 and PRD-1 using the following equation for the settling rate of a sphere in a viscous fluid:

\[ v_s = \frac{d^2 g (\rho - \rho_w)}{18 \mu} \]  

where \( v_s \) is the settling velocity, \( d \) is the diameter of the particle, \( g \) is the gravitational constant, \( \rho \) is the density of the particle, \( \rho_w \) is the density of water, and \( \mu \) is the dynamic viscosity of water (Kane and Sternheim, 1988). For PRD-1 and MS-2, with diameters of 0.062 and 0.026 μm, respectively, and a density of approximately 1.385 g cm\(^{-3}\) (Olsen et al., 1974), the calculated settling velocities in water at 20°C were approximately 70 and 12 μm d\(^{-1}\). For the estimated bacteriophage residence times corresponding to first arrival of the tracers (Table 2), the maximum settling distance for PRD-1 ranged from 0.4 μm at the highest flow rate to 41 μm at the slowest flow rate. For MS-2, the settling distances ranged from <0.1 to 7 μm for the fastest and slowest flow rates, respectively. Settling distances corresponding to the residence time for arrival to plateau concentrations (Table 2) were substantially larger, ranging from 4 to 1700 μm for PRD-1, and 0.8 to 300 μm for MS-2. Since the calculated equivalent fracture aperture value for the sample was only 130 μm, it appears plausible that gravitational settling could influence bacteriophage retention, especially for PRD-1 at the slower flow rates. However, it would be very difficult to distinguish this from electrostatic or hydrophobic attachment to fracture walls (see below), which also tends to be greater at slow flow rates.

Electrostatic or hydrophobic attachment of the bacteriophage to the walls of the fractures appears to be one of the dominant processes for retention in the saprolite. This is based partly on a process of elimination, since the other retention processes discussed above appear insufficient to cause the observed degree of retention. The larger bacteriophage losses observed at slower flow rates are also consistent with a conceptual model of electrostatic or hydrophobic attachment, because at slower flow rates there is a greater probability of diffu-
sion-controlled collisions with the walls of fractures (Yao et al., 1971; Elimelech and O’Melia, 1990). The diameters of PRD-1 and MS-2 (0.062 and 0.026 μm, respectively) differ by a factor of two, and because diffusion coefficients for colloidal particles are related to the square of their diameter (Einstein and Furth, 1926), the smaller particle (MS-2) should have a substantially higher probability of colliding with a fracture wall. This is consistent with the experimental data, which indicated higher losses of MS-2 relative to PRD-1 at the slower flow rates, but about the same amount of loss at the highest flow rate. Both of the bacteriophage strains have diameters substantially smaller than the optimum particle size of 0.5 to 1.0 μm determined for latex microspheres in shale saprolite by Haun (1998) and Cumbe and McKay (1999), which indicates that they are probably in the size range where hydrophobic and electro-
static attachment dominate over gravitational settlement and straining. However, this evaluation is not conclusive, because there are other factors that influence retention of bacteriophage, such as differences in attraction to soil minerals and organic matter. In experi-
mental studies in granular materials, Kinoshita et al. (1993) observed greater losses of PRD-1 than MS-2, and concluded that attachment of PRD-1, which is slightly more hydrophobic than MS-2, was more strongly influenced by the organic content of the soil than by pH of the solution. The effluent from the saprolite column was chemically well buffered, with a fairly constant pH of 4.3, which was slightly above the isoelectric point of both PRD-1 (3–4 [Bales et al., 1991], <4.5 [Powelson et al., 1993]) and MS-2 (near 3 [Zerda, 1992], 3.9 [Powelson et al., 1993]). Based on this, both PRD-1 and MS-2 would be neutral or have weak negative charges, which should result in low attraction to the mineral surfaces.

The experiments, which involved repeated injections of bacteriophage tracers in the same column, show little evidence of “blocking” or “ripening,” which are changes in the tendency of particles to be retained due to the presence of previously retained particles. In all but the slowest flow rate experiment, the bacteriophage tracers reached a relatively constant plateau concentration early in the injection, which could not have occurred if significant blocking or ripening was occurring during the time frame of the individual experiments. In Experiment 1, which was at the slowest flow rate, there were gradual increases in bacteriophage concentration after the initial rapid rise in concentration, but it is not clear whether this was due to blocking or to other factors such as migration in smaller pore classes.

Changing flow rates during the flush portion of the experiment (Fig. 3) appears to have some influence on remobilization of retained bacteriophage, especially for PRD-1, but only a very small fraction of the retained bacteriophage were released by increasing flow rate. Particles initially retained by either gravitational settling or electrostatic–hydrophobic attachment could be re-
mobilized by an increase in flow rate. Larger particles would be subjected to greater changes in shear forces, and hence would have a greater tendency to dislodge.
This is consistent with the experimental data, which showed greater remobilization of the larger bacteriophage, PRD-1.

**CONCLUSIONS AND IMPLICATIONS**

Transport and losses of both MS-2 and PRD-1 in a fractured shale saprolite were strongly influenced by the rate of saturated flow in fractures and macropores. In the case of MS-2, the relationship between the amount of loss and the flow rate was nearly linear. For PRD-1, there appeared to be a threshold flow rate, in this case about 0.039 to 0.19 m d⁻¹, above which losses were independent of flow rate. Flow rate also weakly influenced the release of attached bacteriophage. First arrival of all the tracers occurred at pore volumes much less than one, indicating that flow is dominated by fractures or macropores. Estimates of fracture flow velocity, calculated using the cubic law (Snow, 1969) with measured values of hydraulic conductivity and fracture spacing, were very similar to transport velocities determined from first arrival of the bacteriophage tracers, which confirms the importance of fracture or macropore flow.

Several retention mechanisms, including electrostatic or hydrophobic attachment to fracture or macropore walls, gravitational settling, and physical straining (lodging in fractures or pore throats smaller than the diameter of the bacteriophage), all appeared to contribute to losses of bacteriophage during the experiment. About 50% of the bacteriophage were retained at the highest flow rate, which indicates that processes that are independent of flow rate, for example physical straining, play a significant role. However, because the diameter of the bacteriophage particles was 2000 to 5000 times smaller than the cubic law estimated fracture aperture size (130 μm), it is likely that other retention processes are also significant at high flow rates.

This study shows that very rapid transport of microorganisms (and probably other colloidal contaminants or contaminants attached to mobile colloids) can occur in fractured shale saprolite. These soils are widespread in humid, temperate to tropical climates and in many areas that are used for disposal of wastes through burial (landfills) or infiltration (septic fields and sewage ponds). It is possible that microbial contamination from these sources could affect nearby ground water and surface water supplies. The study also has implications for bioremediation efforts in these types of materials, because Harvey. R.W. 1991. Parameters involved in modeling movement of bacteriophage in groundwater. p. 89–114. In Modeling the environmental fate of microorganisms. Am. Soc. Microbiol., Washington, DC.

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