NATIONAL WATER RESEARCH INSTITUTE

Final Report

Project Number:	HRA 699-514-92
Project Title:	Groundwater Transport of Viruses
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Date Report Prepared:	February 24, 1996

Technical Accomplishments:

The research conducted over the project period, January 1992 to December 1994, studied virus transport at three experimental scales: small-scale columns, intermediate-scale aquifer tank, and field-scale injections. The small-scale column studies improved our understanding of the interactions between viruses and mineral surfaces. The intermediate-scale aquifer tank experiments tested the role of physical and geochemical heterogeneities on virus transport in a reproducible, controlled setting. The field-scale experiments, conducted at the U.S. Geological Survey Cape Cod Toxic Substances Research Site in Massachusetts, revealed the effect of organic matter on virus removal by attachment and inactivation in a real aquifer. In the field test, the use of radiolabeled bacteriophage provided the *unique* opportunity to study the transport of both the total and infective virus populations.

Small-Scale Columns: Bacteriophage Attachment and Release Studies

The small-scale column studies, conducted by graduate research assistant Jonathan Loveland, examined the mechanisms of bacteriophage PRD1 attachment and release in porous media. The bacteriophage PRD1 is a good surrogate for animal viruses considered pathogenic to humans. Using a special technique called *static columns*, Loveland tested the effect of varying solution pH on the reversibility of virus attachment to quartz grains and ferric oxyhydroxide-coated quartz grains. Static columns provide a simple means of measuring attachment and release in a batch-like experiment where the solid/solution ratio is similar to that found in porous media. The porous media materials were chosen and synthesized because they represent the type of porous media found at the Cape Cod field site.

Two types of static column studies were performed: equilibrium and kinetic. The equilibrium studies revealed that the PRD1 attachment is reversible only when the pH of the solution is about 2.5 to 3.5 pH units above the isoelectric point of the mineral surface. Attachment of PRD1 to the mineral surfaces was irreversible at lower pH values. For example, the isoelectric point of the synthesized ferric oxyhydroxide-coated quartz was 5.1; therefore, PRD1 were irreversibly attached at pH values of less than about 8.0. The equilibrium experiments also revealed that the PRD1

irreversibly attached at a lower pH value could be released if the column were flushed with a solution of higher pH, again about 2.5 to 3.5 pH units above the mineral isoelectric point. The reversibility of virus attachment is an important issue in modeling the transport of viruses in groundwater. These results were published in *Colloids and Surfaces A: Physicochemical and Engineering Aspects*.

The kinetic experiments measured the rates of virus attachment and release as a function of solution pH. The kinetics of PRD1 attachment and release were modeled according to the reversibility of the process. These experiments showed that decreases in the electrostatic repulsion between the PRD1 and the mineral surfaces resulted in faster attachment and slower release. The kinetic rate coefficients for attachment and release were successfully related to the changes in the intersurface potential energy between the PRD1 and mineral surfaces. These results are currently being written up as a manuscript for submission to the *Journal of Colloid and Interface Science*.

Intermediate-Scale Aquifer Tanks: Physical and Geochemical Heterogeneities

The intermediate-scale aquifer tank experiments, conducted by graduate research assistant Jeffrey Aronheim, studied the effects of physical and geochemical heterogeneities on virus transport. Using the 10-m long aquifer tank in Tissa Illangasekare's Hydraulics Laboratory, Aronheim created slices of aquifers consisting of homogeneous fine sand, physically heterogeneous fine and coarse sand, and geochemically heterogeneous fine quartz sand and ferric oxyhydroxide-coated quartz sand. The goal of these aquifer tank tests was to recreate the types of heterogeneities encountered at the Cape Cod field site.

Preliminary one-dimensional flow-through column (0.5 m length) experiments were performed with each of these materials to provide basic hydraulic (e.g., hydraulic conductivity) and PRD1 transport (e.g., first-order attachment and release rate coefficients) parameters. These experiments provided PRD1 transport parameters that agreed well with the static column experiments described above.

The three aquifer tank experiments were conducted by injecting radiolabeled PRD1 (^{35}S, ^{32}P) and sodium bromide tracers at one end of the tank and monitoring their progress in arrays of sampling ports along a total of 8 m of transport. The tracer data were used to estimate the dispersivity in the aquifer tank, a measurement that must be estimated at the length scale of the experiment. The PRD1 data showed that viruses respond to heterogeneities similarly to dissolved solutes. In the physically heterogeneous porous media, the PRD1 moved through the coarse sand more rapidly than through the fine sand. In the geochemically heterogeneous porous media, the PRD1 moved through the fine quartz sand, but were attenuated in the ferric oxyhydroxide-coated sand.

The two-dimensional PRD1 transport behavior was successfully modeled using the MODFLOW transport code adapted to handle first-order attachment and release kinetics. Parameters determined in the one-dimensional column experiments were used in the model. The results of these experiments are currently being written up as a manuscript for submission to *Ground Water*.

Field-Scale Experiments: PRD1 Transport in the Cape Cod Aquifer

To test the effects of solution chemistry, mineral surface chemistry, and organic matter on virus

attachment and inactivation in an aquifer, graduate research assistant Annie Pieper orchestrated three injections of ^{32}P-labeled bacteriophage PRD1 into the sewage-contaminated aquifer at the U.S. Geological Survey Cape Cod Toxic Substances Research Site in Massachusetts. This site provided the ideal forum to compare the effect of sewage contamination on virus transport because the U.S. Geological Survey has installed thousands of sampling points above and within the sewage plume emanating from infiltration beds on the Massachusetts Military Reservation.

Permission to inject the radiolabeled viruses, secured by the U.S. Geological Survey site coordinator, Denis LeBlanc, allowed us to compare the transport of the total PRD1 population (measured by radioassay) to the infective PRD1 population (measured by plaque assay). With these two measures of PRD1 transport, we could distinguish the separate contributions of attachment and inactivation on PRD1 removal. Such information has only infrequently been obtained in the laboratory and never in the field.

In addition to the PRD1, we injected linear alkylbenzene sulfonates (LAS) and tracers into the two aquifer zones: (1) contaminated (suboxic, elevated organic matter) and (2) uncontaminated (oxic, low organic matter). The injections were made over a period of 70 days during the summer of 1994 and required a Herculean effort of Annie Pieper in organizing the field crew: three undergraduate research assistants, two graduate research assistants, a USGS research associate, and two of the project PIs (Joe Ryan and Ron Harvey). In addition, the injections were coordinated with injections of bacteria and protozoa by researchers from the University of New Hampshire.

The injected PRD1, LAS, and tracers were monitored over a 4-m distance. In the unaltered groundwater, PRD1 traveled more rapidly through the contaminated zone than through the uncontaminated zone. The injection of LAS remobilized the attached PRD1 in the contaminated zone, but not in the uncontaminated zone. Co-injection of the LAS with PRD1 further diminished PRD1 transport in the uncontaminated zone. In the contaminated zone, LAS initially caused near-complete attenuation of total and infective PRD1; however, nearly all of the total PRD1 broke through later without infective PRD1. The lack of infective PRD1 in the retarded breakthrough suggests that attachment accelerated the rate of PRD1 inactivation in this aquifer.

The results of these experiments indicate that virus transport models should be improved to account for irreversible attachment, porous medium surface heterogeneity, and virus inactivation on porous media surfaces. A manuscript describing these results was just submitted to *Water Resources Research.*

Opportunities Resulting from this Research

We are currently planning to conduct a similar virus transport experiment at the Cape Cod site in conjunction with a colloid mobilization and transport experiment funded by a cooperative agreement with the U.S. Environmental Protection Agency's R.S. Kerr Environmental Research Laboratory in Ada, Oklahoma.

We have submitted two virus transport proposals, one in response to the U.S. Environmental Protection Agency's request for proposals on Risk Reduction in April 1995 and one to the American Water Works Association Research Foundation in July 1995; however, both of these proposals were unfunded. Owing to the unique information we gained in the field test using the radiolabeled viruses, we have just been invited to submit a proposal to the R.S. Kerr laboratory for further field work on virus transport.

Project Publications

A. Journal Papers

Loveland J.P., Ryan J.N., Amy G.L., and Harvey R.W., 1996. The reversibility of virus attachment to mineral surfaces. *Colloids and Surfaces A. Physicochemical and Engineering Aspects* **107**, 205-222.

Ryan J.N. and Elimelech M., 1996. Colloid mobilization and transport in groundwater. *Colloids and Surfaces A. Physicochemical and Engineering Aspects* **107**, 1-56.

Pieper A.P., Ryan J.N., Amy G.L., Illangasekare T.H., Harvey R.W., and Metge D.W., Effect of linear alkylbenzene sulfonates on the natural-gradient transport of bacteriophage PRD1 through contaminated and uncontaminated zones of a sewage-contaminated aquifer. Submitted to *Water Resources Research*, February 1996.

Loveland J.P., Ryan J.N., Amy G.L., and Harvey R.W., in preparation. The kinetics of bacteriophage PRD1 attachment and release in quartz and ferric oxyhydroxide-coated quartz porous media. To be submitted to *Journal of Colloid and Interface Science*.

Ryan J.N., Illangasekare T.H., and Aronheim J.S., in preparation. Transport of bacteriophage PRD1 through physically and geochemically heterogeneous porous media: Experiments and two-dimensional modeling. To be submitted to *Ground Water*.

B. Conference Proceedings

Ryan J.N., Loveland J.P., Amy G.L., and Harvey R.W., 1993. Effect of (bio)colloid size on the kinetics of detachment from packed bed surfaces. *Eos, Transactions of the American Geophysical Union* **74**, 132. Presented at Spring Meeting, American Geophysical Union, Baltimore, MD, May 1993.

Loveland J.P., Amy G.L., Harvey R.W., and Ryan J.N., 1994. Effect of chemical perturbations on virus attachment and detachment: Relating kinetics to intersurface potential energy. *American Chemical Society Meeting Abstracts, Division of Environmental Chemistry* **34(1)**, 445-449. Presented at 207th American Chemical Society National Meeting, San Diego, CA, March 1994.

Loveland J.P., Ryan J.N., Amy G.L., and Harvey R.W., 1995. Attachment and release of viruses from mineral surfaces: The role of secondary minimum attachment. *American Chemical Society Meeting Abstracts, Division of Environmental Chemistry* **35(1)**, 517-520. Presented at 209th American Chemical Society National Meeting, San Diego, CA, April 1995.

Pieper A.P., Ryan J.N., Amy G.L., Illangasekare T.H., Harvey R.W., and Metge D.W., 1995. Effect of linear alkylbenzene sulfonates on the natural-gradient transport of bacteriophage PRD1

through contaminated and uncontaminated zones of an unconfined sand aquifer. *Eos, Transactions of the American Geophysical Union. EOS Trans. Amer. Geophys. Union* **76**, F200. Presented at the Fall Meeting of the American Geophysical Union, San Francisco, CA, December, 1995.

C. Conference Presentations

Aronheim J.S., Illangasekare T.H., Ryan J.N., Amy G.L., Harvey R.W., Loveland J.P., and Pieper A., 1994. Biocolloid transport through saturated porous media: A two-dimensional pilot-scale study. Presented at the Conference on Hazardous Waste Remediation, Bozeman, MT, June 1994.

Ryan J.N., Amy G.L., Illangasekare T.H., and Harvey R.W., 1996. Virus transport in groundwater: Laboratory and field experiments. Invited presentation at American Chemical Society, Rocky Mountain Regional Meeting, Environmental Chemistry Symposium, Lakewood, Colorado, June 1996.

D. Theses and Dissertations

Aronheim J.S., 1995. Virus Transport in Groundwater: Modeling of Bacteriophage PRD1 Transport Through One-Dimensional Columns and a Two-Dimensional Aquifer Tank. M.S. Thesis, Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado, 368 pp.

Loveland J.P., 1995. Virus Transport in Groundwater: Describing Bacteriophage PRD1 Interactions with Mineral Surfaces Using Intersurface Potential Energy. M.S. Thesis, Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado, 119 pp.

Pieper A.P., 1995. Virus Transport in Groundwater: A Natural-Gradient Field Experiment in a Contaminated Sandy Aquifer. M.S. Thesis, Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado, 134 pp.