A Field Example of Bacteriophage as Tracers of Fracture Flow

Larry D. McKay* and John A. Cherry

Waterloo Center for Groundwater Research, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Roger C. Bales

Department of Hydrology and Water Resources, University of Arizona, Tucson, Arizona 85721

Moysar T. Yahya and Charles P. Gerba

Department of Microbiology and Immunology, University of Arizona, Tucson, Arizona 85721

Two bacteriophages, MS-2 and PRD-1, were added to water to an initial concentration of \(10^5-10^6\) pfu mL\(^{-1}\) in a trench-to-trench lateral groundwater flow and tracer migration experiment in the upper 5.5 m of a weathered and fractured clay-rich till. Phage were detected in water from seepage collectors set in the wall of a downgradient (gradient = 0.24) trench, located 4 m from the source trench, between 1 and 2 days after the start of the injection. Peak phage concentrations in the collectors were typically \(10^8-10^9\) pfu mL\(^{-1}\), and detectable phage (>0.1 pfu mL\(^{-1}\)) persisted for up to 5 days. In contrast, the travel time for bromide in the same trenches was several months. The colloidal phage are believed to have moved mainly through fractures with little diffusion into pores of the clay matrix. Phage attenuation was due to inactivation and either attachment to the fracture walls or diffusion into the larger pores. At the temperature of the current experiment (10-12 °C), phage inactivation rates were sufficiently low to permit their use as groundwater tracers over periods of several days.

Introduction

The transport velocity by groundwater of nonreactive solute tracers in a fractured fine-grained porous medium, such as weathered clay, is retarded relative to flow in the fractures by diffusion into the porous matrix (1). The degree of solute retardation, which is a critical factor in assessing contaminant migration, is difficult to determine because of the inability to directly measure flow velocity. Colloidal particles should not be as strongly retarded by matrix diffusion because they are excluded from much of the porous matrix by virtue of their size. Hence, colloidal-sized tracers can be used as an indicator of the lower limit of flow velocity. In cores of fractured tuff from Nevada, the travel time of bacteriophage on the order of 20-30 nm in size has been shown to be about 1.5-2.5 times as long as estimated for flow in fractures alone, but approximately 3 times faster than a nonreactive solute subject to significant diffusion from fractures into the porous but low-permeability matrix (2). Virus (2) and bacteria (3) have also been observed to move ahead of nonreactive salt tracers in unconsolidated media, and it is expected that the use of bacteriophage in tracer experiments can shed light on the behavior of these organisms in groundwater systems.

Four concerns in choosing colloidal particles for use as tracers in fractured geologic media are (i) loss due to diffusion from the fractures into and out of the porous matrix (i.e., matrix diffusion), (ii) retardation due to sorption, (iii) loss due to decay or inactivation, and (iv) detection limit. Bacteriophage are in the size range of 20-80 nm and are large enough to be excluded from the porous matrix in many cases of interest in fractured media (2). An individual species or strain of phage is also very uniform in size and shape. Many bacteriophage are sufficiently hydrophobic that they attach and adhere poorly to mineral surfaces (4,5). A more hydrophobic phage would be expected to be retarded to a greater extent in groundwater than would a less hydrophilic phage. In media with a high mass fraction of organic carbon, the opposite may occur. The rate of phage inactivation in the environment, or loss of ability to infect a host, depends largely on temperature (6). Below about 10-12 °C, phage inactivation is sufficiently slow to permit their use in week-long to month-long experiments (6). Below about 5-7 °C, inactivation is minimal. The detection limit for most bacteriophage is one plaque-forming unit (pfu), corresponding to one phage in a sample. With about 10 mL as a reasonable upper limit on sample size, the detection limit is about 0.1 pfu mL\(^{-1}\) or 100 pfu L\(^{-1}\). Phage can be concentrated to about \(10^{13}\) pfu mL\(^{-1}\); \(10^{13}-10^{14}\) phage is a reasonable injection dose considering preparation time and cost. This allows dilutions over several orders of magnitude to be detected in field samples.

The objectives of the work reported in this paper were (i) to determine the magnitude of the flow velocity in the shallow groundwater zone of a typical weathered and fractured clay till deposit using bacteriophage as a tracer and (ii) to compare the velocity indicated by phage tracers to solute transport velocities. A secondary objective was to demonstrate the utility of phage as tracers in fractured media under field conditions.

Field Site

The field site was located at the Laidlaw industrial waste treatment and disposal facility, near Sarnia in southwestern Ontario. The site was chosen to evaluate the potential for rapid movement of groundwater and contaminants in the visibly weathered zone. The combination of high lateral hydraulic gradients adjacent to landfill mounds, road ditches, or drainage tiles together with the presence of fractures as possible conduits for groundwater flow provide the potential for rapid groundwater movement in the visibly weathered zone. The site is underlain by 40 m of clay-rich (25-45%) glacial deposits, the upper 6 m of which are visibly weathered and fractured, and it was in this shallow zone that the tracer experiments were performed. In the weathered zone, the clay-sized materials...
The experimental setup consisted of three parallel trenches, each approximately 7 m long, 1.8 m wide, and 5.5 m deep. Water-containing solute or colloid tracers was added to the central (or source) trench, and tracer migration was monitored by sampling of piezometers or seepage collectors set into the walls of the receiver trenches, at distances of 4 and 6.2 m from the source (Figure 1). The hydraulic gradient between trenches was maintained at 0.24 by adding water to the source trench and pumping from the receiver trenches. Each trench was back-filled with 1-cm diameter well-sorted pea gravel and covered with a roof.

A 2-year duration solute tracer experiment was begun in August 1988 using bromide as the principal nonreactive tracer (11). The source trench was maintained as a constant-concentration, constant-head boundary throughout the experiment, and migration of the bromide was monitored in the piezometers and seepage collectors. Near the end of the solute tracer experiment, but while it was still in progress, two bacteriophages, MS-2 and PRD-1 were added to 17 000 L of the bromide tracer solution in the steel “injection” tank adjacent to the source trench to obtain an initial concentration of 10^5-10^6 pfu mL^-1. Iodide (as NaI) was also added as a nonreactive tracer to obtain an initial concentration of 9 mg L^-1. The iodide was expected to migrate slowly (due to matrix diffusion) and was used mainly to indicate whether there was any cross-contamination due to the sampling procedure. Makeup water for the tracer solution came from a municipal distribution system that draws its water from Lake Huron, and the water is treated with chlorine. Residual chlorine levels were monitored with a colorimetric field test 1 week prior to addition of the bacteriophages, with all samples being below the detection limit of 0.02 mg L^-1, except one which was at the limit. To ensure that no residual chlorine remained, 2 mg L^-1 of sodium thiosulfate was added to both the source trench and the injection tank 1 day prior to adding the phage. The injection tank and source trench water had pH's of 6.8-7.4 and temperatures of 9.5-12.5 °C.

Approximately 14 000 L of the tracer solution was flushed through the 17 500-L pore volume of the source trench and was then mixed by pumping between wells set in the gravel. Over the following 9 days, tracer solution was added to the source trench at a rate of approximately 250 L day^-1 to maintain a constant water level. Following the initial flushing and mixing, the concentrations of MS-2 and PRD-1 in the source trench were both 2 x 10^5 pfu mL^-1 and the iodide was 7 mg L^-1.

In the receiver trenches RT1 (6.2 m from the source trench) and RT2 (4.0 m from the source), 32 of the 70 seepage collectors were sampled for bacteriophage before the experiment and every 1-2 days during the first 7 days of the tracer injection. Samples were also taken from the steel injection tank and the source trench. Samples were collected in 15-mL polystyrene vials and refrigerated on site at 4 °C. Every 2-3 days the samples were packed in ice and shipped by overnight delivery to the University of Arizona Microbiology and Immunology Laboratory. Assays for both MS-2 and PRD-1 were begun immediately after receipt. Both phage were assayed by the plaque-forming-unit method described by Adams (12). Serial dilutions were made from the water samples in sterile Tris buffer, added to test tubes containing 3 mL of molten overlay agar and 1 mL of 3-6-h culture of the host bacteria, and poured onto TSA plates. The plates were incubated for 18-24 h at 37 °C, after which the plaques were counted.

Samples for determination of bromide and iodide concentration (in polyethylene bottles) were taken from the trenches/collectors and from piezometers located between the trenches. The bromide and iodide analyses were performed by ion chromatography at the University of Waterloo Aqueous Geochemistry Laboratory.

**Description of Bacteriophage Tracers**

Bacteriophage MS-2 is an icosahedral phage with a diameter of 0.020-0.026 µm (13) and pH_{biol} of 3.9 (14). The surface of MS-2 contains hydrophobic and hydrophilic portions (14). MS-2 (ATCC 15597 B-1) was obtained from the University of Arizona Department of Microbiology and Immunology culture collection. Bacteriophage PRD-1 is an icosahedral lipid phage with a diameter of 0.062 µm.
Background concentrations of the tracers bromide, iodide, PRD-1, and MS-2 were all below their respective detection limits before the experiments began. During the 2-year duration of the solute-tracer portion of the experiment, bromide concentrations in the source trench remained nearly constant at 148 mg L\(^{-1}\). Initial breakthrough of the bromide tracer (at \(C/C_0 = 0.01\)) was observed in all piezometers and seepage collectors in the highly fractured zone (upper 3 m) within 30–200 days of the start of the experiment (11). Solute transport velocities (at \(C/C_0 = 0.01\)) range from 0.01 to 0.07 m day\(^{-1}\), and a typical bromide breakthrough curve for one of the seepage collectors is shown in Figure 2.

Phage concentrations in the injection tank remained relatively constant over the 5 days of the injection (Figure 3). This low rate of inactivation was consistent with phage survival experiments, which showed declines of 11.5 and 10.2% (0.03 and 0.047 log unit) per day for PRD-1 for the June 21 and June 28 samples, respectively; corresponding log reductions for MS-2 were 19.9 and 10.1% (0.096 and 0.046 log unit) per day. The 95% confidence intervals were ±1.2% and ±0.9% for PRD-1 and ±0.8 and ±2.0% for MS-2 for the two dates.

Phage concentrations in the source trench declined about 90% (1 log unit) per day (Figure 3), apparently due to a combination of inactivation and attachment to gravel. Because of this decline, the trench provided a phage tracer slug rather than a constant-concentration boundary. Iodide concentrations in the source trench remained constant at approximately 7 mg L\(^{-1}\).

Phage were detected in RT2 (4 m from the source trench) within 1 day of beginning the injection (Figure 4). The first arrival was observed in a seepage collector at a depth of 2.1 m and in a standpipe in the gravel back-fall at a depth of 1.6 m. By the second day, phage arrival was observed in 7 of the 18 seepage collectors sampled in RT2.
Discussion

The phage breakthroughs correspond to transport velocities ranging from 2 to >5 m day⁻¹. Concentrations peaked in most of the collectors at 1.76 days and remained above detection limits up to 6 days after starting the injection. The highest PRD-1 concentrations detected in seepage collectors were 10²–10⁴ pfu mL⁻¹, which represents a 10²–10⁴-fold attenuation of the original input concentration. Lower concentrations in subsequent samples reflected decreases in source trench concentrations and any losses in the 4-m travel distance between the two trenches. MS-2 concentrations in the seepage samples were about 10 times lower than were PRD-1 concentrations. No phage were detected in the second trench, RT1, which was 6.2 m from the source trench.

As expected, none of the samples from the receiver trenches or the piezometers (taken up to 13 days after the start of the injection) had detectable (>0.02 mg L⁻¹) concentrations of the iodide tracer that was added with the phage.

Discussion

The large contrast in transport velocity (Table I) between the phage (2–5 m day⁻¹) and the bromide (0.01–0.07 m day⁻¹) is believed to be due to extensive diffusion of the solutes but not the phage into the pore water of the clay matrix. Measurements of solute concentration in matrix pore water from core samples taken during the experiment confirmed that most of the bromide was in the matrix rather than in the fractures (11). The phage are expected to be physically excluded from much of the matrix by virtue of their large diameter relative to the size of many of the matrix pore throats. Based on mercury porosimetry measurements in reconstituted and undisturbed clay samples from a site in the same clay till several kilometers away, 30–50% of the matrix pore throats are smaller than 0.065 μm (diameter of PRD-1) and 20–40% are smaller than 0.026 μm (diameter of MS-2) (17, 18).

However, the phage are small relative to hydraulically determined fracture apertures, 80% of which fall within a 5–30-μm range and could be transported at velocities approaching those of flow in the fractures (9).

The volume of tracer solution flushed through the field cells (2.4 m³ over a 9-day period) is equivalent to approximately 60 times the fracture pore volume (0.04 m³) but only 3% of the matrix pore volume (86 m³) of the portion of the field cells below the water table.

The 10³–10⁴ attenuation of phage between source and receiver trench at 4-m distance was several fold greater than can be accounted for by inactivation. This attenuation corresponds to approximately 1 log cycle per meter of travel. Attachment of phage to the fracture walls and limited diffusion into the larger pores are believed to be the principal causes of the decrease. Part of the 10-fold difference between the MS-2 and PRD-1 breakthrough concentrations in RT2 could be due to greater matrix diffusion of the smaller phage.

The attenuation of the solute (bromide) relative to the phage, by a factor of approximately 100, is much greater than that observed for laboratory experiments with fractured tuff from Nevada (attenuation factor of 3) (2). The lesser attenuation in the fractured tuff may be partly due to the larger aperture, 57–133 μm, and to the slightly larger matrix pore throats, two-thirds of which were greater than 0.1 μm.

The lack of detectable phage in RT1 is expected to be due to further attenuation over the longer travel distance (6.2 m for RT1 versus 4 m for RT2). At the attenuation rate observed in RT2 of approximately 1 log cycle per meter of travel, the phage would have been at or below the detection limit upon reaching the 6.2-m trench. Transport of the phage may also have been influenced by minor differences in the two flow systems. Measured values of hydraulic conductivity, hydraulic gradient, and fracture spacing indicate that the flow systems between the different trenches were very similar (9), but the influence of hydrogeological differences cannot be ruled out.

Conclusions

Bacteriophage behaved markedly different from dissolved tracers in water-saturated fractured clay, with phage travel times on the order of 0.5–1% of those for a conservative solute (bromide). The colloidal phage were thought to move mainly through fractures with minimal diffusion into the porous matrix of the clay, analogous to size-exclusion chromatography. Colloids were attenuated, however, suggesting either attachment to the fracture walls or limited diffusion into larger pores. This experiment provides the first reliable indication of the magnitude of flow velocity in fractures in clay and, even given the uncertainty concerning phage attenuation, it is clear that flow must be at least as fast as the movement of the phage (generally 2–5 m day⁻¹).

The experiment shows that there is potential for rapid migration in fractured clays of colloid-sized contaminants and of contaminants adhering to colloids. In such deposits, which are often of low hydraulic conductivity, contaminants are usually expected to migrate very slowly.

Under the temperature of the current experiment (10–12 °C), phage inactivation rates were sufficiently low to permit their use as groundwater tracers over several days. A longer experiment would require daily to biweekly additions of phage solution to mitigate inactivation in the source water or the use of higher input concentrations. Given preparation times on the order of 1–2 days for the phage stock used in this experiment, further use of bacteriophage as a sensitive, flexible field tracer appear quite feasible.

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